From DEPARTMENT OF MEDICINE, SOLNA
Karolinska Institutet, Stockholm, Sweden

UNDERSTANDING THE ROLE OF NON-CODING RNAs IN SKIN HOMEOSTASIS AND CANCER

KUNAL DAS MAHAPATRA

Karolinska
Institutet

Stockholm, 2020

All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by Eprint AB, 2020
© Kunal Das Mahapatra, 2020

ISBN 978-91-7831-900-8

Understanding the role of non-coding RNAs in skin homeostasis and cancer

THESIS FOR DOCTORAL DEGREE (Ph.D.)

The thesis will be defended on August 28, 2020

Location: Visiongatan 18, CMM Lecture Hall, Ground Floor, L8:00
Center for Molecular Medicine, Solna, Stockholm

By

KUNAL DAS MAHAPATRA

Principal Supervisor:
Andor Pivarcsi, Associate Professor
Karolinska Institutet
Department of Medicine, Solna
Division of Dermatology and Venereology

Co-supervisor(s):
Mona Ståhle, Professor
Karolinska Institutet
Department of Medicine, Solna
Division of Dermatology and Venereology

Leonard Girnita, Associate Professor
Karolinska Institutet
Department of Oncology-Pathology

Johan Hansson, Professor
Karolinska Institutet
Department of Oncology-Pathology

Opponent:
Wim Declercq, Professor
Ghent University
Department of Biomedical Molecular Biology

Examination Board:
Gonçalo Castelo-Branco, Associate Professor
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Hans Törmä, Professor
Uppsala University
Department of Dermatology and Venereology

Elisabet Nylander, Professor
Umeå University
Department of Public Health and Clinical Medicine

ABSTRACT

The epidermis is a stratified epithelium with continuous self-renewing capacity. As the outermost layer of our body, it provides a protective barrier against external trauma, produces pigmentation, and keeps the skin hydrated. Keratinocytes are the primary constituent cells type within the epidermis. A fine balance is maintained between keratinocyte cell proliferation and differentiation to sustain a functional epidermis. The interplay between multiple signaling pathways, transcription factors, epigenetic modulators, and non-coding RNAs is the key to maintaining this balance. Disrupted epidermal homeostasis can cause various diseases, including cancer. Cutaneous squamous cell carcinoma (cSCC) is one such keratinocyte-derived cancer that begins with the accumulation of somatic mutations and genetic abnormalities. The pigment-producing melanocytes within the epidermis can undergo oncogenic transformation due to numerous genetic and environmental factors, and give rise to malignant melanoma. In this thesis, we have explored the role of non-coding RNAs in epidermal homeostasis and the development of skin cancers.

Paper I: In this study, we investigated the role of miR-203 in cSCC, and found that its expression was negatively correlated with the differentiation grade of the tumors. Functionally, miR-203 inhibited cell cycle progression, self-renewability, motility and proangiogenic-activity of cSCC cells *in vitro*, and reduced xenograft tumor growth and angiogenesis *in vivo*. We identified c-MYC as a potential upstream regulator of the transcriptomic changes caused by miR-203 overexpression, and subsequently demonstrated that c-MYC is a direct target of miR-203 in cSCC. In line with these findings, overexpression of c-Myc rescued the growth-inhibitory effect of miR-203 in cSCC cell lines.

Paper II: In this study, we analyzed the small RNA-seq data from the skin cutaneous melanoma (TCGA SKCM-cohort) and found that miR-203 is the most downregulated miRNA in metastatic melanoma. Moreover, high miR-203 abundance seems to confer longer overall survival to patients with metastatic melanoma. Methylome data from patient samples, together with results from *in vitro* experiments, suggested that promoter hypermethylation could suppress miR-203 expression in metastatic tumors. Functionally, miR-203 acted as a tumor suppressor by inhibiting cancer/metastatic hallmarks such as cell migration, invasion, self-renewal, and angiogenesis. SLUG, an essential regulator of epithelial-mesenchymal transition, was found to be a direct target of miR-203. *In vivo*, miR-203 effectively suppressed melanoma metastasis to the inguinal lymph nodes and the lungs.

Paper III: In this study, we investigated the changes in the coding and non-coding landscape in cSCC using RNA-seq. We identified a large number of differentially expressed coding transcripts linear lncRNAs and circRNAs. Representative transcripts from each group were validated using an extended cohort. We found that several transcription factors regulating skin development and cSCC oncogenesis were altered at mRNA level. In addition to various lncRNAs with potential oncogenic function, we identified a set of skin-specific lncRNAs, which were mostly downregulated in cSCC. We observed a global downregulation of circRNA abundance in cSCC. Apart from previously annotated circRNAs, novel skin-enriched circRNAs were also identified and validated.

Paper IV: In this study, we characterized a skin-specific lncRNA located at the Epidermal Differentiation Complex (EDC) on human chromosome 1. It is highly induced during the late stages of keratinocyte differentiation and localized to the granular layer of the human epidermis. We showed that transcription factor YY1 suppresses its expression in the progenitor keratinocytes. CRISPR-mediated activation of this lncRNA locus led to an increased expression of late differentiation marker genes. In contrast, loss-of-function experiment in a 3D organotypic skin model resulted in impaired terminal differentiation program and formation of thinner cornified envelope. Due to its functional requirement in late differentiation, we have renamed this lncRNA as ELDAR (Epidermal Late Differentiation Associated RNA).

LIST OF SCIENTIFIC PAPERS

I. **MicroRNA-203 inversely correlates with differentiation grade, targets c-MYC, and functions as a tumor suppressor in cSCC**

   Warangkana Lohcharoenkal, Masako Harada, Jakob Lovén, Florian Meisgen, Ning Xu Landén, Lingyun Zhang, Jan Lapins, **Kunal Das Mahapatra**, Hao Shi, Liisa Nissinen, Veli-Matti Kähäri, Mona Ståhle, Enikö Sonkoly, Dan Grander, Marie Arsenian-Henriksson and Andor Pivarcsi

   Journal of Investigative Dermatology. 2016 Dec 1;136(12):2485-94.

II. **Genome-wide screen for microRNAs reveals a role for miR-203 in melanoma metastasis**

   Warangkana Lohcharoenkal, **Kunal Das Mahapatra**, Lorenzo Pasquali, Caitrin Crudden, Lara Kular, Yeliz Z. Akkaya Ulum, Lingyun Zhang, Ning Xu Landén, Leonard Girnita, Maja Jagodic, Mona Ståhle, Enikö Sonkoly and Andor Pivarcs

   Journal of Investigative Dermatology. 2018 Apr 1;138(4):882-92

III. **A comprehensive analysis of coding and non-coding transcriptomic changes in cutaneous squamous cell carcinoma**

   Kunal Das Mahapatra, Lorenzo Pasquali, Jonas Nørskov Søndergaard, Jan Lapins, István Balazs Nemeth, Eszter Baltás, Lajos Kemény, Bernhard Homey, Liviu-Ionut Moldovan, Jørgen Kjems, Claudia Kutter, Enikö Sonkoly, Lasse Sommer Kristensen and Andor Pivarcsi

   Scientific Reports. 2020 Feb 27;10(1):1-2.

IV. **LncRNA ELDAR acts as a key regulator of late epidermal differentiation program in the human epidermis**

   Kunal Das Mahapatra, Lorenzo Pasquali, Christian Ziegler, Johannes Graf, Nicole Hemmer, Li Chen, Eniko Sonkoly, Markus Kretz, Andor Pivarcsi

   Manuscript

PUBLICATIONS NOT INCLUDED IN THE THESIS

Chromatin interactions in differentiating keratinocytes reveal novel Atopic Dermatitis and Psoriasis genes

Pelin Sahlén, Rapolas Spalinskas, Samina Asad, **Kunal Das Mahapatra**, Pontus Höjer, Jesper Eisfeldt, Ankit Srivastava, Pernilla Nikamo, Anaya Mukherjee, Kyu-Han Kim, Otto Bergman, Mona Ståhle, Enikö Sonkoly, Andor Pivarcsi, Carl-Fredrik Wahlgren, Magnus Nordenskjöld, Fulya Taylan, Maria Bradley and Isabel Tapia-Páez

Submitted

The keratinocyte transcriptome in psoriasis: pathways related to immune responses, cell cycle and keratinization

Lorenzo Pasquali, Ankit Srivastava, Florian Meisgen, **Kunal Das Mahapatra**, Ping Xia, Ning Xu Landén, Andor Pivarcsi, Enikö Sonkoly

Acta Derm Venereol. 2019 Feb 1;99(2):196-205.

MicroRNA-130a acts as a tumor suppressive miRNA in cutaneous squamous cell carcinoma and regulates the activity of the BMP/SMAD1 pathway by suppressing ACVR1

Warangkana Lohcharoenkal, Chen Li, **Kunal Das Mahapatra**, Jan Lapins, Bernhard Homey, Enikö Sonkoly and Andor Pivarcsi

Submitted

Contents

LIST OF ABBREVIATIONS 1
1 INTRODUCTION 3
1.1 The Skin 3
1.1.1 The epidermis 4
1.1.2 Epidermal differentiation and barrier formation 4
1.1.3 The Epidermal differentiation complex 6
1.1.4 Regulation of epidermal differentiation 8
1.1.4.1 Signaling pathways 8
1.1.4.2 Transcription factors 10
1.1.4.3 Non-coding RNAs in epidermal homeostasis 11
1.2 Cutaneous squamous cell carcinoma 13
1.2.1 Epidemiology 13
1.2.2 Risk factors 13
1.2.3 Molecular pathogenesis 14
1.2.4 Treatment 16
1.3. Cutaneous melanoma 17
1.3.1 Epidemiology 17
1.3.2 Cell of origin 17
1.3.3 Risk factors 17
1.3.4 Molecular pathogenesis 18
1.3.5 Treatment 20
1.4 Non-coding RNAs 21
1.4.1 MicroRNAs 22
1.4.1.1 MiRNA biogenesis and mode of function 22
1.4.1.2 Regulation of miRNA biogenesis and function 23
1.4.1.3 MiRNAs in cancer 25
1.4.1.4 MiRNAs in cutaneous squamous cell carcinoma 26
1.4.1.5 MiRNAs in cutaneous malignant melanoma 27
1.4.2 Long non-coding RNAs 29
1.4.2.1 Genomic location 29
1.4.2.2 Subcellular localization and functional classes 30
1.4.2.3 LncRNAs in epidermal differentiation and cSCC 34

1.4.3 Circular RNAs 37
1.4.3.1 CircRNA biogenesis and turnover 37
1.4.3.2 Modes of functions 38
2 AIMS 39
3 MATERIALS AND METHODS 40
4 RESULTS AND DISCUSSION 49
4.1 MiRNA-203 inversely correlates with differentiation grade, targets c-MYC, and functions as a tumor suppressor in cSCC 49
4.2 Genome-wide screen for miRNAs reveals a role for miR-203 in melanoma metastasis 54
4.3 A comprehensive analysis of coding and non-coding transcriptomic changes in cSCC 60
4.4 Characterization of lncRNA ELDAR as a critical regulator of late epidermal differentiation 68
5 CONCLUDING REMARKS 75
6 ACKNOWLEDGMENTS 78
7 REFERENCES 82

LIST OF ABBREVIATIONS

ADAR	Adenosine deaminase acting on RNA
ALOX	Arachidonate 12-lipoxygenase
cAMP	cyclic Adenosine monophosphate
ATF	cAMP-dependent transcription factor
AURKA	Aurora kinase A
CDSN	Corneodesmosin
CREB	cAMP response element-binding protein
DGCR8	DiGeorge syndrome chromosomal region 8
ECL	Enhanced chemiluminescence
EDC	Epidermanl differenitation complex
EDTA	Ethylenediaminetetraacetic acid
EdU	5-Ethynyl-2´-deoxyuridine
EGFR	Epidermal growth factor receptor
ENCODE	Encyclopedia of DNA Elements
ERK	Extracellular receptor kinase
FANTOM	Functional annotation of the mamalian genome
FAR2	Fatty acyl.-CoA reductase 2
FBS	Fetal bovine serum
FDA	Food and drug adminstration
FIRRE	Functional intergenic repeating RNA element
FUS	RNA binding protein-FUS
GFP	Green fluoroscent protein
GRHL3	Grainyhead-like 3
HBEGF	Heparin binding EGF-like growth factor
HEPES	N-2-hydroxyethylpiperazine-N-ethanesulfonic acid
HGF	Haematopoietic growth factor
HOTAIR	HOX transcript antisense RNA
HUVEC	Human umbilical vein endothelial cell
KIF	Keratin intermediate filament
LB	Lamellar body
LCE	Late cornified envelope

MAF	Musculoaponeurotic fibrosarcoma oncogene homolog
MALAT1	Metastasis associated lung adenocarcinoma transcript 1
MAX	MYC Associated Factor X
MEK	Mitogen-activated protein kinase kinase
MET	Hepatocyte growth factor receptor or tyrosine-protein kinase
MITF	Melanocyte Inducing Transcription Factor
MYC	MYC Proto-Oncogene
NEAT1	Nuclear enriched abundant transcript 1
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NRF1	Nuclear respiratory factor 1
NSG	NOD scid gamma
PBS	Phosphate buffer saline
PCNA	Proliferating cell nuclear antigen
PD-1	Programmed cell death protein 1
PDCD4	Programmed cell death 4 (Neoplastic transformation inhibitor)
PFA	Paraformaldehyde
Poly-HEMA	Poly-2-hydroxyethyl methacrylate
PTEN	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein
QKI	QKI, KH domain containing RNA binding
RBP	RNA binding protein
RPKM	Reads per kilobase million
SMAD	Mothers against decapentaplegic homolog
SP1	Specificity protein 1
SPINK5	Serine peptidase inhibitor Kazal type 5
STAT	Signal transducer and activator of transcription
TARBP	Transactivating RNA-binding protein 1
TGM	Transglutaminase
UTR	Untranslated region
UV	Ultra violet
WNT	Wingless-related integration site
XIST	X-inactive specific transcript
ZEB	Zinc finger E-box-binding homeobox

1 INTRODUCTION

1.1 The Skin

The skin is the largest organ of our body. Operating as the outermost physical, chemical and immunological barrier, it not only protects our body from external trauma, harmful chemicals, and microorganisms but also prevents the loss of vital fluids and nutrients (Abdallah, Mijouin, and Pichon 2017; Simpson, Patel, and Green 2011). Besides the protective responsibilities, the skin also functions as the primary sensory organ. In order to perform this plethora of sophisticated functions, the skin has a highly organized and layered structure, along with key ancillary components such as hair follicles, nails, sweat and sebaceous gland. From inside to outside, three morphologically distinct layers of the skin are present; hypodermis, dermis and epidermis (*Figure 1*) (Abdo, Sopko, and Milner 2020). Hypodermis majorly consists of adipose tissues and provides thermal insulation, source of energy storage and a protective cushion to the inner organs. Being the middle layer, the dermis contains connective tissues, blood and lymph vessels, nerves, hair follicles, sweat and sebaceous glands; and provides physical support, oxygen, and nutrient supply to the epidermal cells. Between dermis and epidermis, there is a basement membrane, which provides an anchorage for the basal epidermal cells and a connecting interface between the two separated layers (Simpson et al. 2011).

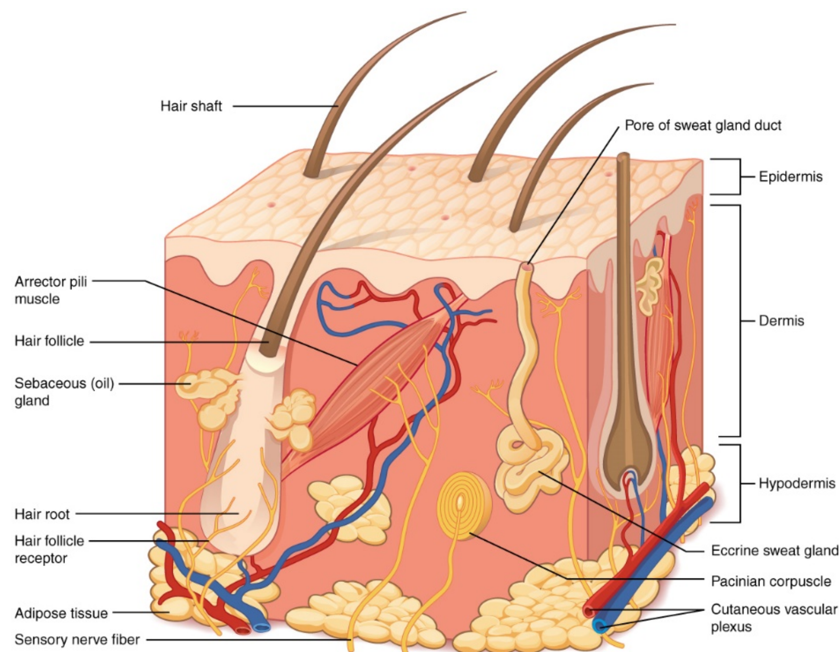

Figure 1: *Anatomy of the human skin with different layers and appendages. Downloaded from http://openstax.org/books/anatomy-anad-physiology/pages/1-introduction. Creative Commons Attribution 4.0 International license.*

1.1.1 The epidermis

The outermost layer of the skin is the epidermis, a multilayered squamous epithelium. Apart from providing a physical barrier against external insults, it also serves as a permeability barrier against subcutaneous water and nutrient loss. Keratinocytes are the major constituent cell types (~90%) within the epidermis. The remaining cells are the pigment-producing melanocytes, specialized cells of the immune system, and the mechanoreceptor Merkel cells. The epidermis itself is divided into four different layers with distinct states of differentiation (*Figure 2*) (Simpson et al. 2011). The innermost strata is the basal layer- a single layer reservoir of mitotically active progenitor keratinocytes. As the basal progenitor cells detach from the basement membrane and move upwards, they withdraw from cell cycle, and undergo a highly ordered terminal differentiation program to form the uppermost-cornified layer, via transitioning through two other intermediate layers (Blanpain and Fuchs 2009). The spinous layer, which is located just above the basal layer, consists of 8-10 layers of keratinocytes expressing intermediate keratin filaments and tightly anchored by the desmosomes. In the subsequent granular layer (3-5 layers), the cells start expressing the substrates for the barrier formation. Finally, in the cornified layer (squames), the enucleated and metabolically inactive cells undergo a unique process of cell death (cornification) while being wrapped up in an insoluble lipid envelope (Eckhart et al. 2013; Kalinin, Marekov, and Steinert 2001).

1.1.2 Epidermal differentiation and barrier formation

At the core of epidermal homeostasis is a finely orchestrated interplay between proliferation, differentiation and cell death. There are two proposed models to define how basal layer keratinocytes maintain a constant pool of progenitor cells, while also generating another group of cells programmed to undergo the terminal differentiation program (Fujiwara, Tsutsui, and Morita 2018; Ghadially 2012). In the classical model, a small population of long-lived, self-maintaining keratinocyte stem cells gives rise to short-lived transit-amplifying cells (TA), which in turn commit to the differentiation program after a few rounds of cell division (Jones and Simons 2008). An alternative model contradicts the stem cells/TA model and hints at a constant pool of progenitor keratinocytes undergoing asymmetric and symmetric cell division at a fixed probability (Clayton et al. 2007; Lim et al. 2013). While symmetric cell division maintains the progenitor keratinocyte pool, the daughter cells from the asymmetric cell division are fated to undergo terminal differentiation after receiving various cues such as increasing

calcium concentration (both intracellular and extracellular), altered cell-cell communication and activation of differentiation-promoting signaling pathways (Clayton et al. 2007; Schepeler, Page, and Jensen 2014).

Epidermal keratinocytes express various differentiation stage-specific keratins- the primary structural proteins that assemble as obligate heterodimers into an intricate network of 10-nm intermediate filaments (KIFs) (Candi et al., 2005). For instance, keratin 5 (K5) and keratin 14 (K14) is typically expressed in the progenitor keratinocytes within the basal layer where they form stable dimers or tetramers (*Figure 2*) (Natsuga 2014). As the differentiating cells move towards the suprabasal layer, K5 and K14 pairs are replaced by keratin 1 (K1) and keratin 10 (K10) filaments (Natsuga 2014). Later, the keratinocytes within the granular layer express other structural proteins such as loricrin (LOR), trichohyalin (THH), small proline-rich proteins (SPRRs), involucrin (IVL), and profilaggrin (precursor for filaggrin monomers) which are accumulated within the keratohyalin granules (KHGs) (*Figure 2*) (Eckhart et al. 2013). Another feature of the granular layer architecture is the cytoplasmic accumulation of lamellar bodies, which contain pro-barrier lipids (ceramides, free-fatty acid and cholesterol), crucial enzymes for cornification and structural proteins for corneodesmosomes as cargo (Menon, Lee, and Lee 2018).

As the keratinocytes transition into the cornified layer, profilaggrin is released from the KHGs, followed by dephosphorylation and proteolytic cleavage into filaggrin monomers (Eckhart et al. 2013). Free filaggrin monomers aggregate and compact the keratins into tight bundles, causing a complete collapse of the KIF cytoskeleton. As a result, the cellular structure becomes more flat, one of the key characteristics of this layer (Natsuga 2014). Subsequently, filaggrin monomers and other structural proteins such as loricrin, SPRRs, involucrin, periplakin and envoplakin are covalently cross-linked by a number of transglutaminases (TGM1, TGM3 and TGM5) to form a rigid cornified envelope (5-10 nm thick) beneath the plasma membrane (Candi et al., 2005, 2016). The process of cornification is also closely accompanied by enzymatic degradation of cellular organelles such as nuclei, mitochondria and endoplasmic reticulum (Candi et al. 2016; Lippens et al. 2005). In the final step, the lamellar bodies fuse with the plasma membrane to extrude their lipid content to the extracellular matrix that forms an impermeable lipid lamellae (Eckhart et al. 2013; Menon et al. 2018). This densely packed layer of lipid lamellae and corneocytes, which are tightly connected by corneodesmosomes, plays a decisive role in preventing the loss of water and electrolytes through the epidermal barrier (Candi et al. 2005a; Proksch, Brandner, and Jensen 2008). The cells of the cornified

layer are continuously sloughed off and regularly (~4 weeks) replaced by the basal keratinocytes committed to terminal differentiation (Blanpain and Fuchs 2009).

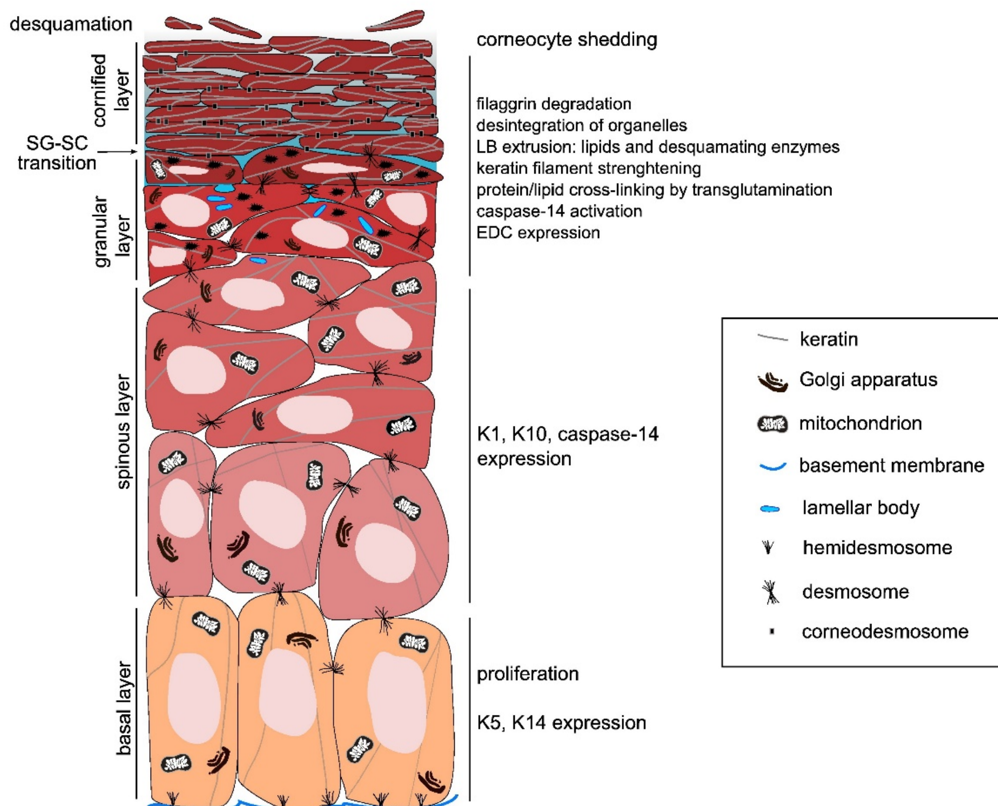

Figure 2: *Key structural components and the fundamental molecular events associated with epidermal stratification and differentiation. Modified from* (Eckhart et al. 2013).

1.1.3 The Epidermal differentiation complex

Majority of the genes encoding for essential structural proteins for the terminal differentiation of keratinocytes are clustered together in a 2-Mb long genomic region on the human chromosome 1q21 (Abhishek and Palamadai Krishnan 2016; Marenholz et al. 2001). This region, known as the Epidermal Differentiation Complex (EDC) contains as many as 63 different genes and remains under tight epigenetic and transcriptional governance to ensure their timely and coordinated expression. This region remains conserved in terms of the linearity and synteny of the clustered genes in primates, rodents, marsupials and birds (Oh and de Guzman Strong 2017). Interestingly, EDC also turns out to be one of the fastest evolving locus as seen from a number of comparative genomics based analysis. EDC-genes in mammals (including primates) have been found to have a clear signature of lineage-specific positive

selections across all the mammals, including apes and humans (Goodwin and de Guzman Strong 2016; Waterson et al. 2005).

Genes within the EDC, except for loricrin and involucrin, are grouped into four gene families based on their structural and functional similarity (*Figure 3*). The boundary of this gene cassette is marked by the presence of the genes coding for EF-hand calcium-sensing proteins of the S100 family. In the middle, there are three tandem clusters of gene families, namely, S100-fused type proteins (filaggrin, filaggrin-2, hornerin, repetin, trichohyalin and trichohyalin-like-1), late cornified envelope proteins (LCEs), and proteins with small proline rich regions (SPRR) (Kypriotou, Huber, and Hohl 2012; Marenholz et al. 2001).

The EDC-genes are expressed in a coordinated manner and play many important roles in terminal differentiation and cornification. For instance, members of the S100 protein family, upon calcium-binding, undergo conformational changes and interact with a wide array of target proteins and nucleic acids to regulate protein degradation, cytoskeletal assembly, cell locomotion, innate immune response, calcium homeostasis and differentiation (Donato et al. 2013). Prior to cornification, filaggrin monomers bind to the KIFs, leading to their aggregation and flattening of the cellular structure. Of note, loss-of-function mutations in filaggrin have been identified to be a causative factor for skin barrier disorders like ichthyosis and atopic dermatitis (Sandilands et al. 2009). Loricrin, which is expressed at the granular layer, accounts for almost 80% of the cornified envelope. Loricrin deficient mice showed delayed barrier formation during the mice embryonic development (Koch et al. 2000). Mutation in human loricrin is proven to be the underlying reason behind congenital skin disorders (e.g. loricrin keratoderma), which are marked by hyperkeratosis and epidermal barrier defect (Schmuth et al. 2004). EDC's functional significance can also be surmised from the presence of several skin disorders-associated (e.g. psoriasis, atopic dermatitis, ichthyosis vulgaris) genetic variants in this region (Hoffjan and Stemmler 2007; Oh and de Guzman Strong 2017). Although filaggrin, loricrin, involucrin, and S100 proteins are well characterized in the context of terminal differentiation, the function of the SPRR and LCE group of proteins remains largely elusive.

Apart from the protein coding genes, there are numerous uncharacterized pseudogene and non-coding RNA locus within the EDC, particularly within the disease susceptibility loci (Tsoi et al. 2015). A number of recent studies show that the evolutionarily conserved non-coding genomic elements within the EDC can regulate the dynamic chromatin architecture and tissue-specific expression of the EDC genes (Oh and de Guzman Strong 2017). However, systemic

characterization of these lncRNA loci and the functional impact of their transcriptional output will be necessary to gain a comprehensive understanding of the regulation of EDC gene expression in terminal differentiation.

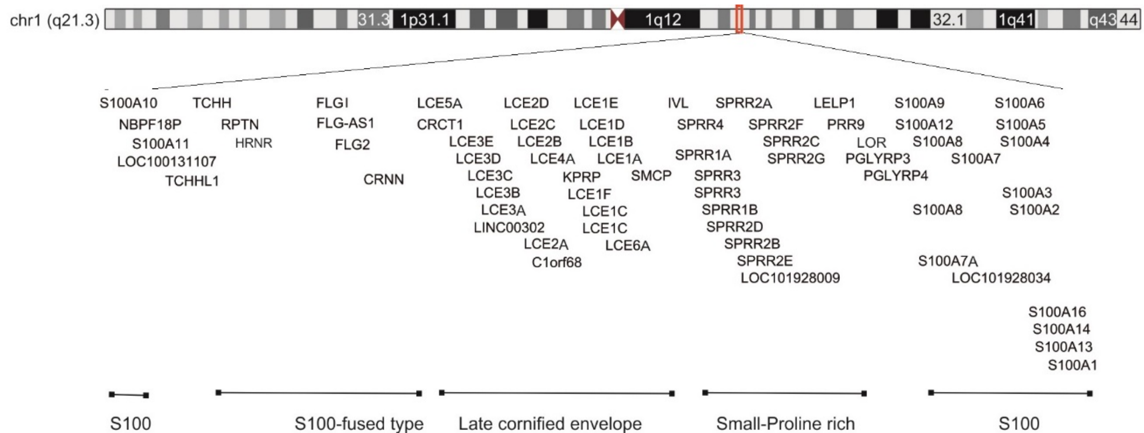

Figure 3: *Schematic representation of EDC on human chromosome: Figure extracted and modified from UCSC genome browser using hg38 assembly and Refseq gene predictions from NCBI Homo sapiens annotation (release 109.20190905).*

1.1.4 Regulation of epidermal differentiation

Regulation of epidermal differentiation is a multilevel process involving a coordinated interplay of signaling pathways, transcription factors, epigenetic modifiers and non-coding RNAs. This section will enumerate some of the essential regulatory units and outline their functional relevance in the process of epidermal development.

1.1.4.1 Signaling pathways

Among various signaling pathways, calcium signaling acts as the pivotal point in keratinocyte differentiation (Tu and Bikle 2013). Briefly, the binding of calcium ions to the calcium-sensing receptors present on the keratinocytes activates the calcium-signaling pathway that leads to E-cadherin mediated cell-cell interaction and transcriptional activation of various structural genes. Besides, increase in the extracellular calcium leads to activation of phospholipase C (PLC), which converts phosphatidylinositol bisphosphate to inositol trisphosphate, which mediates the release of intracellular calcium from Golgi and endoplasmic reticulum (ER) and an increased calcium influx mediated through calcium channels (Bikle, Xie, and Tu 2012). Loss of intracellular calcium results in ER stress signaling, and activating ER-stress associated transcription factor XBP, which in turn promotes epidermal differentiation (Celli et al. 2011).

Calcium sensing also activates protein kinase C (PKC) that further leads to activation of pro-differentiation transcription factor AP-1 (Welter et al. 1995a).

In conjunction with calcium signaling, several other signaling pathways such as Notch, mitogen-activated protein kinase (MAPK), transforming growth factor beta (TGF-β), and nuclear factor-κB (IKK/NF-κB) play important roles in epidermal differentiation by activating downstream transcription factors (Klein et al. 2017; Klein and Andersen 2015; Segre, Bauer, and Fuchs 1999:4; Sen et al. 2012).

Multiple lines of evidence show that Notch signaling plays crucial role both in cell cycle withdrawal and in induction of terminal differentiation of keratinocytes. Conditional Notch1 deletion resulted in keratinocyte hyperproliferation and depleted spinous and granular layer in mice epidermis (Moriyama et al. 2008; Watt, Estrach, and Ambler 2008). Using Notch1 knockout mice, Rangarajan et al., showed that Notch1 activates p21 to induce growth arrest in differentiating keratinocytes (Rangarajan et al. 2001). A subsequent study from the same group showed Notch1 can also suppress p63, a master regulator of epidermal differentiation, through negative regulation of the components of interferon signaling (IRF3 and IRF7) (Nguyen et al. 2006).

A number of studies have linked IKK/NF-κB signaling pathway to epidermal homeostasis and skin immune response. First, in a knockout mice model of IκB kinase α, an important non-canonical initiator of the NF-κB pathway, the epidermis failed to differentiate and remained hyperproliferative (Hu et al., 1999). A later study reported that independent of NF-κB pathway, IKKα functions as a co-factor of SMAD2/3 to induce the inhibitors of c-MYC, which in turn leads to the suppression of keratinocyte proliferation (Descargues, Sil, and Karin 2008). A functional characterization of the effectors of canonical NF-κB pathway by double deletion of transcription factor *RelA* and *c-Rel* (in a TNF-α-deficient background) showed reduced proliferative and clonogenic capacity of the progenitor cells as well as defective epidermal differentiation during mice embryogenesis (Gugasyan et al. 2004).

Different branches of the canonical MAPK signaling cascade are shown to be indispensable for maintaining the balance between cell proliferation and differentiation/cell death. Using dominant-negative mutants or kinase inhibitors, several studies have outlined the importance of PKC and p38 MAPK pathway in promoting keratinocyte differentiation via transcriptional activators such as AP-1, SP1 and C/EBP (Eckert et al. 2002; Efimova, Broome, and Eckert 2003). In a contrasting manner, MEK1- a mitogen activated protein kinase, which operates

within the MAPK pathway, has shown anti-differentiation characteristics by increasing cell proliferation and β1 and β4 integrin expression, via ERK phosphorylation in human and mice epidermis (Scholl, Dumesic, and Khavari 2004).

1.1.4.2 Transcription factors

In the past decade, a plethora of transcription factors (e.g. p63, KLF4, AP-1, GRHL3, ZNF750, GATA3, MYC, C/EBPα/β, E2F1) have been identified as the primary effector molecules driving both the spatial and temporal changes in gene expression for maintaining epidermal homeostasis (Dai and Segre 2004; Miyai et al. 2016). Transcription factor p63, a homolog of tumor suppressor gene *p53*, plays the role of a master regulator in maintaining the progenitor pool and establishing the basal-spinous switch for epidermal homeostasis (Blanpain and Fuchs 2007). Strikingly, *p63*$^{-/-}$ mice completely failed to form stratified epithelia and died at birth due to dehydration, underscoring its indispensable role in ectodermal differentiation (Mills et al. 1999). Although p63 has many isoforms, a later study using a knockout mouse model of deltaNp63 isoform reproduced the severe effect showed by a complete *p63* knockout on epithelial stratification and limb morphogenesis (Romano et al. 2012). Similar to the mice models, p63 and deltaNp63 loss-of-function studies in human organotypic skin model resulted in the impairment of keratinocyte stratification and differentiation (Truong and Khavari 2007). As the part of an extensive transcriptional network, p63 also exerts its pro-differentiation function through transcriptional activation of *ZNF750,* which in turn activates *KLF4* (Sen et al. 2012).

KLF4, a zinc finger containing transcription factor, has been extensively characterized in the context of epidermal differentiation. The first line of evidence about its functional significance came from the whole body *KLF4*$^{-/-}$ mice, which died after birth due to barrier loss and dehydration (Segre et al. 1999). Complementing this finding, epidermis-specific ectopic overexpression of KLF4 in the basal layer resulted in an accelerated terminal differentiation and early barrier acquisition during mice embryogenesis (Jaubert, Cheng, and Segre 2003). A follow up study from the same group revealed that KLF4 and differentiation-inducing corticosteroids activate an overlapping set of pro-differentiation genes during embryonic barrier acquisition (Patel et al. 2006). Using luciferase-reporter assay, this study demonstrated that KLF4 binds to the promoter region of several genes (e.g. ALOX12B, CDSN, SPINK5, FAR2) associated with different steps in barrier acquisition (Patel et al. 2006).

AP-1 family of transcription factors is composed of multiple jun and fos members that are triggered upon a wide range of extracellular stimuli (Eckert et al. 2013). Upon activation, they regulate various biological pathways such as cell proliferation, differentiation or apoptosis (Hess, Angel, and Schorpp-Kistner 2004). Many of the epidermal structural proteins have AP-1 binding sites within their promoter, indicating a potentially regulatory relationship (Eckert et al. 2013; Welter et al. 1995b). Understanding the function of AP1 transcription factors is quite challenging because dimerization with different partners (fos-fos, jun-fos or jun-jun) can alter their activity in different developmental context (Eckert et al. 2013). A number of studies using conditional knockout or embryonic knockout of individual members of AP-1 family provided some insights into their potential role in skin development. The epidermis-specific c-Jun knockout mice exhibited no defect in skin development. However, the epidermal keratinocytes, when isolated and cultured *in vitro,* displayed reduced proliferative capacity, as well as increased differentiation (Zenz et al. 2003). A second study demonstrated that overexpression of TAM67, a dominant negative mutant of c-Jun that inactivates all AP-1 transcription factors, specifically in the suprabasal layer, causes impaired epidermal differentiation and hyperkeratosis (Young et al. 2017).

1.1.4.3 Non-coding RNAs in epidermal homeostasis

Non-coding RNAs provide an additional layer of regulation for epidermal homeostasis (*Figure 4*). One of the earliest evidences of such non-coding regulators came with the investigation of miR-203 in skin morphogenesis. A number of complementary studies, using primary human keratinocytes and transgenic mice model, reported that PKC and AP-1 signal cascade induces miR-203 expression during terminal differentiation, and it plays a pro-differentiation role through suppression of cell proliferation in the differentiating layers by directly targeting p63 (Lena et al. 2008; Sonkoly et al. 2010; Yi et al. 2008). Phenotypically, miR-203-ablated epidermis exhibited signs of basal cell depletion and severe impairment of suprabasal layer (Yi et al. 2008). In an opposite manner, deletion of miR-205, a progenitor-restricted miRNA in epidermis, resulted in an upregulation of phosphatidylinositol 3-kinase (PI3K) pathway and a premature exit of epidermal stem cells from cell cycle (Wang et al. 2013).

A little after the miRNAs, lncRNAs entered this regulatory landscape of epidermal development. As one of the first lncRNAs to be characterized in the context of epidermal homeostasis- terminal differentiation-induced ncRNA (TINCR) was reported to promote

epidermal differentiation by directly binding and stabilizing the mRNAs of pro-differentiation genes (Kretz et al. 2013a). Conversely, anti-differentiation ncRNA (ANCR) whose expression is strictly restricted to the progenitor layer suppresses differentiation within the basal layer possibly by employing repressive chromatin marks to differentiation-promoting genes (Kretz et al. 2012). The lncRNA-transcription factor interplay came to the foreground, when a follow-up investigation revealed that ANCR and TINCR could link up on to a common regulatory network, also joined by p63, to regulate the pro-differentiation function of the transcription factors MAF and MAFB (Lopez-Pajares et al. 2015). Disrupted functionality of these regulatory components at genetic, transcriptional or post-transcriptional level can lead to aberrant epidermal homeostasis, and ultimately manifests into cutaneous disorders such as ichthyosis, epidermolysis bullosa, psoriasis, basal cell carcinoma and squamous cell carcinoma (Lopez-Pajares et al. 2013).

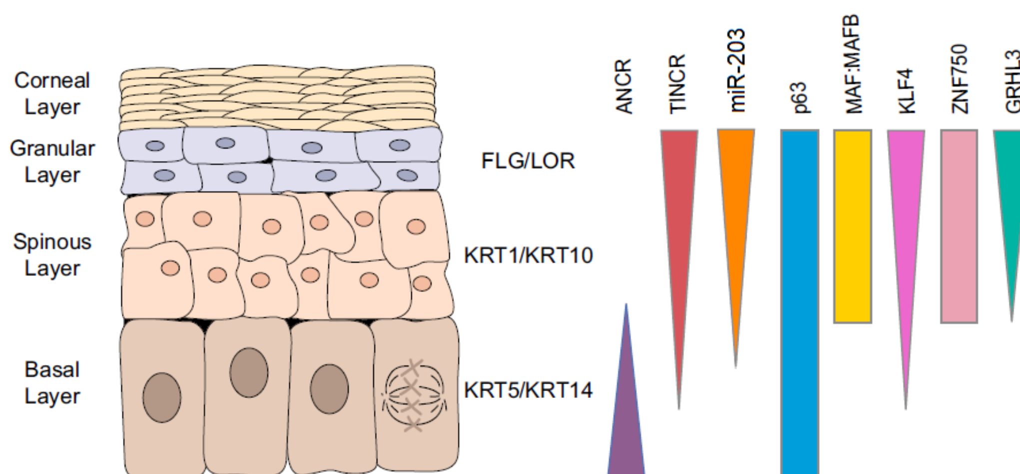

Figure 4: *Spatial expression of key non-coding RNAs and transcription factors in the different layers of the epidermis. Modified from* (Klein and Andersen 2015)

1.2 Cutaneous squamous cell carcinoma

1.2.1 Epidemiology

Non-melanoma skin cancer (NMSC) represents one-third of all the malignancies diagnosed worldwide (Alam and Ratner 2001). Although there are many subtypes of NMSC, keratinocyte-derived cancers, especially basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC) roughly account for 80% and 20% of the diagnosed cases (Alam and Ratner 2001; Rogers et al. 2015). Worldwide, cSCC incidence is increasing steadily among light-skinned people, with an estimated annual incidence over a million only in the US (Rogers et al. 2015). The trend is no different in Sweden, as we see a yearly increase of 6.5% for men and 5.5% for women, making cSCC the second most rapidly increasing malignancy among the Caucasian population (Christensen et al. 2019). CSCC poses a significant health hazard owing to its aggressive nature; occurrence in multiple locations and in some cases it's capacity to metastasize to regional and distant organs (Kwa, Campana, and Moy 1992). Although excision/cryosurgery is the first-line effective treatment for indolent primary tumors (95%), patients with metastatic cSCC (5%) face a remarkably high risk of mortality (5-year survival rate of 25-35%) due to the inefficacy of systemic chemotherapy (Li et al. 2015; Rowe, Carroll, and Day 1992). At present, there are only a few approved therapy available for metastatic cSCC, owing to our poor understanding of cSCC molecular pathogenesis.

1.2.2 Risk factors

Like numerous other cancer types, an individual's risk for developing cSCC is governed by the interaction of phenotypic, genetic, epigenetic and environmental factors (Thompson et al. 2016). However, it is well understood that the single most important risk factor for the development of cSCC is the cumulative exposure to ultraviolet (UV) radiation (Xiang et al. 2014). Although both UV-A and B radiation have been implicated in cSCC, UV-B (290 to 320 nm) from sunlight is the most influential risk factor (Marks 1995; Schmitt et al. 2011). Cumulative UV-B exposure induces non-sense mutation to the tumor suppressor gene p53, which primes the skin for acquiring additional mutations for cSCC development (Brash et al. 1991). Consistent with this, history of sunburn, skin color and age are some of the secondary risk factors for cSCC (Alam and Ratner 2001; Que, Zwald, and Schmults 2018). Notably, organ transplant recipients (OTRs) who have taken immunosuppressive drugs are strongly

predisposed to develop cSCC (60-100 times higher risk than immunocompetent controls), highlighting an important role for the immune system in the protection against cSCCs (Hofbauer, Bavinck, and Euvrard 2010). Historically**,** human papillomavirus (HPV) infection remains a highly debated risk factor for cSCC (Chahoud et al. 2016). Patients with a history of skin injury or chronic diseases such as radiation dermatitis and long-standing ulcers are also at higher risk to develop recurrent cSCC (Longobardi, Sullivan, and Mansour 2011). Genome-wide association studies have also identified numerous cSCC-susceptibility-loci, some related to skin pigmentation and photodistribution, highlighting the heritable phenotypic risk of cSCC (Chahal et al. 2016; Sarin et al. 2020).

1.2.3 Molecular pathogenesis

CSCC, which develops mostly on the sun-exposed skin, proceeds as a progressively invasive malignancy (*Figure 5, 6*). It may arise from precancerous actinic keratosis (AK) or cSCC *in situ* (cSCCIS), and subsequently progress into invasive cSCC and its metastatic variant (Alam and Ratner 2001; Dlugosz, Merlino, and Yuspa 2002). The transformation of a normal keratinocyte to an SCC cell is caused by the accumulation of genetic changes due to cumulative exposure to UV-irradiation (Pickering et al. 2014). The inactivation of p53 is one of the earliest events leading towards oncogenic genomic instability (*Figure 5*) (Jonason et al. 1996; Martincorena et al. 2015). Additionally, loss of function mutation in *NOTCH1* and activating mutations in *HRAS* have been commonly identified in sun-exposed skin, as well as at the onset of cSCC (Martincorena et al. 2015; South et al. 2014). Remarkably, almost 90% of cSCC lesions contain signature of UV-B induced mutation (C to T or CC to TT) in the *p53* gene (BLACK and OGG 2003). Mutation of *p53* can be found already in precancerous lesions and in sun-damaged skin along with the selective expansion of the mutated clones. Inactivation of p53 leads to altered cell cycle regulation and decreased apoptosis, all cumulatively leading towards hyperproliferation of keratinocytes (Nakazawa et al. 1994). A $p53^{-/-}$ mice model illustrated this outcome, as they were prone to develop more papillomas (equivalent to AK) and SCC upon UV-B exposure (Jiang et al. 1999).

Loss-of-heterozygosity (LOH) is another common event in AK and cSCC pathogenesis (*Figure 5*). Chromosomal locations, often corresponding to different tumor-suppressor gene loci, such as Chr: 3p, 13q, 17p, 17q, 9p, and 9q are frequently lost in AK. A far extensive LOH is observed in case of cSCC, with recurrent loss of Chr: 9p, 3p, 2q, 8p and allelic gain of chr3q and 8q

(Ratushny et al. 2012; Rehman et al. 1996). A study based on somatic mutation and copy number analysis of invasive cSCC samples shows a recurrent loss of chr 9p (*CDKN2A* and *2B),* 7p, 11q and frequent allelic gain in Chr 7p (*EGFR*), 8q (*c-Myc*) and 19q (*NOTCH3*), contributing to alteration of Ras pathway, Cell cycle and epidermal differentiation (Li et al. 2015).

Activating mutations in the Kirsten/Harvey Rat Sarcoma 2 viral oncogene homolog (*KRAS* or *HRAS* gene are common features of AK and cSCC. According to the catalogue of somatic mutations in cancer (COSMIC), 21% of cSCCs contain Ras activating mutation, with *HRAS* mutation contributing the most (9%) (Bamford et al. 2004). Besides activating mutations, genomic amplification and sequence rearrangement can also contribute to Ras upregulation and subsequent activation of MAPK pathways (Pierceall et al. 1991). Aberrant activation of EGFR by genomic amplification or increased expression of its ligands have been shown to downregulate p53 and notch1 signaling to promote epidermal oncogenesis (Kolev et al. 2008). A prognostic relevance was uncovered when Cañueto et al. showed that EGFR overexpression in the primary cSCC indicated a strong association with invasiveness (Cañueto et al. 2017). Notch signaling that has been characterized extensively as a tumor suppressor in keratinocytes is also downregulated in cSCC due to low expression of p53, a transcriptional activator for Notch1 (Lefort et al. 2007). Notch1 inactivation has been shown to induce oncogenic Wnt signaling by aberrantly activating beta catenin (Sherwood and Leigh 2016). Activation of signal transducer and activator of transcription 3 (STAT3), a downstream target of EGFR, has also been reported to promote cSCC tumorigenesis in a transgenic mice model, by regulating genes involved in cell cycle regulation and apoptosis (Kim et al. 2009). In addition to the oncogenic mutations in protein coding genes, cSCC pathogenesis could be acutely regulated by the changes in the expression and functionality of various non-coding RNAs.

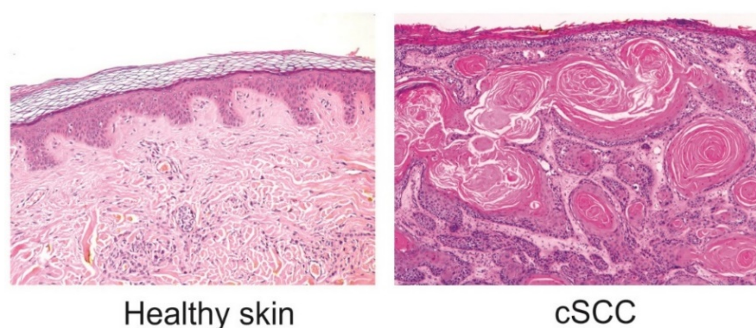

Figure 5: *Normal skin (right) vs cutaneous squamous cell carcinoma (left). In the cSCC specimen, the tumor cells have invaded into the dermis. However, nest of well-differentiated keratinocytes (keratin-pearl) within the dermis can be seen in the histology (left).* (Yanofsky, Mercer, and Phelps 2011)

1.2.4 Treatment

Electrodessication and curettage are some of the effective treatments against low-grade cSCC. For invasive tumors, Mohs micrographic surgery and surgical excision are expensive but extremely potent options. Patients, who are unable to undergo surgical process, can take radiation therapy in case of low-grade tumors. It can also be used as an adjuvant therapy along with surgery against aggressive tumors (Alam and Ratner 2001). For patients with unresectable tumors, two other drugs are proven promising in clinical trials. The first one, an EGFR inhibitor called cetuximab was used in phase II clinical trial in a cohort containing 36 advanced cSCC patients for 6 weeks, resulting in a disease control rate of 69%, with 2 cases of complete and 8 cases of partial remission with the minimum side effect (Maubec et al. 2011; Montaudié et al. 2020). The second drug cemiplimab (PD-1 inhibitor), has been approved by FDA, after it produced a promising and durable response in patients with metastatic or locally advanced cSCC (Falchook et al. 2016; Research 2019). In this regard, various non-coding RNAs, due to their widespread involvement in growth signaling pathways, p53-coordinated gene networks and maintenance of epidermal homeostasis, can provide additional targets for treatment (Gomez et al. 2013; He et al. 2007).

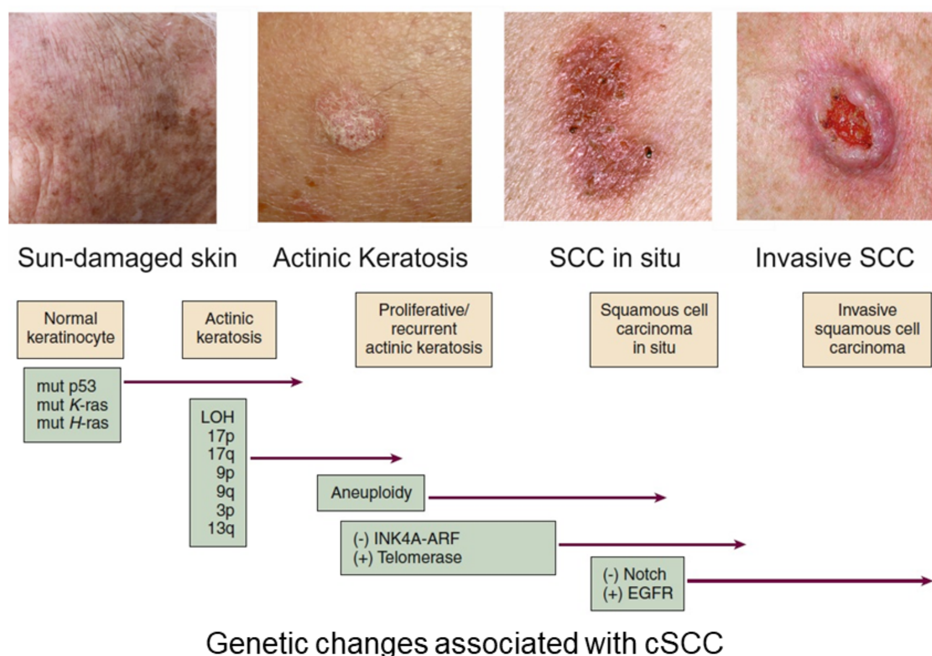

Figure 6: *Progression of cSCC from sun-damaged skin. Key mutations and chromosomal aberrations associated with cSCC progression are shown below the intermediate events. Modified from (Dlugosz et al. 2002).*

1.3. Cutaneous melanoma

1.3.1 Epidemiology

Melanoma, a malignancy of melanocytes, accounts for 5% of all skin cancers, and causes almost 80% of skin cancer-related deaths (Erdmann et al., 2013). Melanoma is more prevalent in the western world, particularly among the fair-skinned Caucasian population (Domingues et al. 2018). The incidence of melanoma is increasing rapidly across the world, with about 100,000 predicted cases in the US only, for 2020 (American Cancer Society, 2020). Although surgery is proven effective against early-stage melanomas, invasive and metastatic melanoma, localized to distant organs (lymph node, lungs, and brain), have a much worse prognosis (not more than 19% survival rate for 5 years after diagnosis) (Sandru et al., 2014).

1.3.2 Cell of origin

Cutaneous melanoma is a malignancy of the pigment-producing cells known as melanocytes. During the course of development, melanocytes, which are neural crest-derived cells, localize to the skin, eye, ear, GI tract, oral and genital mucous membranes. Within the skin, melanocytes are located in epidermis and hair follicles (Mort, Jackson, and Patton 2015). In the epidermis, they are located at the basal layer, interspersed between progenitor keratinocytes (one melanocyte per ten keratinocytes). In response to UV-induced DNA damage in keratinocytes, melanocytes proliferate and synthesize melanin, a pigment molecule capable of absorbing and scattering UV radiation. Upon synthesis, it is transported back to keratinocytes to provide protection against UV induced DNA damage (Cichorek et al. 2013).

1.3.3 Risk factors

Melanoma development involves a complex interplay between a number of environmental and genetic factors. Epidemiological studies have shown that UV-exposure is one of the primary risk factors for malignant melanoma (Lawrence et al. 2013). Therefore, individuals with history of major sunburn are at greater risk to develop melanoma in the end. The mutagenic effect of UV-B radiation becomes more evident from high frequency of C to T and CC to TT mutational signatures in cutaneous melanoma samples (Sample and He 2018). Besides UV-induced mutations, BRAF, an oncogene acting in MAPK pathway, is recurrently reported to acquire an activating mutation in approximately 40-60% of all melanoma cases (Hayward et al., 2017; Hodis et al., 2012; Krauthammer et al., 2012). Constitutively active BRAF promotes

uncontrolled cell proliferation and growth to potentiate tumor progression. *NRAS*, a GTPase belonging to the RAS-RAF-MAPK pathway, is also frequently mutated (15-20%) in melanoma patients (Sample and He 2018). In terms of mutational burden, melanoma displays the most mutated genome among all cancer types, as measured by the number of mutations per megabase genome (Akbani et al., 2015). Presence of a high number of melanocytic nevi (birthmark or moles), a benign neoplasm with high number of melanocytes, is also associated with melanoma predisposition. Hereditary causes like light hair, fair skin, eye color, along with mutations in cyclin-dependent kinase 4 (*CDK4*) and cyclin dependent kinase inhibitor 2A (*CDKN2A*), have also been identified as risk factors from various genome wide association studies of malignant melanoma (Leonardi et al. 2018). A recent whole genome study by Hayward et al; shows that non-coding loci are also heavily mutated in cutaneous melanoma, further strengthening the potential functional role of various small and long noncoding RNAs (Hayward et al. 2017) .

1.3.4 Molecular pathogenesis

The oncogenic transformation of melanocytes to metastatic melanoma has been classically (the Clark model) described as a multistep process involving many histopathological intermediates such as benign nevus, dysplastic nevus, melanoma *in situ* in radial growth phase (RGP), invasive melanoma in vertical growth phase (VGP) and metastatic melanoma (Miller and Mihm 2006). However, a growing number of clinical evidence has refuted the linearity of this progression, revealing that invasive melanoma can arise without progressing incrementally through the intermediate steps (*Figure 7*) (Damsky, Theodosakis, and Bosenberg 2014; Varrone and Caputo 2020).

A number of studies indicated strongly that cutaneous melanoma originates from the tumorigenic conversion of benign nevi (Bastian 2014), arising from cutaneous melanocytes that have acquired initiating mutations in *BRAF* and *NRAS* (Pollock et al. 2003). This leads to an aberrant activation of the serine/threonine kinase and its downstream MAPK pathway. Permanent activation of MAPK pathway contributes fundamentally towards increased cell proliferation and suppression of apoptosis (Pollock et al. 2003; Shain et al. 2018). In some cases, benign nevi converts to dysplastic nevi, a premalignant lesion with structural atypia (Miller and Mihm 2006). Pathogenic mutations in genes acting in the MAPK pathway and cell cycle regulator *CDKN2A* loss are reported in dysplastic nevi (Goldstein et al. 2000). Activating

mutations at *TERT* promoter and loss-of-function mutations within *PTEN* are also commonly associated with this stage (Shain and Bastian 2016). Activated *TERT* enhances self-renewability and cell proliferation of tumor cells (Reyes-Uribe et al. 2018). *PTEN* inactivation leads to induction of PI3K/Akt pathway, leading towards decreased apoptosis, increased cell proliferation and protein synthesis (Kwong and Davies 2013). Only a fraction of dysplastic nevi further progress into the early phase of melanoma (radial-growth-phase). In this phase, the tumor cells grow in a localized manner between the epidermal layers without forming any nodule (Miller and Mihm 2006).

In the vertical-growth-phase, the lesions undergo a nodular growth, and breach the basement membrane to invade deep into the dermis. Invasive melanoma carry forwards important drivers like activating mutations for *BRAF, TERT* and exhibit frequent deactivating mutations in the tumor suppressor *CDKN2A* (Shain et al. 2015). As the malignancy acquires invasiveness, tumor cells show reduced expression of E-cadherin and increased expression of N-cadherin (Danen et al. 1996; My et al. 2000); one of the key molecular changes associated with epithelial to mesenchymal conversion. As the malignancy becomes metastatic, disseminated tumor cells first colonize to local lymph nodes and eventually to distant organs such as liver, lungs and brain (Damsky et al. 2014).

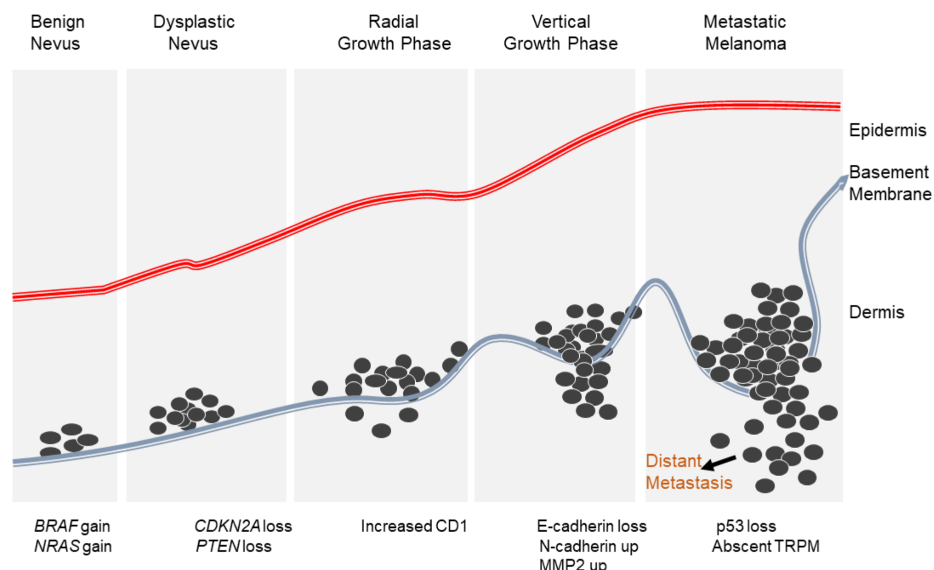

***Figure** 7: Key molecular events associated with malignant melanoma and different intermediates*

1.3.5 Treatment

For low-grade cutaneous melanoma, surgical removal of the tumor, followed by adjuvant therapy, is an effective treatment. For a long time, chemotherapeutic drugs (e.g. dacarbazine and temozolomide) were used as the standard treatment with limited efficacy against advanced melanoma (Luke and Schwartz 2013). However, with the advent of the target-based approach and immunotherapy, chemotherapeutic drugs have taken a back seat.

When it comes to targeted therapeutics, inhibitors of MAPK pathway are the primary drugs of choice for patients with *BRAF*-mutant melanoma. FDA-approved drugs targeting BRAF (vemurafenib and dabrafenib) and MEK (trametinib), have shown great efficacy when used in combination (Maverakis et al. 2015). Recently, the new generation of inhibitors of BRAF (Encorafenib) and MEK (Binimetinib), due to their increased half-life and enhanced anticancer activity, have exhibited further improvement in prolonging the anti-tumor response and disease-free survival (Sullivan et al. 2020). However, acquired and intrinsic resistance against BRAF inhibitors is a key roadblock in melanoma treatment (Luebker and Koepsell 2019).

Over the past decades, rapid progress in the field of immunotherapy has revolutionized melanoma treatment. At present, the most commonly used immunotherapeutics are checkpoint inhibitors, which effectively remove the intracellular brake over T-cell activation. Ipilimumab, the first FDA-approved checkpoint inhibitor for treating advanced melanoma patients, inhibits CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) to promote T-cell activation and expansion (Darvin et al. 2018). Subsequently, FDA approved two other antibodies, namely nivolumab and pembrolizumab, which are currently used in the clinic. These drugs block the interaction between PD-1 and its ligand PD-L1, resulting in the activation of T-cell mediated anti-tumor immune response (Grywalska et al. 2018). Many of these drugs, such as nivolumab and ipilimumab, are more effective when used in combination than monotherapy (Varrone and Caputo 2020). Similar to targeted therapy, acquired resistance is a major problem in establishing a long-term efficacy of immunotherapeutics (Restifo, Smyth, and Snyder 2016). In this scenario, non-coding RNAs have emerged as exciting novel druggable targets which could contribute to overcoming drug-resistance (Caporali et al. 2019).

1.4 Non-coding RNAs

The human genome is made up of approximately 3 billion base pairs, but only a small fraction (<2%) of them can code for proteins(Lander et al. 2001). Initially, most of the non-protein coding genome was considered "junk DNA" that lacks any functional contribution to the survival of the species. Even though ribosomal RNAs (rRNAs) and transfer RNA (tRNAs) were proven to play important catalytic roles in protein synthesis (since 1960), it took a long time to fully explore the idea of a pervasive RNA-mediated mechanism for regulating gene expression that can contribute to the species-complexity (*Figure 8*) (Kruger et al. 1982). With the discovery of catalytically active ribozymes and small nuclear RNAs (snoRNAs), it started to appear that RNA, rather than being just a passive intermediate molecule in the scheme of the central dogma, can also provide an additional layer to gene expression regulation. However, the biggest impetus in the field of RNA biology came through the discovery of a small 21 nt long RNA molecule- lin-4 (now classified under miRNAs) in a *C. elegans*, where it downregulates the expression of LIN-14 protein through partial base pairing with the corresponding mRNA (Li et al., 1993). The similarity between lin-4 and other small RNA mediated regulation (RNA-interference) in plants and worms further fueled the interest of finding and studying small RNAs exhaustively in higher eukaryotes. Since then, the field of RNA biology has undergone a thorough transformation. We have come across many novel classes of non-coding RNAs such as – small nucleolar RNA (snoRNAs), which are responsible for chemical modifications of rRNAs and tRNAs, and PIWI-interacting RNAs (piRNAs) - responsible for preserving the genomic integrity by suppressing mobile genetic elements (Bachellerie et al. 1995; Houwing et al. 2007). With the inception of high throughput transcriptomic studies in the recent years, it became apparent that a large portion of the eukaryotic genome is actively transcribed (Djebali et al. 2012). These studies gave rise to another class of non-coding RNAs (long non-coding RNAs) with a transcript length of more than 200 nucleotides. A growing number of studies suggest that they play fundamental roles in cellular processes including X-chromosome inactivation, epigenetic regulation of transcription, imprinting, chromatin remodeling, and regulation of translation (Marchese, Raimondi, and Huarte 2017).

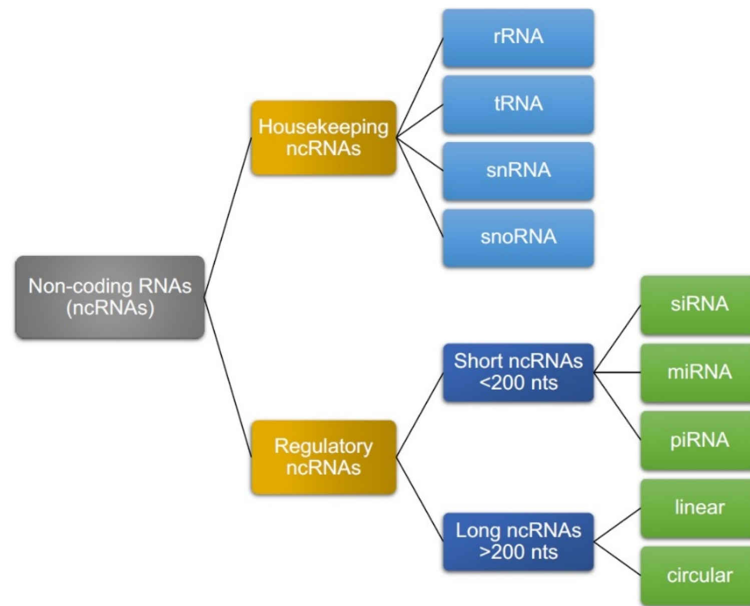

Figure 8. *Different classes of non-coding RNAs in the eukaryotes.*

1.4.1 MicroRNAs

MicroRNAs (miRNAs) are a class of evolutionarily conserved small ncRNAs (21-23 nt) that have emerged as an important player in posttranscriptional gene regulation. Since their discovery in 1993, miRNAs have been implicated in virtually every biological process, and disease progression in the plant and animal kingdom (Lee, Feinbaum, and Ambros 1993). Primarily, miRNAs exert their silencing effect by binding to a short complementary region in the 3'-UTR of an mRNA, leading to either its degradation or inhibition of translational (Guo et al. 2010).

1.4.1.1 MiRNA biogenesis and mode of function

The majority of canonical miRNA genes are intragenic (intronic or exonic). Intragenic miRNAs are transcribed from either the host promoter or an independent promoter by RNA polymerase II (*Figure 9*) (Lee et al. 2004). However, RNA polymerase III transcribes most intergenic miRNAs from an independent promoter (Borchert, Lanier, and Davidson 2006). Upon transcription, human miRNAs undergo a multistep step processing to produce a mature miRNA transcript. In the first step, a microprocessor complex composed of Drosha – an RNase III endonuclease, and its cofactor DGCR8 cleaves the primary transcript to produce a 60-70

nucleotide stem looped precursor (Lee et al. 2003). This precursor miRNA (pre-miRNA) is transported from the nucleus to the cytoplasm by Exportin 5 (XPO5) and Ran-GTP protein complex (Okada et al. 2009). In the cytoplasm, Dicer – another ribonuclease III in association with a double-stranded RNA binding protein-TARBP further cleaves the pre-miRNA into a shorter, imperfect duplex, with 2-3 nt overhang (Chendrimada et al., 2005). This duplex RNA comprises of the mature miRNA and a passenger strand that comes from the complementary part of the pre-miRNA transcript (Chendrimada et al. 2005). Subsequently, the matured miRNA is incorporated into an Argonaute2 (AGO2) protein that forms an effector complex: RNA induced silencing complex (RISC) while the passenger strand is degraded (Hutvagner and Simard 2008). Once loaded onto the RISC, the matured miRNA directs target mRNA silencing depending on the degree of sequence complementarity between miRNA seed sequence (nucleotide 2-8 of 5' end) and 3'-UTR of target mRNA (Bartel 2009). A perfect complementarity leads to the degradation of target mRNA, while an insufficient complementarity promotes translational blocking (Brennecke et al. 2005; Doench and Sharp 2004) (Figure 4). Apart from the miRNA-mRNA seed pairing, length, secondary structure and the number of target sites within the 3'-UTR can influence miRNA-mediated regulation (Bartel 2009). Possibly, through an act of co-evolution, matured miRNA sequence and corresponding 3'-UTRs have remained evolutionarily conserved (Friedman et al. 2009; Lewis, Burge, and Bartel 2005). Depending on the similarity between functionally crucial seed sequences or matured sequence, miRNAs are grouped into different families, with overlapping functions between the family members (Altuvia et al. 2005). Given that miRNA-mRNA interaction is short (6-8 nt), each microRNA can have multiple target mRNAs within the cell. Although the silencing effect is modest because of having multiple targets, most of the miRNAs play an important role in cellular networks by simultaneously regulating various pathways (German et al. 2008). In alignment with one miRNA-multiple targets postulate, more than half of the human transcriptome is estimated to be regulated by miRNAs, underscoring the importance of the miRNome in virtually every possible gene network (Rigoutsos 2009).

1.4.1.2 Regulation of miRNA biogenesis and function

Due to the presence of TATA box sequences in the promoter region, initiation elements, and various regulatory histone marks, miRNAs are often subjected to transcription factor or epigenetic factor mediated regulation (Ozsolak et al. 2008). For example, c-MYC has been

shown to induce the expression of miR-17-92 cluster by binding to both the host gene TSS and an intronic promoter (Chang et al. 2008). Another study demonstrated that transcription factor Zinc Finger E-Box Binding Homeobox (ZEB1 and ZEB2) transcriptionally suppresses the miR-200c and miR-141 by directly binding to the putative promoter regions (Bracken et al. 2008). Through a conjoint effort, epigenetic modifier HDAC2 is recruited by MYCN to activate miR-183 expression by promoter acetylation in neuroblastoma (Lodrini et al. 2013). Promoter hypermethylation has been proven a frequent mode of inhibitory mechanism for miRNA expression. For example, miR-34a is frequently downregulated in various cancer types, through promoter-associated CpG hypermethylation (Suzuki et al. 2012). Another recent study demonstrated that methyl CpG binding protein 2 (MECP2), by binding to the methylated CpGs within the miRNA gene body, impedes RNA pol II elongation and miRNA biosynthesis (Glaich et al. 2019). MiRNA expression and function is also regulated post-transcriptionally, effectively during every step of its biogenesis. For instance, LIN28 has been proposed to recruit TUTase4 (TUT4) for uridylation of let-7 precursor (or other miRNAs with TUT4 binding site). This in turn inhibits dicer mediated processing of the precursors and leads to their degradation (Heo et al. 2009).

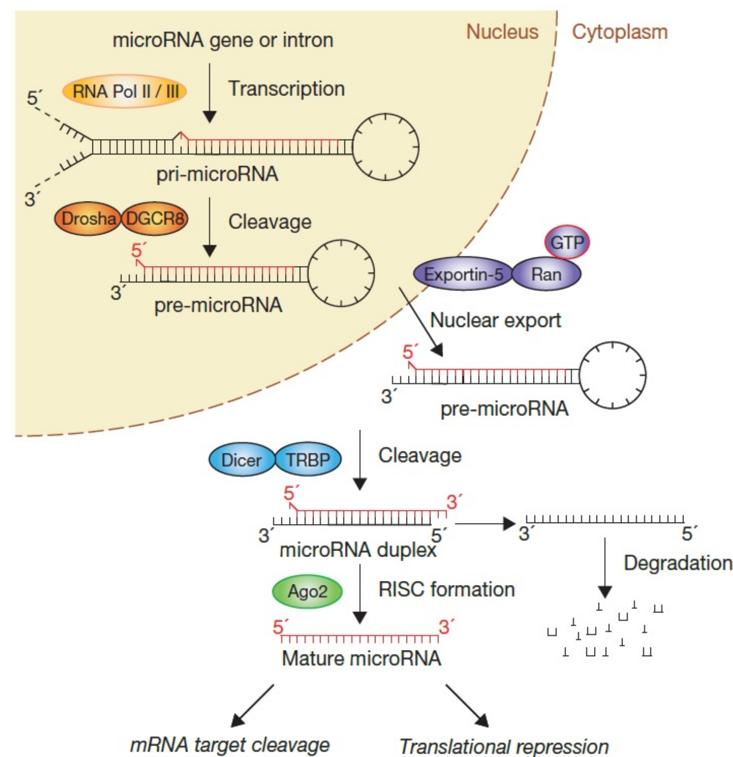

***Figure 9**: The canonical miRNA biogenesis pathway and modes of target repression, by either mRNA degradation or inhibition of protein synthesis. Adapted and modified from* (Mulrane et al. 2013).

1.4.1.3 MiRNAs in cancer

Considering that miRNAs function as fine-tuners of gene expression to maintain cellular homeostasis or as regulators of cell responses, especially towards developmental cues, it is not a surprise that they have been regularly implicated in a complex developmental disorder like cancer (Ebert and Sharp 2012; Peng and Croce 2016). The first reported case of miRNA alteration in cancer came in 2002, where miR-15 and miR-16 genes were found to be deleted in Chronic Lymphocytic Leukemia (CLL), causing an increase in their direct target- B-cell lymphoma 2 (Bcl-2) (Calin et al., 2002). Since then, miRNAs have become the cardinal regulators of cancer progression by modulating target genes involved in cell proliferation, immune cell evasion, tumor growth, migration, invasion, and neo-angiogenesis (*Figure 10*) (Di Leva, Garofalo, and Croce 2014; Peng and Croce 2016). Moreover, many miRNAs have been identified as biomarkers for predicting disease progression, classification, patient survival, and treatment outcome (Rosenfeld et al. 2008; H. Wang et al. 2018).

MiRNAs with functional roles in cancer can be broadly grouped into tumor-suppressive or oncogenic miRNAs (Figure 5). One of the most well-characterized miRNAs with tumor suppressive function is miR-34. All three members of miR-34 family are downregulated in different cancer types such as lung, prostate and breast cancer (Zhang, Liao, and Tang 2019). They are transcriptionally regulated by tumor suppressor p53, while regulating cell survival and proliferation by directly targeting oncogenic transcription factors such as c-MYC and MET (Hermeking 2010; Zhang et al. 2019). Let-7 miRNA family members have also been demonstrated to act as a tumor suppressor by directly targeting oncogenic HRAS in breast cancer (Johnson et al. 2005). Therapeutically, lipid nanoparticle-mediated delivery of miR-34 (stand-alone or in combination with let-7) mimic had provided promising early results in several solid and hematological tumor models in mice and moved to phase I clinical trial (NCT01829971) (Stahlhut and Slack 2015; Wiggins et al. 2010). However, due to multiple immune-related adverse effects, this trial was terminated in 2016 (Van Roosbroeck and Calin 2016). Some other well-characterized miRNAs with tumor suppressive role are- miR-200, miR-203 and miR-520 (Gregory et al. 2008; Keklikoglou et al. 2012; Saini et al. 2011). Among the oncogenic miRNAs, miR-21 is upregulated in breast, and colorectal cancer, and directly targets pro-apoptotic protein PDCD4 to trigger tumor metastasis (Asangani et al. 2008; Si et al. 2007). Some other well-characterized oncogenic miRNAs are miR-155, miR17-92 and miR-221/222 (Fuziwara and Kimura 2015; Galardi et al. 2011; Gironella et al. 2007). Several miRNAs within the cell or in cell-free circulation have been also proven useful as biomarkers. In one such

example, a five-miRNA signature (miR-92a-3p, miR-342-3p, miR-16, miR-21 and miR-199a-5p) was identified for accurately diagnosing triple-negative breast cancer (Shin et al. 2015). A study by Yokoi et al., has identified a signature of 10 serum-derived miRNAs in ovarian cancer, that can be used as a biomarker (Yokoi et al. 2018).

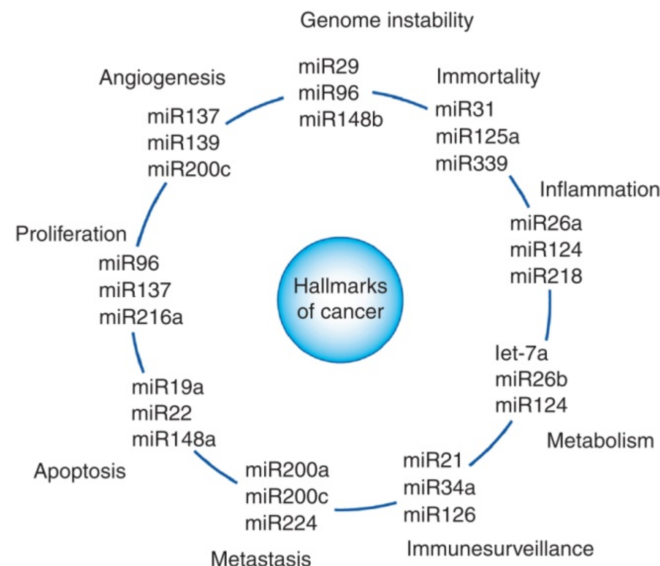

Figure 10: *MiRNAs regulating the hallmarks of cancer. Modified from* (Pichler and Calin 2015)

1.4.1.4 MiRNAs in cutaneous squamous cell carcinoma

Several studies involving tissue-specific ablation of miRNA processing enzymes highlighted that miRNAs play a crucial role in skin morphogenesis and change in their expression can lead to abnormal epidermal morphology and altered differentiation program (Andl et al., 2006; Yi et al., 2006). Additionally, miRNA expression is differentially regulated in different layers of epidermis, during different stages of differentiation, further underscoring the importance of miRNA in skin morphogenesis and homeostasis (Sonkoly et al. 2010; Wei et al. 2010; Yi et al. 2008). A great many studies have demonstrated extensively that the expression of miRNAs is altered in chronic inflammatory skin diseases as well as non-melanoma skin cancers (García-Sancha et al. 2019; Timis and Orasan 2018). Among one the earliest studies about miRNAs in cSCC, Wang et al. demonstrated that miR-31 is differentially upregulated in cSCC tumors, and it causes increased cell proliferation, migration, and invasion (A. Wang et al. 2014). Later, Xu et al. demonstrated that miR-125b acts as a tumor-suppressor in cSCC, by limiting cell

proliferation and motility via targeting matrix metalloprotease 13 (MMP13) in human cSCC cell lines (Xu et al. 2012). Pan-cancer tumor-suppressor miR-34a was also found to be downregulated in cSCC. Besides, the same work also reported that miR-34a is induced during keratinocyte differentiation, and modulates p53-mediated Notch1 expression to regulate differentiation (Lefort et al. 2013). Using patient-derived cSCC cell lines, Mizrahi et al. demonstrated that miR-497 is a stage-specifically altered oncogenic miRNA in cSCC and it influences epithelial to mesenchymal transition by targeting serpin family E member 1 (SERPINE1), a serine protease inhibitor (Mizrahi et al. 2018).

Much of our understanding about the multi-stage development of epithelial cancer comes from the studies using a mice model where the sequential application of carcinogenic DMBA (7,12-dimethylbenz[a]-anthracene) and tumor-promoting ester TPA (12-Otetradecanoylphorbol-13-acetate) on mice skin leads to the development of skin squamous cell carcinoma (Neagu et al. 2016). The importance of miRNAs in the pathobiology of SCC development in this model was highlighted in a study, which revealed differential expression of 112 miRNAs in the primary tumors. Among others, miR-193b/365a cluster seemed to have an inhibitory effect on cSCC progression, by directly targeting KRAS and MAX (Gastaldi et al., 2014). Following a similar approach, Riemondy et al. has demonstrated using a knockout mice model, that miR-203 suppresses the expansion of oncogenic $HRAS^{G12V}$ transformed tumor-initiating cells, resulting in fewer papillomas. MiR-203 exert this tumor-suppressive function, partly by targeting regulators of cell division: HBEGF (Heparin Binding EGF Like Growth Factor) and POLA (DNA polymerase alpha 1, catalytic subunit) (Riemondy et al. 2015). Another recent study using $p63^{-/-}$ mice has uncovered a regulatory axis involving tumor suppressor p63, two miRNAs (miR-30c-2* and miR-497) and AURKA in keratinocyte derived malignancy (Davis et al., 2020). These two miRNAs were found to be downregulated in the $p63^{-/-}$ mice and exhibited strong tumor growth suppressive effect *in vivo*, while chemical inhibition of AURKA (a direct target of miR-497) phenocopied the miRNA overexpression (Davis et al. 2020).

1.4.1.5 MiRNAs in cutaneous malignant melanoma

A growing body of literature suggests that miRNAs can play a vital role in melanocyte homeostasis and melanoma pathogenesis. For instance, MITF - the master regulator in melanocytes, has been reported to transcriptionally activate DICER during melanocyte differentiation, which in turn processes pre-miR-17-92. Successively, members of the miR-17

cluster inhibit pro-apoptotic factor BIM (Bcl-2-like protein 11) to promote melanocyte survival (Levy et al. 2010). The first indication about the involvement of miRNAs in melanoma came from a miRNA-gene-specific DNA copy number analysis, which contained a high proportion of copy number alterations (85%) in melanoma patients (Zhang et al. 2006). Another miRNA expression profiling, using NCI-60 panel of tumor-derived cell lines (including melanoma), revealed a tumor tissue-specific miRNA enrichment when compared with cancer cell lines derived from non-melanoma tumors (Gaur et al. 2007). In the following years, many miRNAs were characterized in-depth in stage-specific melanoma progression. In one such study, multiple members of the let-7 family are downregulated in primary melanoma tissues when compared to melanocytic nevi with a potential role in melanoma progression. Functionally, let-7b downregulates pro-oncogenic cyclins 2 and 3, and CDK4, and suppresses melanoma progression (Schultz et al. 2008). MiRNAs are also reported to play vital role in regulating MITF during melanoma development. Haflidadottir et al. delineated a mechanism, where miR-137 and miR-148 directly target MITF and modulates melanoma progression (Haflidadóttir et al. 2010). In addition to the functional roles, miRNA-signatures are proven equally useful for predicting melanoma progression or long-term survival of the patients. A study by Stark et al. detected a panel of seven miRNAs (miR-16, 211, 4887, 4706, 4731, 509-3p, and 5p) which are more efficient than the available serological markers in predicting early recurrence and patients' survival (Stark et al. 2015). With a steady increase in our knowledge about the role of miRNAs in melanomagenesis, we can clearly envisage their diagnostic and therapeutic potential (Varrone and Caputo 2020). However, as always, further research is required to fine-tune the treatment and avoid potential pitfalls of non-specific targeting.

1.4.2 Long non-coding RNAs

In recent years, long non-coding RNAs (lncRNAs) have emerged as fundamental regulators of various eukaryotic cellular pathways, both in homeostatic and disease conditions. According to the present nomenclature of lncRNAs, they can be broadly defined as a functionally diverse group of regulatory RNAs without protein coding capacity and having transcripts longer than 200 nucleotides (Kapranov et al. 2007). A paradigm shift in the field came with the systematic identification of more than 20,000 lncRNAs locus through cDNA clone analysis by ENCODE and FANTOM consortium (Carninci et al. 2005; Derrien et al. 2012). In terms of biogenesis, most annotated lncRNAs are transcribed by RNA polymerase II, undergo splicing, and frequently get 5'-capped and 3'-poly-adenylated similar to the protein-coding genes (Carninci et al. 2005). Due to their relatively lower abundance and lack of sequence conservation, lncRNAs were initially considered as spurious byproducts of leaky transcription (Struhl 2007). Later studies revealed that the expression of lncRNAs is stringently regulated in a spatiotemporal manner, in particular developmental contexts (Mercer et al. 2008). The functional significance of cross-species lncRNAs appears to stem from not only sequence conservation but also from the conserved secondary structure and shared synteny (Johnsson et al. 2014). A number of genome-wide studies revealed a conserved signature of secondary structure and splicing pattern for a large number of lncRNAs, underscoring the multilayered conservation in lncRNAs (Ponjavic, Ponting, and Lunter 2007; Torarinsson et al. 2006).

1.4.2.1 Genomic location

The present nomenclature of uncharacterized lncRNAs is based on their location in relation to the proximal protein-coding gene(s) (*Figure 11*). LncRNAs that are transcribed and spliced from the intron of a protein-coding gene are termed Intronic lncRNAs (IT), while lncRNAs locus that are located between two protein coding genes are intergenic in nature and presented with a prefix- LINC (long intergenic non-coding RNAs). A bidirectional lncRNA transcribed from a common promoter of a protein-coding gene has a prefix AU (antisense upstream). An antisense lncRNA (AS) is transcribed from the opposite strand of a protein coding gene, while the sense lncRNA can exist as an overlapping transcripts (OT) or intronic transcript (Harrow et al. 2012).

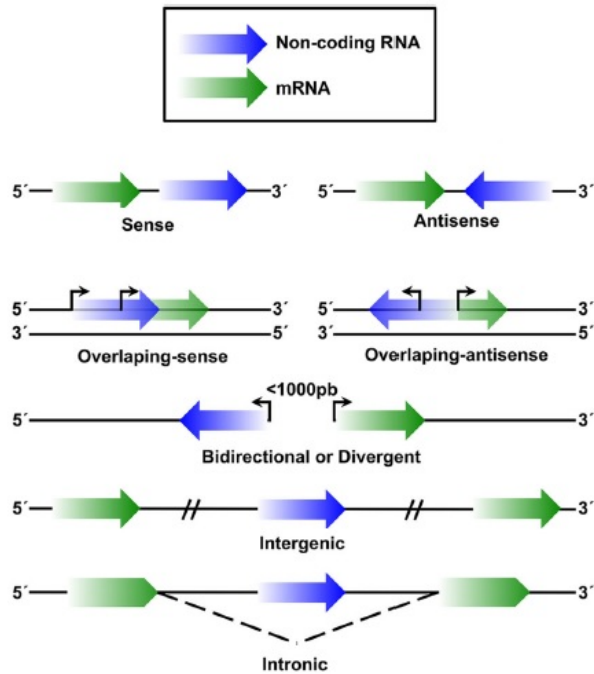

***Figure 11**: Different classes of lncRNAs (Blue) according to their relative location to the proximal protein-coding gene/s (Green). Modified from* (Herrera-Solorio et al. 2017).

1.4.2.2 Subcellular localization and functional classes

Several studies focusing on the subcellular localization of lncRNAs revealed that they predominately exhibit three different kinds of expression pattern- i) enriched in the nucleus ii) localized to the cytoplasm and iii) comparable abundance in both subcellular compartments (Cabili et al. 2015; Mas-Ponte et al. 2017). Subcellular localization can be a useful indicator of lncRNA functionality (*Figure 12*). Nuclear enriched lncRNAs are found to regulate gene expression at the transcriptional level by interacting with the transcriptional machinery, epigenetic modulators and genomic DNA (Sun, Hao, and Prasanth 2018). On the contrary, lncRNAs accumulated in the cytoplasm majorly function as posttranscriptional regulators by interacting with the mRNA processing enzymes and the translation machinery (Chen 2016). LncRNAs, which can shuttle between the two compartments, often exhibit dual functional attributes (Miao et al. 2019).

Regulation of transcription

Nuclear localized lncRNAs can regulate transcriptional dynamics either by chromatin remodeling or by recruiting transcription factors to the promoter of a proximal (*cis*) or distal (*trans*) gene locus (Wilusz, Sunwoo, and Spector 2009). Such regulation can be a direct effect

of the lncRNA transcript or just a byproduct of transcription initiation at the lncRNA locus, which in turn alters the local chromatin accessibility (Kung, Colognori, and Lee 2013). In one such instance, the activity of human growth hormone (hGH) enhancer- HSI was reported to be influenced by the transcription of a downstream lncRNA locus. Transcription termination experiment, in combination with the restoration of enhancer activity by insertion of an unrelated bacteriophage RNA, highlighted an RNA independent transcriptional regulatory function of this lncRNA locus (Yoo, Cooke, and Liebhaber 2012). Conversely, a lncRNA transcribed upstream of the dihydrofolate reductase gene (*DHFR*) was found to repress the transcription of *DHFR* by physically interacting with transcription factor TFIIB, leading to the dissociation of the preinitiation complex from *DHFR* promoter (Martianov et al. 2007). Interaction with transcription factors and epigenetic modulators are often dictated by lncRNAs containing distinct structural domains, acting as docking modules for the interacting proteins. This archetype mechanism is also common among enhancer-associated lncRNAs with activators like function (eRNA and ncRNA-a) function (Ørom et al. 2010). In one such study, Lai et al reported that ncRNA-a7, a lncRNA with transcriptional activator-like function, interacts with the mediator complex to enhance the expression of neighboring genes- snail family transcriptional repression 1 (SNAI1) and aurora kinase A (AURKA) (Lai et al. 2013).

Modulation of epigenetic regulation

A number of studies have extensively explored the interactive nature between nuclear lncRNAs and epigenetic modulators to activate or repress genomic loci. One of the most well-characterized lncRNAs- XIST coats the X chromosome in female cells and recruits polycomb repressive complex 2 (PRC2)-a methyltransferase that trimethylates lysine 27 of histone 3 (H3K27me3) across the whole chromosome, causing a transcriptionally inert heterochromatin formation (Zhao et al. 2008). Similarly, *HOTAIR*, a lncRNA proximally located to the *HOXC* locus (human chromosome 12), guides PRC2 complex to transcriptionally repress a distal locus of *HOXD* (human chromosome 2) (Rinn et al. 2007). While it interacts with PRC2 via its 5'-end, HOTAIR can also interact (via 3'-end) with lysine-specific demethylase 1 (LSD1), which is responsible for demethylation of lysine 4 and 9 at histone 3, and gene silencing (Tsai et al. 2010). Consistent with these findings, a study about breast cancer pathogenesis reported that HOTAIR overexpression leads to a genome-wide change in PRC2 recruitment, causing suppression of vital tumor suppressor genes (Gupta et al. 2010).

Maintenance of nuclear architecture

Recent studies have highlighted that nuclear-retained lncRNAs play fundamental role in the establishment and maintenance of nuclear domain structures. Within the nucleus, paraspeckles are highly dynamic non-membranous structures enriched with RBPs that can regulate RNA splicing and editing (Spector 2001). Paraspeckles also contain a nuclear-enriched lncRNA NEAT1. Clemson et al., using gain and loss of function approach illustrated that both NEAT1 transcript and its active transcriptional foci are indispensable for paraspeckles formation (Clemson et al. 2009). In addition, was also reported to control the nuclear retention of *Alu* elemenets containing mRNAs. These mRNAs undergo frequent adenosine to inosine editing event at the *Alu* regions, which in turn alters their half-life (Bond and Fox 2009). MALAT1 (alternatively called NEAT2), another nuclear-retained lncRNA, is found to be associated with serine/arginine splicing factors (SR) to regulate alternative splicing by dictating their activity and overall distribution within the nuclear speckle domains (Tripathi et al. 2010). Apart from maintaining interchromatin granules, lncRNAs can also facilitate interchromosomal interactions. LncRNA Firre, together with nuclear matrix protein hnRNPU, anchors several multi-chromosomal interactions in three-dimensional space with high specificity, allowing co-regulation of genes that are involved in energy metabolism, adipogenesis and pluripotency pathways in mice and human embryonic stem cells (Hacisuleyman et al. 2014).

Regulation of mRNA stability

Cytoplasmic lncRNAs are capable of regulating mRNA stability via interacting with specific RBPs. Staufen1 (STAU1) is a double-stranded RBP that governs mRNA degradation via STAU1 mediated decay (SMD) pathway. STAU1 has a binding preference towards double-stranded structure formed within the 3'UTR of a target mRNA, either by intramolecular base pairing or by imperfect base pairing with lncRNAs containing Alu repeat elements, which primes them for SMD mediated decay (Gong and Maquat 2011). A disparate function of STAU1 was uncovered when Kretz et al reported that TINCR, a cytoplasmic lncRNA expressed during keratinocyte differentiation, interacts directly with STAU1 to stabilize a host of mRNAs expressed during epidermal terminal differentiation in an *Alu* independent manner (Kretz et al. 2013b). Another study revealed that lncRNA gadd7 (growth arrested DNA-damage inducible gene 7) binds to TAR DNA binding protein (TDP-43) and impairs its interaction with the 3'UTR of cyclin-dependent kinase 6 (CDK6) mRNA, causing its degradation and ultimately affecting G1/S phase entry upon UV irradiation (X. Liu et al. 2012).

Regulation of translation

Yoon et al. showed that cytoplasmic lincRNA-p21, when associated with HuR, undergoes rapid degradation by let-7 miRNA. However, in the absence of HuR, lincRNA-p21 accumulates in the cytoplasm, and selectively targets JUNB and CTNNB1 mRNA, and facilitates their interaction with translational repressor Rck- a DEAD box RNA helicase and FMRP in HeLa cells (Yoon et al. 2012). Another study using mice neuronal cell line uncovered the translation modulating function of Uchl1-AS, a lncRNA antisense to ubiquitin c-terminal hydrolase L1 (UCHL1) and highly enriched in mice ventral mid-brain. The authors showed that the regulatory feature of Uchl1-AS is dependent on an inverted SINEB2 repeat and a 73 nt long complementary sequence overlapping to the 5'-end of Uchl1 mRNA. Uchl1-AS, which is nuclear under physiological condition, translocates to the cytoplasm upon activation of cellular stress response and base pairs with Uchl1 mRNA to recruit active polysomes, enhancing 5'-cap-dependent translation (Carrieri et al. 2012).

MiRNA sponges

Several cytoplasmic lncRNAs act as miRNA sponges (alternatively called competing endogenous RNAs-ceRNAs) owing to miRNA binding sites along their transcripts (Ebert and Sharp 2010). Therefore, they can indirectly regulate the miRNA-targets by titrating away the corresponding miRNA-RISC complex. For instance, oncogenic phosphatase and tensin homolog (PTEN) has a pseudogene PTENP1 that acts as a molecular decoy for miRNAs such as miR-17, miR-19, miR-21 and miR-26. By hijacking these miRNAs, PTENP1 can fine-tune the expression of their target – PTEN, and exert a tumor-suppressive effect (Poliseno et al. 2010). More recently, another group characterized a novel gall bladder cancer de-regulated lncRNA PAGBC (prognosis-associated gallbladder cancer lncRNA) that exerts its pro-metastatic function by sponging away tumor-suppressor miR-133b and miR-511. Suppression of these miRNAs increases the expression of Phosphatidylinositol 3-kinase regulatory subunit gamma (PI3KR3), which in turn activates the Akt/mTOR pathway in gallbladder cancer (Wu et al. 2017).

Encoding small functional peptides

Although most of the lncRNAs do not contain large ORFs, recent evidences from ribosome-profiling studies however revealed that several cytoplasmic lncRNAs have similar profiling characteristics of the 5'-end of coding mRNAs (Ingolia et al. 2014; Ji et al. 2015). Not only

unstable byproducts, these translational events can also give rise to functional micropeptides (< 100 aa). In one such instance, a skeletal muscle-specific micropeptide - MINION (microprotein inducer of fusion) was proposed to regulate muscle fusion in mice and humans (Q. Zhang et al. 2017). In another recent study, CASIMO1 (Cancer-Associated Small Integral Membrane Open reading frame 1)- a 10 kDa micropeptide overexpressed in hormone receptor-positive breast cancer, was shown to interact with squalene epoxidase (SQLE) and regulate downstream ERK phosphorylation, to drive tumor cell motility and proliferation (Polycarpou-Schwarz et al. 2018).

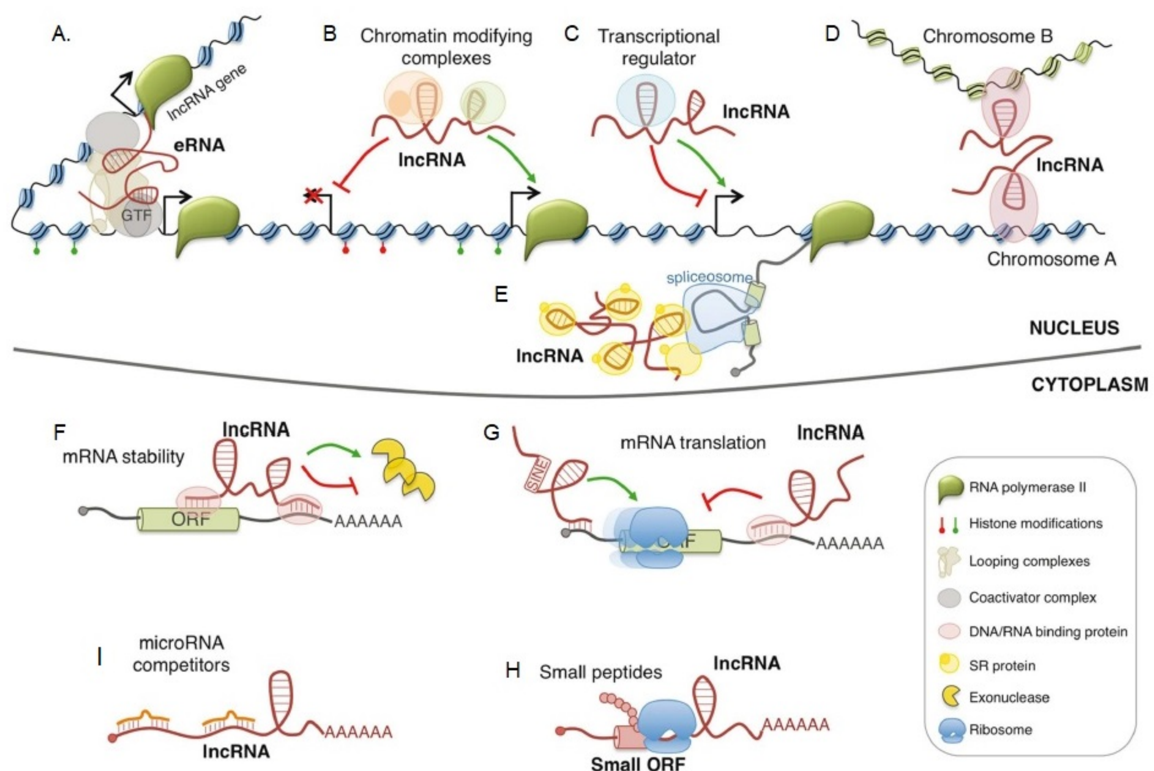

Figure 12. *Summary of various modes of function of nuclear and cytoplasmic long non-coding RNAs. From left to right (top section), nuclear lncRNAs can act (A) as enhancer RNAs (B) recruit chromatin modifiers to activate or repress gene expression (C) regulate transcription factors, (D) alter spatial interchromosomal interactions or (E) regulate mRNA splicing. Cytoplasmic lncRNAs (bottom section) can influence (F) mRNA stability, (G) protein synthesis, (H) produce functional peptides, (I) miRNA activity and abundance by acting as sponge. Modified from* (Morlando, Ballarino, and Fatica 2015).

1.4.2.3 LncRNAs in epidermal differentiation and cSCC

Due to their cell-type-specific expression pattern in particular developmental context, lncRNAs have promptly made their way into the regulatory jigsaw of tissue development and organogenesis. The earliest evidence of a lncRNA being associated with keratinocyte biology

/skin disorder was provided by Sonkoly et al. through the identification of PRINS (Psoriasis Susceptibility-Related RNA gene Induced by Stress), a lncRNA upregulated specifically in the non-lesional epidermis, and induced by various stress signals in keratinocytes (Sonkoly et al. 2005). Almost 5 years later, another study using custom-made expression array identified hundreds of differentially expressed lncRNAs upon TPA mediated differentiation of primary keratinocytes (Ørom et al. 2010). Shortly after, two related studies uncovered hundreds of dynamically expressed lncRNAs during calcium-induced differentiation of primary keratinocytes, and further characterized two of them having antagonistic roles in epidermal differentiation. While intergenic lncRNA ANCR was found to be downregulated during terminal differentiation, expression of TINCR was induced. Since ANCR depletion led to ectopic expression of differentiation, it seems to enforce an undifferentiated state in the progenitor compartment (Kretz et al. 2012). On the contrary, TINCR - an abundant lncRNA in terminally differentiated layers of the epidermis, promoted keratinocyte differentiation by stabilizing differentiation-specific mRNAs in a STAU1 mediated mechanism (Kretz et al. 2013a). Another study, using primary human keratinocytes, demonstrated that lncRNA H19 sponges miR-130b-3p, and thereby releases its inhibitory effect on a differentiation-promoting factor Desmoglein1 (Dsg1) (Li et al. 2017). In a separate study, progenitor enriched LINC00941 has been shown to repress differentiation in the basal layer of the human epidermis, partly by suppressing SPRR5- a novel EDC derived transcript (Ziegler et al. 2019:5). In the latest example of a lncRNA regulating epidermal differentiation, an ultra-conserved region containing lncRNA uc.291 was reported to aid epidermal differentiation by titrating away actin-like 6A (ACTL6A)- an epigenetic regulator that prevents the binding of SWItch/Sucrose Non-Fermentable (SWI/SNF) complex to the promoters of vital differentiation genes such as filaggrin and loricrin (Panatta et al. 2020:2).

Since lncRNAs play important regulatory roles in epidermal homeostasis; it is hardly surprising to speculate their potential involvement in keratinocyte derived malignancies that are marked with altered epidermal homeostasis (*Figure 13*). An array-based lncRNA profiling first reported about thousands of differentially expressed lncRNAs in cSCC (Sand, Falk G Bechara, et al. 2016). Recently, another deep sequencing-based investigation has identified several uncharacterized SCC-misregulated non-coding transcripts (SMRTs), some being regulated by oncogenic KRAS and expressed inversely between epidermal differentiation and cSCC (Lee et al. 2018). Among the limited number of characterized lncRNAs in cSCC, p38-inhibited cutaneous squamous cell carcinoma associated lncRNA (PICSAR) was proposed to activate the

MAPK pathway to promote tumor growth (Piipponen et al. 2016). Consistent with the previous findings, TINCR, a highly expressed lncRNA in various epithelial tissues (including the skin), has been shown to suppress esophageal SCC via a ZNF750 dependent regulatory network (Hazawa et al. 2017). Finally, a recent finding has identified a p53-suppressed lncRNA (PRECSIT), which promotes cSCC progression via activation of STAT3 pathway (Piipponen, Nissinen, Riihilä, et al. 2020). These findings simultaneously form the framework of our understanding of ncRNA functionality and provide a resource for future elucidation of uncharacterized transcripts in relation to keratinocyte biology.

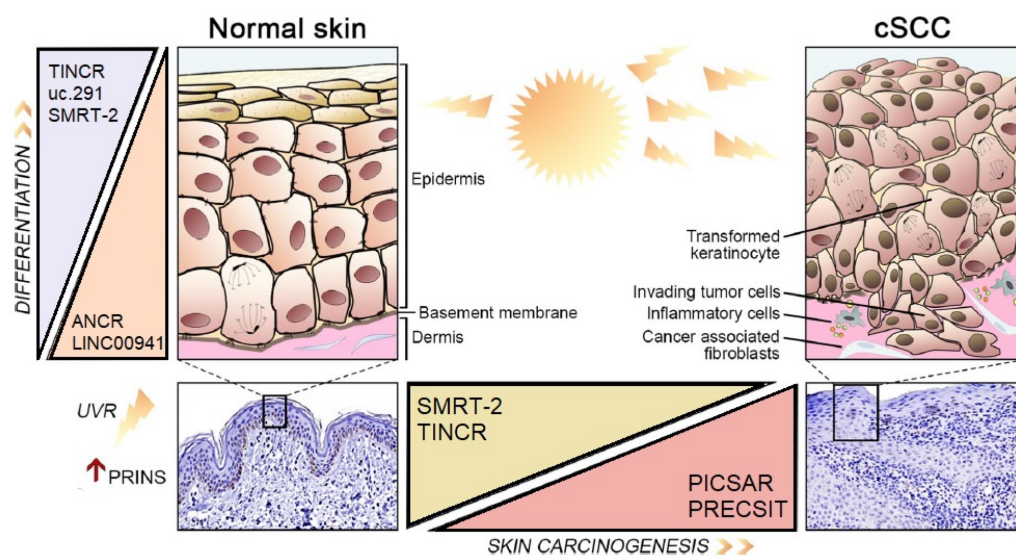

***Figure 13**: A summary of lncRNAs with regulatory role in epidermal homeostasis and/or cutaneous squamous cell carcinoma. Modified from* (Piipponen, Nissinen, and Kähäri 2020).

1.4.3 Circular RNAs

Circular RNAs (circRNAs) are a novel class of single-stranded, covalently closed regulatory RNA. Just like the early years of lncRNAs, circRNAs were initially considered as junk byproducts of faulty splicing (Cocquerelle et al. 1993). However, with the advent of deep sequencing, and the development of robust bioinformatics platforms, we have seen an exponential increase in the discovery of circRNAs in various eukaryotes.

1.4.3.1 CircRNA biogenesis and turnover

The primary mode of circRNA biogenesis is through back splicing of a 5'-splice donor site to a 3'-splice acceptor site of a single or multiple exon(s). This process is often promoted by the physical proximity of the 5'-donor and the 3'-acceptor site by the formation of a loop structure (*Figure 14*) (Memczak et al. 2013). This loop can be formed by either complementary base pairing, due to the presence of inverted repeated elements (e.g. *Alu* elements), or dimerization and binding of trans-acting RNA binding proteins (e.g. QKI and FUS) to specific RBP-binding motifs present in the flanking introns (*Figure 14*) (Conn et al. 2017; Errichelli et al. 2017; Kristensen et al. 2019). Another study has proposed that looping could be influenced by the presence of splicing factors such as hnrnps and SR splicing factors (Kramer et al. 2015). Alternatively, circRNAs can be generated from lariats – a canonical splicing intermediate that can undergo internal back splicing (Barrett, Wang, and Salzman n.d.; Kristensen et al. 2019). Subsequently, the circRNAs are exported into the cytoplasm by dead-box RNA helicases (e.g. DDX39A and DDX39B) in a size-dependent manner (Huang et al. 2018).

Post-biogenesis processing and degradation of circRNAs are still not well understood. Due to their lack of free 5' 7-methylguanosine cap and 3'-polyA tails, circRNAs are remarkably stable, and can only be cleaved internally via endonucleases. Like mRNAs, circRNAs are also targeted by miRNAs for RISC-mediated degradation. For instance, miR-671 has been demonstrated to mediate the degradation of circRNA CDR1as by complementary base pairing and loading into the Argonaute-2 (Ago2) complex (Hansen et al. 2011). Another recent study has uncovered a group of circRNAs with highly structured 3'-end, which undergoes enzymatic degradation in a sequence-independent manner by two key RBPs with helicase function, UBP1 and G3BP1 (Fischer et al. 2020). Apart from active degradation, circRNAs can also be cleared from intracellular space via vesicle-mediated exportation (Lasda and Parker 2016).

1.4.3.2 Modes of functions

Expression profiling data showing tissue/cell-specific expression pattern and altered abundance of circRNAs in different disease conditions give a strong indication about their functional relevance. Additionally, more than often, circRNA expression does not correlate well with their host gene, which can imply a functional divergence (Kristensen et al. 2019). A large number of studies have reported that circRNAs can function as miRNA sponges. CDR1as (alternatively ciR-7), which contains as many as 70 conserved binding sites for miR-7, has been demonstrated to sponge in various tissues (Hansen et al. 2013). Another study in human embryonic stem cells reveal that circBIRC6 and circCORO1C titrate pluripotency regulating miRNAs (miR-34a and miR-145) (Yu et al. 2017). Besides sponging miRNAs, ciRNAs can also directly interact with RBPs to exert their biological function. For instance, circMBNL1 has binding sites for MBNL1; a splicing factor encoded by its own host gene *muscleblind*. Through an autoregulatory loop, whenever free MBNL1 is excess within the cell, it can downregulate its own pre-mRNA splicing by binding to circMBL1 (Ashwal-Fluss et al. 2014). Apart from its non-coding mode of functions, ORF-containing circRNAs can be translated into functional/non-functional peptides via internal ribosome entry site (IRES) mediated translation. This has been illustrated by FBXW-185aa, a peptide derived from circFBXW7 (F-Box and WD Repeat Domain Containing 7), which can act as a tumor suppressor by preventing deubiquitinylation of Myc in glioblastoma (Yang et al. 2018).

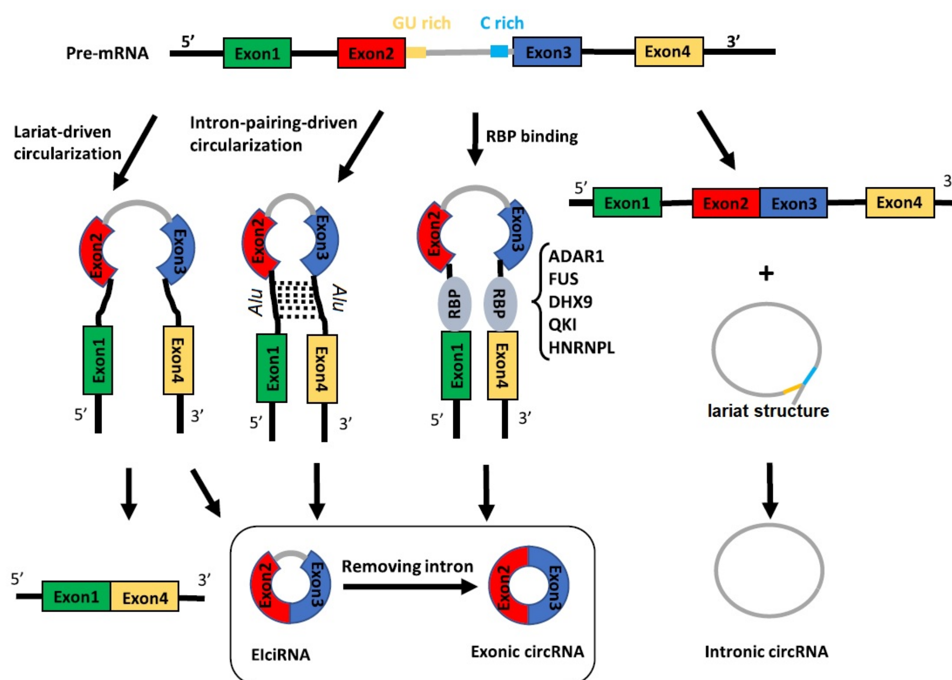

***Figure 14**: The different modes of circRNA biogenesis. Modified from* (Shang et al. 2019)

2 AIMS

In this thesis, we have aimed to characterize the role of different classes of non-coding RNAs in epidermal differentiation and skin cancers.

The objective of the individual studies are-

I. To investigate the functional significance of miR-203 in cSCC (**Paper I**)

II. To investigate the functional significance of miR-203 in cutaneous melanoma with a focus on metastasis (**Paper II**)

III. To dissect the changes in coding and non-coding transcriptome in cSCC (**Paper III**)

IV. To characterize the role of skin-specific lncRNA ELDAR in epidermal differentiation (**Paper IV)**

3 MATERIALS AND METHODS

Cell culture, treatments, transfections, and nucleofection

Human cSCC cell lines UT-SCC-7 (a kind gift from Veli Matti Kahari, University of Turku) and A431 (ATCC) were cultured in DMEM (Gibco) containing 10% FBS (Gibco) and 1% streptomycin/penicillin (Gibco) (**Paper I**). An additional 1% HEPES (Gibco) and 1% non-essential amino acids (Gibco) were added as supplements for the UT-SCC-7 culture medium. Melanoma cell lines BE, DFB and Sk-Mel-28 (kindly provided by Rolf Kiessling and Lars-Gunnar Larsson from Karolinska Institutet) were cultured in RPMI1640 (Gibco) supplemented with 10% FBS and 1% Penicillin/Streptomycin (**Paper II**). For DNA demethylation experiments using melanoma cell lines, we used 10 nM 5-aza-2-deoxycytidine (InvivoGen), and harvested the cells after 48 hrs of incubation in 5-aza containing medium (**Paper I** and **II**). Transfection with miRNA mimics or siRNAs was performed using Lipofectamine 2000 (ThermoFisher Scientific) according to the manufacturer's instruction (**Paper I** and **II**). Stable miR-203 or negative control-overexpressing UT-SCC-7 or BE cell lines were established by lentiviral vector pEZX-MR03-eGFP (GeneCopoeia). Transduced cells were sorted based on GFP expression using FACS after 48 hrs (**Paper I** and **II**). Sorted cells were once again selected with Puromycin (1 µg/ml, Gibco). Primary keratinocytes from adult donors (ThermoFisher Scientific) were cultured in EpiLife medium supplemented with growth factors (ThermoFisher Scientific). Differentiation of primary keratinocytes was induced by switching to growth factor-depleted EpiLife containing 1.5 mM Calcium Chloride (Sigma) (**Paper IV**). All the experiments with primary keratinocytes were performed when they were in passage 3. LncRNA knockdown was executed using an siRNA pool of 11–30 different siRNAs (siTOOLs Biotech). For the nucleofection, 5 million primary keratinocytes were electroporated with 1 nmol siPool using the Amaxa human keratinocyte Nucleofector kit (Lonza) using the program T-018 (**Paper IV**). Before seeding them into a 15 cm plate, nucleofected cells were immediately transferred to warm complete medium and incubated at 37°C (for 30 mins) to increase cell viability.

CRISPR-mediated transcriptional activation

In **paper IV**, for the transcriptional activation of the lncRNA locus, we used the CRISPR/Cas9 Synergistic Activation Mediator (SAM) system, which uses a dCas9 devoid of endonuclease activity. The core component of this system is three plasmids - sgRNA (MS2) cloning backbone

(Plasmid #61424), MS2-P65-HSF1_GFP (Plasmid #61423) and dCAS9-VP64_GFP (Plasmid #61422), which were purchased from Addgene. Online tool at Crispr.mit.edu was used to design the sgRNAs targeting a 500 nt long sequence upstream of the TSS. Individual sgRNA expression plasmid was constructed by first annealing the oligonucleotides pair and then ligating them to BbsI-linearized sgRNA (MS2) backbone (Konermann et al. 2015). Primary adult keratinocytes, at passage 3, with 70% confluence were transfected with all three plasmids using Lipofectamine 2000 (ThermoFisher Scientific). Transfected cells were harvested after 24 hrs for RNA extraction, or subjected to calcium chloride-mediated differentiation up to 96 hrs.

RNA extraction and qRT-PCR

Total RNA from primary keratinocytes and cancer cell lines growing in monolayer was isolated using TRIzol reagent (Invitrogen) and Chloroform (Merck) according to the manufacturer's instructions (**Paper I, II** and **IV**). For **paper III**, snap frozen tumor or healthy skin biopsies were homogenized using Tissuelyzer LT (Qiagen), followed by addition of QIAzol lysis reagent (Qiagen) to the pulverized material. From this point onwards, the protocol for miRNeasey (Qiagen) mini kit was followed to extract total RNA. For **paper IV**, total RNA from organotypic skin tissues was isolated using the RNeasy Plus mini kit (Qiagen) according to the manufacturer's instruction. RNA quantity was measured by NanoDrop One (ThermoFisher Scientific). For subsequent microarray or RNA-seq based expression analysis, RNA integrity was measured using an RNA 6000 Nano kit (Agilent Technologies) in a Bioanalyzer-2100 instrument (Agilent Technologies). RNA samples with an integrity score more than 8 were used for whole transcriptome analysis.

For quantifying mRNAs and lncRNAs by quantitative PCR, first, the RNA samples were reverse transcribed using a RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific) (**Paper I, II** and **IV**). For miRNA expression quantification, the reverse transcription was performed using a miRNA RT kit (Fisher Scientific) (**Paper I** and **II**). Gene expression was quantified by SYBR Green expression assays (ThermoFisher Scientific) or TaqMan Real-Time PCR assays using QuantStudio 7 Real-time PCR system (ThermoFisher Scientific) and normalized to housekeeping gene 18S (for mRNAs and lncRNAs) or U48 snoRNA (for miRNAs) using the delta-delta Ct-method.

Methyl-sensitive restriction enzyme-qPCR

For **paper II**, we performed a methyl-sensitive restriction enzyme-qPCR (MSRE-qPCR) analysis to quantify the demethylation in miR-203 promoter upon 5-aza treatment of the melanoma cell lines. Methylation-sensitive digestion of the CpGs within the promoter region of miR-203 was carried out with the EpiJET DNA Methylation Kit (ThermoFisher Scientific), which uses different preference for digestion substrates of *Msp*I and *Hpa*II (ThermoFisher Scientific). When an internal tetranucleotide sequence (CCGG) of a CpG is methylated, it undergoes digestion by *Msp*I (cuts both methylated and unmethylated sequence) but not by *Hpa*II (cuts only unmethylated). We used 150 ng of genomic DNA for the digestion reaction for 1 hr at 37°C. Digestion was followed by heat-inactivation at 90°C for 10 mins. The methylation levels were quantified by qPCR using primers flanking the CCGG site. qPCR data were analyzed by 2-ΔCt method, and presented as the ratio of *HpaII*-digested methylated DNA and input/non-digested DNA.

Bisulfite Pyrosequencing

The basis of bisulfite pyrosequencing is the differential conversion of unmethylated cytosines to uracils upon sodium bisulfite treatment, while the methylated cytosines remain unchanged. For the pyrosequencing analysis in **paper II**, CpGs within the miR-203 promoter were targeted using primers (a forward primer primer, biotinylated reverse primer, and a sequencing primer) designed by PyroMark software (Qiagen). Total 500 ng of genomic DNA was bisulfite-converted using EpiTect Bisulfite Kit (Qiagen) and eluted in a volume of 15 µl, quantified using a NanoDrop, and 20 ng of this bisulfite-converted DNA was PCR amplified using PyroMarks PCR kit (Qiagen). Epitect control DNA (human), which was either completely unmethylated or methylated (Qiagen), was used as a control to assess the efficiency and sensitivity of the PCR reaction. In the next step, utilizing a PyroMark Gold 96 reagent kit (Qiagen), we used the entire PCR product along with 4 pmol of the sequencing primer, and streptavidin sepharose beads (GE) for setting up the pyrosequencing reactions on a PSQ 96 system (Qiagen). PyroMark CpG software 1.0.11 (Qiagen) was used for data analysis.

Microarray

Expression profiling of RNA extracted from monolayer cultures or organotypic skin equivalents was performed using Affymetrix Genechip system at the Bioinformatics and Expression analysis core facility of Karolinska Institute (BEA) (**Paper I** and **IV**). Briefly, 100 ng of total RNA with RNA integrity value more than 8 were used to prepare cDNA following the Affymetrix 3′IVT Express Kit (ThermoFisher Scientific) labeling protocol. Standardized array processing procedures recommended by Affymetrix, including hybridization, fluidics processing, and scanning, were followed. Transcriptome Analysis Console (TAC, Affymetrix) was used for differentiation expression analysis. Statistical filters for individual studies were described in the respective articles.

RNA sequencing

For **paper III**, RNA extracted from the cSCC and healthy skin biopsies were sent to Exiqon (now part of Qiagen) for cDNA library preparation, and sequencing. Before cDNA preparation, 100 ng of total RNA was depleted of rRNAs using ribo-zero gold kit (Illumina Inc.), and enzymatically fragmented. Subsequently, the fragmented RNA was used for first strand and second strand cDNA synthesis, followed by DNA purification, end-reparation, 3'-adenylation and adaptor ligation. In the next step, cDNA library was PCR amplified and assessed for the size distribution using a Bioanalyzer (Agilent Technologies). After quality control, these libraries were sequenced using a NextSeq-500 instrument using v2 reagent kits, according to the manufacturer instructions (Illumina Inc.). Alignment of sequencing read was done by Exiqon/Qiagen using their XploreRNA pipeline based on the Tuxedo suit (Trapnell et al. 2012). After data filtering and read-mapping using Bowtie2 and Samtools, we aligned the transcripts using Cufflinks suite against the canonical chromosomes from GRCh37.p13. The identified transcripts were separated into protein coding and non-coding transcripts based on their ENSEMBL biotypes using BiomaRt. A trimmed Mean of M-values (TMM) normalization method was utilized to account for the cDNA library size variation before performing the differential expression analysis using edgeR (Bioconductor) (Robinson, McCarthy, and Smyth 2010). All the comparisons were corrected for multiple hypothesis correction by Benjamini-Hochberg approach. All the heat-maps and hierarchical clustering were made using Multiple Experiment Viewer (MeV).

NanoString nCounter assay

For the NanoString-based quantification in **paper III**, a custom CodeSet was designed targeting the mRNAs, lncRNAs, and backsplicing junctions of circRNAs to be validated. Reference genes RPLP0, PUM1, SF3A1, GUSB, and ALAS1 were used for normalization. Probe-set and RNA hybridization reactions were performed according to the manufacturer's instructions. For quantification, the mean of the negative controls was subtracted while analyzing the data using nSOLVER 3.0 software (NanoString Technologies). Positive control normalization was performed using the geometric mean of all reference genes. A second normalization was done using the geometric mean of the three most stable reference genes (ALAS1, PUM1, and SF3B1).

Enrichment analysis

Enrichment of biological processes (GO Biological process) and oncogenic signatures (MSigDB v7.1) in the differentially expressed genes were assessed using Enrichr (v1.0) (**paper I, II, III** and **IV**) (Chen et al. 2013). For the prediction of the potential upstream regulators, we employed the transcription factor module of MetaCore (Thomson Reuters, v19.1). MetaCore uses publicly available data to assess the over-representation of various transcription factor-binding motifs among a set of genes. Enriched terms with $P < 0.05$ upon Multiple-hypothesis correction were considered significant.

Cell proliferation assay

In **paper I**, the cell proliferation assay was performed using Click-iT™ EdU (a modified thymidine analogue) proliferation kit (ThermoFisher Scientific) according to the manufacturer's instructions. 48 or 72 h post-transfection, the cells were incubated in complete medium containing a 10 µM EdU solution for 2 hrs in a 37°C incubator. Subsequently, after aspirating the medium containing Edu, the cells were washed with 1% BSA (in 1X PBS) and fixed with Click-it fixative reagent at room temperature in the dark for 20 mins, followed by three wash with 1% BSA (in 1X PBS). For EdU detection, the cells were incubated with the EdU reaction cocktail for 30 mins on ice in the dark. Finally, EdU-labeled cells were rinsed with 1X PBS, and stained with AlexaFluor-647 before flow cytometric analysis.

Wound healing assay

In **paper I** and **II**, cSCC or melanoma cell lines were seeded and transfected in 6 well plates with miRNA mimic or siRNA. Post-transfection, when the cells were 90% confluent, a scratch was made in the middle of the plate using a 10 ul tip. After a wash with 1X PBS (Gibco), cells were kept in serum-free medium for up to 24 hrs and photographed at regular intervals to assess the closing of the gap made due to the scratch. ImageJ was used for calculating the wound area.

Migration and invasion assay

In **paper I** and **II**, cell migration and invasion assay were performed using the transwell migration chamber (Corning) and growth factor-reduced Matrigel invasion chamber (BD Biosciences). SiRNA or miRNA mimic transfected cells were resuspended in their respective serum-free medium and transferred onto the transwell insert at a density of 1.5×10^4 (migration) and 3×10^4 cells (invasion) per well in a 24-well setup. The lower chamber was filled with complete medium as the chemoattractant. After 24 hrs (migration) or 48hrs (invasion), cells that did not move to the other side of the insert were removed using a cotton swab. Finally, the migrated or invaded cells at the lower surface of the insert were fixed with 4% PFA and stained with crystal violet (Sigma).

Colony formation assay

For assessing the colony forming ability of the cancer cells, after transfection with miR-203 or negative control mimic, cSCC or melanoma cell lines were seeded at a density of 10000 cells/well in 24-well plates in their respective complete media containing 0.3% agarose with 0.6% agarose underlay (**Paper I** and **II**). Approximately, after 10 days, when the colonies are large enough (more than 100 cells), they were stained with Crystal Violet and photographed.

Tumor sphere formation assay

Sphere formation is another way of assessing the self-renewal and proliferative capacity of cancer progenitor cells. For that, after transfection with miR-203 or negative control mimic, cSCC or melanoma cells were resuspended in mixed medium of DMEM/F12 (1:1) (Gibco) supplemented with B27 (ThermoFisher Scientific) and seeded at a density of 4×10^4 cells/well

in 6-well plates coated with poly-HEMA (Sigma). When the spheroids become larger than a diameter of 100 µm, they are counted and quantified (**Paper I** and **II**).

Angiogenesis assay

For the angiogenesis assay in **paper I** and **II**, HUVEC cells (ATCC) were cultured in M200 medium (Gibco) supplemented with low serum growth supplement (LSGS) kit (Gibco). On the day of the experiment, HUVEC cells were trypsinized and counted before preparing a cell suspension of 4.5×10^4 cells/well in a mixed media (1:1) containing a conditioned media (from mimic or siRNA transfected cancer cells) and M200 medium without growth supplement. This cell suspension was plated onto a Matrigel-coated 48-well plate, and cultured for 18-20 hrs at 37°C, before photographing the wells for quantifying the number of well-formed nodes.

Tumor xenograft and metastasis assay

To perform the tumor xenograft assays in **paper I** and **II**, NSG mice (Jackson Laboratory) were purchased and acclimatized for 7 days before injecting 10^7 stable cell lines (BE or UT-SCC-7) subcutaneously into left (negative control) and right flank (miR-203) of each mouse. Prior to the injection, a cell suspension was prepared in 100 µl of ice-cold complete medium and mixed at equal volume with Matrigel (BD Biosciences). Twice a week, the mice were weighed, and inspected for any sign of sickness. Using a caliper, we also measured the size of the tumors on a weekly basis. When the tumor volume reached 1000 mm^3, the mice were sacrificed, and the tumors and organs were harvested and snap-frozen. For the metastasis assay in **paper II**, BE cells overexpressing miR-203-GFP or negative control-GFP tag were injected intravenously into the tail vein of the mice. Weekly monitoring was performed to check for weight loss and signs of sickness. At a predetermined human end-point, considering their health, the mice were sacrificed, and organs such as the brain, lungs, liver, and spleen were harvested. The presence of melanoma cells within the organs was measured by quantifying the GFP-positive cells using flow cytometry.

Organotypic culture for generating epidermis

In **paper IV**, for the generation of lncRNA-depleted organotypic human epidermal tissue, primary human keratinocytes were nucleofected with siPool and allowed to recover for 24 hrs in complete medium. The next day, the cells were trypsinized, counted, resuspended in a

keratinocyte growth medium (66% DMEM, 22% Ham's F-12, 1% Pen-Strep, 1% Antibiotic-antimycotic, 10% FBS, 0.2% Hydrocortisone, 0.2% Adenine, 0.1% Cholera toxin, 0.1% Insulin, 0.1% EGF, 0.1% T/T3) seeded onto the basement membrane side of the devitalized dermis (500,000 cells/dermis) and raised to the air–liquid interface to initiate stratification and differentiation, as described previously (Truong and Khavari 2007). Medium for differentiation was changed every other day. Organotypic tissues were harvested at day 4 of differentiation.

Immunofluorescence analysis of organotypic epidermis

Depending on the protein to be detected, skin sections were fixed in methanol, ethanol, or acetone (acetone- LOR, Col VII, Ki-67; ethanol- FLG, methanol- KRT1). Following blocking with 1% BSA, organotypic tissue sections were incubated overnight at 4°C with the primary antibodies (diluted in 1% BCS in 1X PBS) in a humidified condition (**Paper IV**). The next day, sections were washed thrice with 1X PBS before incubation with the fluorescently labeled secondary antibodies for one hour at room temperature. After the incubation, the slides were gently washed in 1X PBS and incubated with Hoechst solution for nuclear staining for 5 minutes at room temperature. Washed and air-dried slides were mounted using ProLong Gold Antifade Mountant (Thermo Fisher Scientific). Imaging was done with an Axiovert 200 M (Carl Zeiss) microscope at 20x magnification. Details about antibody dilutions can be found in the individual articles.

Western Blot

Cells were washed with 1XPBS and then lysed by RIPA buffer (Cell Signaling Technology) with EDTA (Sigma) and protease inhibitor cocktail (Roche). 20 µg protein of each sample was resolved in 4%-20% SDS-PAGE gel (Bio-Rad), transferred to PVDF membrane and probed with primary antibody. Detection was performed by incubation with HRP-conjugated secondary antibody (Dako) followed by incubation with chemoillumination detection reagent (ECL kit, Amersham). Details about antibody dilutions can be found in the individual articles.

RNAscope *In situ* hybridization

In **paper IV, f**or detecting the lncRNA in tissue and monolayer cell culture, a custom ISH probe was designed for the RNAscope assay (ACD). Cells growing in a monolayer on culture slides (Corning) were fixed in cold 10% neutral buffered formalin solution (Sigma) for 15 minutes. This was followed by sequential dehydration with 50%, 70%, and 100% ethanol. To block the endogenous peroxidases, the cells were treated with hydrogen peroxide (ACDbio) for 5 mins at room temperature. Next, the slides were incubated with Protease III (ACDBio) at room temperature for 20 minutes, followed by hybridization with a target or negative control probes for two hours at 40°C in a HybEZ™ II Hybridization System. This was followed by washing and sequential pre-amplification and amplification using RNAscope® Multiplex Fluorescent Reagent Kit v2 (ACDBio). For ormalin-fixed-paraffin-embedded (FFPE) healthy skin or cSCC specimens, the sections were deparaffinized using X-TRA-Solv and serially hydrated using alcohol of different concentration (100%, 96% and 70%) and water. A heat-induced antigen retrieval was performed with a target retrieval reagent (ACDBio) at 99°C for 10 mins. In the next step, the tissue sections were treated with Protease Plus reagent (ACDBio) at 40°C for 30 minutes, followed by hybridization with the target or negative control probes for two hours at 40°C. After washing, signal amplification was performed with sequential usage of preamplifier and amplifiers and labeling with a suitable fluorophore (TSA plus Fluorescence kit, PerkinElmer). Finally, the slides were air-dried and mounted with ProLong Diamond Antifade with DAPI (Thermo Fisher Scientific). Imaging was done using a Zeiss LSM800 confocal microscopy with 20x or 63x magnification. Confocal images were analyzed using Zen 3.1 (Zeiss) imaging software.

Statistical analysis

For different *in vitro* and *in vivo* experiments presented in the thesis, Student's t-test, Mann-Whitney *U* test, two-way ANOVA were used through Prism 7.0 (Graph Pad Software) to determine the statistical significance of an outcome (P-values < 0.05 were considered to be statistically significant). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and ****$P < 0.0001$.

4 RESULTS AND DISCUSSION

4.1 MiRNA-203 inversely correlates with differentiation grade, targets c-MYC, and functions as a tumor suppressor in cSCC

Rationale

MiR-203 is an epithelial tissue-restricted miRNA with the highest expression in the skin. Previous work from other groups and we have extensively demonstrated that miR-203 is a pro-differentiation miRNA which is highly induced during keratinocyte differentiation (Sonkoly et al. 2010; Wei et al. 2010; Yi et al. 2008). In our earlier study, a tumor-suppressive role of miR-203 was uncovered in a keratinocyte-derived malignancy (BCC) (Sonkoly et al. 2012). This prompted us to investigate the expression and functional role of this miRNA in another common keratinocyte-derived skin cancer, cSCC.

Main findings

MiR-203 is downregulated in poorly differentiated cSCC

Primary cSCC tumors, based on their resemblance to the tissue of origin, are grouped into three categories: well-differentiated (Grade I), moderately differentiated (Grade II) and poorly differentiated (Grade III) (Stenman et al. 2018), where grade III tumors have the highest chance to metastasize. *In-situ* hybridization and quantification demonstrated that compared to healthy skin, miR-203 is significantly downregulated in poorly differentiated cSCC (*Figure 15A, B*). This observation was validated in a separate cohort by qPCR (*Figure 15C*). Along this line, miR-203 abundance was strikingly reduced in patient-derived cSCC cell lines compared to primary human keratinocytes (**Paper I.** Figure 1c).

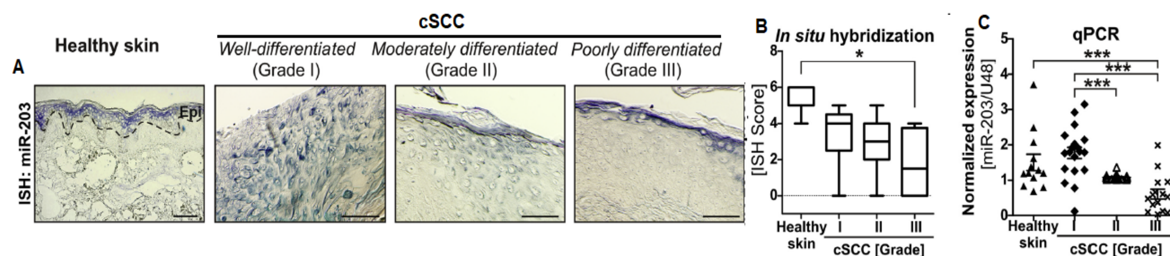

***Figure 15**: (A) Representative images of miR-203 ISH in healthy skin (n = 10), and cSCC with differentiation grades (n = 40). Scale bar= 100 µm. (B) Semi-quantitative ISH score represented in box-*

*plot. (C) Normalized expression of miR-203 in healthy skin (n = 13) and cSCC grade I (n = 20), grade II (n = 20) and grade III (n = 15) tumors. . Student's t test. *P < 0.05, ***P < 0.001.*

MiR-203 inhibits cell cycle transition from G1 to S phase

To gain a broader understanding of the functional relevance of miR-203 in cSCC, we performed transcriptome profiling of a metastatic cSCC cell line (UT-SCC-7), transfected with either miR-203 mimic or a control mimic. The result showed that miR-203 suppresses the expression of a number of genes (e.g. MYC, IL8, c-Jun, HGF) previously reported to play regulatory functions in cSCC (Ratushny et al. 2012; Syed et al. 2011; Toll et al. 2009; Tuong et al. 2019; Zenz et al. 2003). Functional categorization of differentially expressed genes upon miR-203 overexpression resulted in significant enrichment of GO terms such as "Cell proliferation" and "Cell cycle process". To elucidate the effect of miR-203 on cell cycle progression, we performed an EdU assay after overexpressing miR-203 in cSCC cell lines (A431 and UT-SCC-7). The result showed a significant increase in the number of cells in G1 phase and a reduction in the number of cells entering S phase (**Paper I**, Figure 2c), along with a reduction in total EdU positive cells in the miR-203-overexpression group. In line with this observation from cell cycle analysis, miR-203 overexpression also reduced the expression of proliferation markers- Ki-67 and PCNA, at mRNA and protein level.

C-MYC is a direct target of miR-203 and it can rescue miR-203-induced cell cycle arrest

Next, we aimed to identify the potential mediators of the miR-203-regulated transcriptomic changes. MetaCore's TF enrichment analysis predicted that oncogene c-MYC is one of the top upstream regulators of miR-203 regulated genes (*Figure 16A*). Bioinformatic analysis also identified a miR-203 binding site within the 3'-UTR of c-MYC, suggesting that it may be a direct target of miR-203. We validated this predicted miRNA-target interaction by a luciferase assay using reporter constructs containing either wild type or mutated 3-UTR of c-MYC. MiR-203 overexpression significantly reduced the reporter activity of the wild type construct, while the mutated construct almost rescued the effect (*Figure 16B, C*). Moreover, a transcription factor-activity assay using a DNA-binding ELISA kit showed that miR-203-overexpressing cSCC cells have attenuated c-MYC transcriptional activity. This was once again reflected in the downregulation of several c-MYC target genes *(CCND1, CDK1 and ODC1*) after miR-203 overexpression (**Paper I**, Figure 5e, and f). Next, we assessed if c-MYC can rescue the

inhibitory effect of miR-203 on cell cycle. To this end, we transfected cSCC cell lines with miR-203 mimic, either with an empty expression plasmid or expression plasmid containing full-length c-MYC sequence and subjected them to EdU assay. The result demonstrated that the number of cells accumulated in G1 phase due to miR-203 overexpression was significantly decreased upon c-Myc co-expression. Similarly, the number of cells in S phase was increased upon c-Myc co-expression with miR-203 (*Figure 16D*). These results effectively place c-MYC and miR-203 in a common regulatory axis to exert a tumor-suppressive role in cSCC.

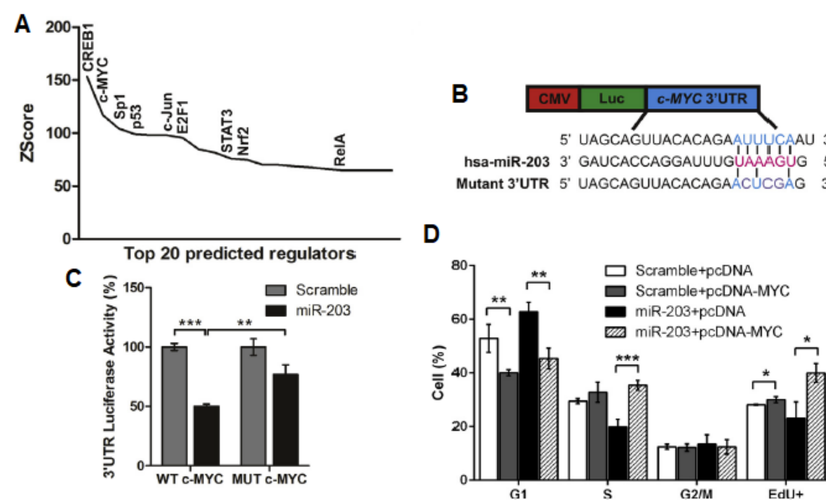

Figure 16: *(A) MetaCore analysis of potential upstream regulators of differentially expressed genes upon miR-203 overexpression. (B) Schematic of c-MYC and miR-203 seed pairing. Mutations introduced to the 3'-UTR for the luciferase assay are highlighted in purple. (C) Luciferase reporter activity measured for c-MYC wild type and mutated 3-UTR, construct in combination with control or miR-203 overexpression. (D) c-MYC rescue in EdU cell cycle assay. Student's t test *P < 0.05, **P < 0.01, ***P < 0.001.*

MiR-203 suppresses cSCC tumor growth in vivo

In addition to cell cycle and proliferation, we demonstrated that miR-203 suppresses a wide array of oncogenic hallmarks such as clonogenicity, cell motility, and angiogenesis *in vitro* (**Paper I**, Figure 2e, 3, 4). These observations prompted us to evaluate whether miR-203 has any suppressive effect on cSCC tumor formation *in vivo*. Therefore, we established UT-SCC-7 cell lines constitutively expressing miR-203 or negative-control using a lentiviral vector system and injected them subcutaneously to the left (negative control) and right (miR-203) flank of NSG mice. Tumor progression in these mice was followed up for five weeks (*Figure 17A*). In terms of tumor volume and weight, miR-203 overexpressing tumors were significantly smaller and lighter (*Figure 17B, C*), and exhibited diminished expression of proliferation

marker Ki-67. Similar to our observation *in vitro*, the expression of c-MYC was downregulated at the protein level in the tumors overexpressing miR-203 (**Paper I**, Figure 6d, and f).

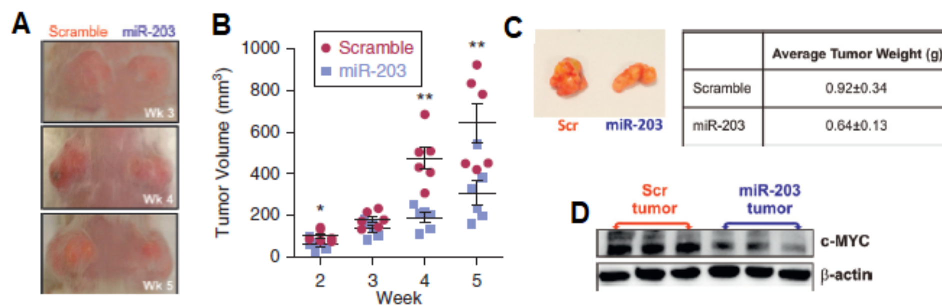

Figure 17: *(A) Representative images of tumor growth in xenograft assay upon subcutaneous injection of UT-SCC-7 cells stably overexpressing miR-203 (right flank) or negative control (left flank), into NSG mice. (B) Parameters for calculating the tumor volume ((width^2 X length)/2) was measured weekly using a caliper. (C) Harvested tumors at humane endpoint. Mean weight ± SD was presented in the table. (D) Western blot analysis of c-MYC expression in the harvested tumors after 5 weeks.*

Discussion

In this study, we have demonstrated that the abundance of miR-203 is progressively decreased from low-risk (grade I) to high-risk (grade III) cSCC. Particularly in the poorly differentiated cSCC, miR-203 expression was significantly downregulated. To corroborate this finding, we also performed a correlation analysis between miR-203 and involucrin, a marker of epidermal differentiation, and observed a positive correlation in the cSCC samples. These observations go consistently with the previous reports about miR-203 being induced specifically during epidermal differentiation and demarcating the highly proliferating basal layer and the spinous layer, which is committed to terminal differentiation (Sonkoly et al. 2010; Yi et al. 2008).

Our *in vitro* functional assays unveiled a tumor-suppressive role of miR-203 in cSCC, where it inhibited cell cycle, clonogenicity, cell motility and angiogenesis *in vitro*, and tumor growth *in vivo*. Consistent with these findings, earlier studies have demonstrated the tumor-suppressive role of miR-203 in various epithelial malignancies (e.g. lung, cervical, head and neck cancer) (Obayashi et al. 2016; N. Wang et al. 2014; Zhu et al. 2013).

Target identification, validation, and rescue experiment demonstrated that miR-203 possibly exerts a part of its tumor-suppressive role by directly inhibiting c-MYC, a well-known oncogene, which is consistently amplified in cSCC (Toll et al. 2009). As a transcription factor, it acts downstream of various growth factor signaling pathways (EGFR, NF-kB, PI3K etc.), and

induces the expression of genes related to cell proliferation, cell cycle progression, apoptosis, metabolism and protein biosynthesis (Gabay, Li, and Felsher 2014). Many of these c-MYC upstream signaling pathways are aberrantly activated in cSCC (Kobielak and Fuchs 2006; Ratushny et al. 2012). Of note, upon miR-203 overexpression, we observed a consistent downregulation of cyclin D1 (CCND1), a key effector of c-MYC (Yue et al. 2017). CCND1 is a cell cycle regulator, and often found amplified/upregulated in various types of cancer, including cSCC (Liao et al. 2007; SHEN et al. 2014). This aberrant expression can contribute to an early exit from G1 phase and uncontrolled cell proliferation during malignant transformation. Therefore, by targeting a critical hub like c-MYC, miR-203 can simultaneously modulate different nodes of an extensive pro-oncogenic network.

Although we primarily focused on c-Myc as a direct target of miR-203 in cSCC, various other targets have been reported, such as p63 in mice and human epidermal development, c-Jun in BCC, and MAPK signaling pathway activator HBEGF in cSCC (Lena et al. 2008; Riemondy et al. 2015; Sonkoly et al. 2012; Yi et al. 2008). Our gene set enrichment and upstream regulator analysis of miR-203-regulated genes could indeed trace the alterations in the genes downstream of these targets (e.g. p63 and c-Jun). A multitude of targets, belonging to a wide array of pro-oncogenic pathways, make miR-203 an ideal candidate for therapeutic application. Apart from standalone therapy, it will be equally interesting to evaluate if miR-203 mimic can supplement the effect of MYC-inhibitor drugs (Allen-Petersen and Sears 2019).

4.2 Genome-wide screen for miRNAs reveals a role for miR-203 in melanoma metastasis

Rationale

Malignant melanoma is one of the deadliest cancer types, with its metastatic variant having a particularly grim survival rate (5-19%) (Sandru et al. 2014). In this study, we wanted to identify the miRNAs associated with metastatic melanoma and explore their functional significance.

Main findings

MiR-203 is downregulated in metastatic melanoma, and correlates with overall survival

To identify the miRNAs deregulated in metastatic melanoma, we performed an *in silico* analysis of the small-RNA expression data of the skin melanoma cohort from The Cancer Genome Atlas (TCGA) database. Differential expression analysis between primary (n = 96) and metastatic tumors (n = 346) revealed that miR-203 is the most downregulated miRNA in the metastatic cohort ($\log_2$ fold change= -3.4086, P= 8.49 x 10^{-28}) (*Figure 18A, B*). Next, we assessed the prognostic potential of miR-203 abundance in the metastatic patients by performing a survival analysis, which showed that the patients with higher miR-203 expression (RPKM > 7) have a significantly longer overall survival compared to the patients with lower miR-203 expression (RPKM < 4) (*Figure 18C*).

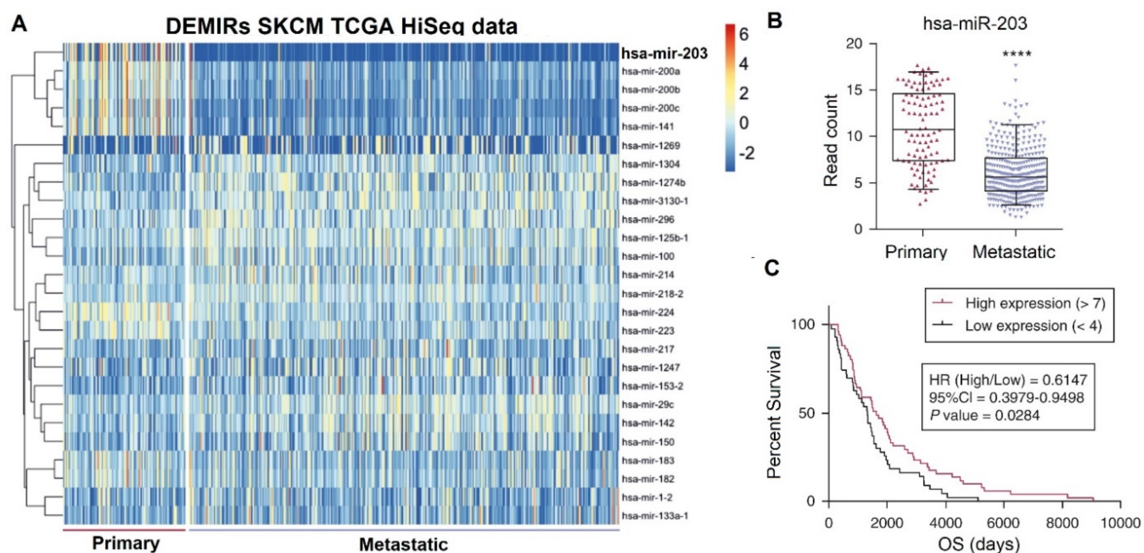

Figure 18: *(A) Unsupervised clustering and heatmap of differentially expressed miRNAs between primary and metastatic melanoma from TCGA SKCM cohort ($\log_2$ fold change ± 0.58, FDR < 0.05). (B) Expression of miR-203 in RPKM in primary and metastatic melanoma. Mann-Whitney U test. **** P < 0.0001. (C) Kaplan-Meier analysis for evaluating the association between miR-203 expression and long-term survival of patients with metastatic melanoma (TCGA).*

Since *MIR203* locus is located within a CpG island (chr14, CpG 179), we postulated that DNA methylation could be a potential regulatory mechanism to suppress miR-203 expression in metastatic melanoma. Therefore, we compared the primary and metastatic tumors in terms of the estimated level of methylation within the CpG island overlapping the *MIR203* promoter and observed significant hypermethylation in the metastatic cohort (**Paper II**, Figure 1e). To substantiate this finding, we performed an *in vitro* demethylation assay using 5-aza-2-deoxycytidine in a panel of melanoma cell lines (BE, DFB, and SK-Mel-28), which resulted in an increased expression of miR-203 (**Paper II**, Figure 1f). Both *MSRE*-qPCR and bisulphite pyrosequencing-based analyses targeting the CpGs within the promoter of *MIR203* unraveled a concomitant hypo-methylation signature in the 5-aza-treated cells (**Paper II**, Figure 1g, Supplementary figure S2).

MiR-203 suppresses oncogenic hallmarks such as cell motility, self-renewability and angiogenesis

Next, we evaluated the functional significance of miR-203 in cutaneous melanoma, by assessing its impact on several hallmarks of cancer and metastatic progression, such as the capacity of the tumor cells for migration, invasion, enhanced clonogenicity, and formation of new blood vessels at the site of dissemination through neoangiogenesis. To this end, we transfected three melanoma cell lines with miR-203 mimic or control mimic, and subjected them to transwell migration and invasion assays, which showed a significant reduction in the number of migrated and invaded cells after miR-203 overexpression (**Paper II**, Figure 2b). MiR-203-overexpressing cells formed smaller and lesser number of spheres in a three-dimensional sphere formation assay, highlighting the inhibitory effect of this miRNA on the self-renewability of melanoma cells in an anchorage-independent growth condition (**Paper II**, Supplementary figure S5). Moreover, in an *in vitro* angiogenesis assay, HUVEC cells treated with the conditioned supernatants from miR-203-overexpressing cells formed a significantly lesser number of capillary nodes compared to the control mimic treated group. (**Paper II**, Figure 3a). These *in vitro* functional data suggested that miR-203 has a tumor-suppressive and potentially anti-metastatic effect in melanoma.

Prometastatic transcription factor SLUG is a direct target of miR-203 in melanoma

In order to identify a potential direct target through which miR-203 can exert its tumor suppressive function, we performed an *in silico* analysis, which identified SLUG (protein SNAI2) with an 8mer binding site in its 3'-UTR. As an essential transcription factor that promotes epithelial-mesenchymal transition, SLUG appeared to be a relevant target of miR-203 in melanoma. Target validation in a luciferase assay, using reporter construct containing either wild type or mutated 3'-UTR of SLUG, demonstrated that miR-203 overexpression significantly reduced the luciferase activity in the wild type construct while the mutated construct could rescue the effect (**Paper II**, Figure 4a, and b). SLUG expression was also markedly reduced at RNA and protein level upon miR-203 overexpression in all three melanoma cell lines. SLUG knockdown phenocopied the effect of miR-203 upregulation on melanoma cell motility (**Paper II**, Figure 5). Finally, we assessed if exogenous SLUG expression can salvage the inhibitory effect of miR-203 on cell motility. To test this, we double transfected melanoma cells with miR-203 mimic and an expression vector containing full-length SLUG sequence (or empty vector as control) and performed a transwell migration assay, which showed that SLUG restoration in miR-203-overexpressing cells increased the number of migrated cells drastically (**Paper II**, Supplementary figure S9).

MiR-203 inhibits primary melanoma growth and metastasis in vivo

To explore the tumor-suppressive effect of miR-203 in an *in vivo* setup, we established BE melanoma cell lines constitutively expressing either miR-203 or negative control. To perform a primary tumor xenograft assay, we injected the miR-203-overexpressing and control cells to the right and left flank of NSG mice and followed the tumor growth for four weeks. At this point, the volume of the miR-203-overexpressing tumors was significantly lesser compared to the control group. The average weight of the miR-203-overexpressing tumors was reduced non-significantly ($P = 0.0571$) as compared to the control group (**Paper II**, Figure 5a, and b). Of note, we noticed visibly enlarged inguinal lymph nodes, a sign of metastasis, only on the side of the mice injected with control melanoma cells. Immunohistochemical analysis of lymph nodes with Melan-A (a marker for melanoma) affirmed the presence of melanoma cells in the enlarged lymph nodes, further strengthening our hypothesis about a potential inhibitory effect of miR-203 on melanoma metastasis (**Paper II**, Figure 6f).

To evaluate a more direct effect of miR-203 on melanoma metastasis, we injected the miR-203 or negative control overexpressing BE cells (also expressing reporter GFP) into the tail vein of NSG mice. These mice were regularly weighed and monitored for any sign of sickness. Surprisingly, while the mice injected with the control cells kept losing weight drastically, the miR-203-overexpression group did not show any sign of sickness or substantial weight-loss (*Figure 19A*). Quantification of GFP-expressing melanoma cells metastasized to different organs (brain, liver, lung, and spleen) from both groups revealed a significantly lower percentage of GFP-positive cells in the lungs of miR-203-injected mice (*Figure 19B*). Histological analysis of lung sections together with immunofluorescence analysis of Melan-A highlighted a lesser abundance of melanoma cells in the group injected with miR-203 expressing BE cells (*Figure 19B*). Taken together, these findings demonstrate that miR-203 suppresses melanoma progression by impeding primary tumor growth and metastasis to regional lymph nodes and lungs.

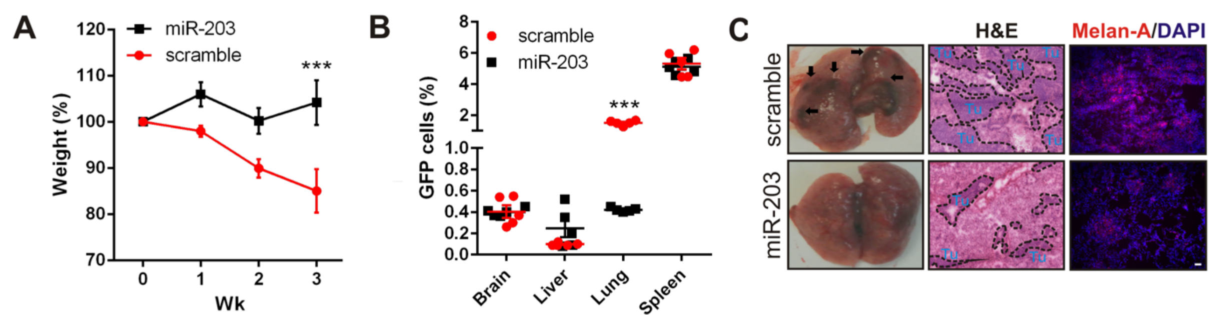

Figure 19: *(A) Change in the body weight of the NSG mice intravenously injected with melanoma cells stably overexpressing miR-203 or negative-control sequence for* ***in vivo*** *metastasis assay. (B) Flow cytometric analysis of GFP-positive cells to estimate the metastatic load in different distant organs where melanoma cells metastasize. (C) Representative images of the harvested lungs from miR-203 or control cell injected mice. Black arrows indicate metastatic nodules (left panel). Hematoxylin-eosin staining (middle panel) and Melan-A IF staining (right panel) in lung tissue sections. Dotted lines in the middle panel highlight the tumor (Tu) stroma boundary. ANOVA or Mann-Whitney U test.* $^{***}P < 0.001$*.*

Discussion

In this study, we reanalyzed the small RNA-seq data from the TCGA SKCM cohort in order to identify the miRNAs associated with metastatic melanoma. We identified 77 differentially expressed miRNAs, which included miR-203, miR-224, miR-146a, and several members of the miR-200 family (miR-200a, miR-200b and 200c). In addition to miR-203, which we have characterized in this article, miR-200c has been identified as a suppressor of EMT and drug resistance in melanoma by targeting BMI1 (S. Liu et al. 2012:1). MiR-224 has been reported to

act as a tumor suppressor in uveal melanoma by attenuating the PIK3R3/Akt3 signaling pathway (J. Li et al. 2019). Previously, miR-146a was shown to be a negative regulator of immune activation in melanoma cells (Mastroianni et al. 2019). In addition to the miRNAs previously implicated in melanoma, we came across many miRNAs with yet to be characterized role in melanoma development.

MiR-203, which is a well-characterized miRNA in epidermal homeostasis and keratinocyte-derived cancers, was found to be the top downregulated miRNA in metastatic melanoma. Additionally, low miR-203 expression correlated with poor survival in metastatic melanoma (Li et al. 2015), underscoring its prognostic value. In this regard, further analysis will be required to evaluate the association between miR-203 abundance and different clinicopathological features of regional and distant metastatic melanoma. Expression analysis in primary cells and melanoma cell lines revealed that miR-203 is expressed in the detectable range in primary melanocytes, although less than the level in differentiating keratinocytes, and it is further downregulated in the melanoma cell lines of different pigmentation status, suggesting a potentially tumor-suppressive role of miR-203.

Next, we set out to explore the tumor-suppressive role of miR-203 in melanoma using different *in vitro* and *in vivo* functional assays where miR-203 inhibited cell migration, invasion, angiogenesis, growth of primary xenograft tumors, and most importantly suppressed metastasis to inguinal lymph and lungs. The anti-metastatic function of miR-203 has been previously explored in head and neck SCC, where it directly targets multiple targets for regulating cytoskeletal rearrangement and ECM remodeling (Benaich et al. 2014). Consistent with our observations, previous studies reported a growth inhibitory and pro-melanogenic effect of miR-203 in primary melanocytes and melanoma cell lines (Noguchi et al. 2012, 2014). In one such investigations, Noguchi et al. had shown that miR-203 promotes pigmentation in melanoma cells by increasing Melan-A and TYR expression through directly targeting kinesin superfamily protein 5b (KIF5b) (Noguchi et al. 2014). However, the metastasis-suppressing role of miR-203 in melanoma is uncovered for the first time through our study.

Altered methylation of tumor suppressor genes is a frequent event in melanoma progression (Wouters et al. 2017). Given that the *MIR203* gene and its promoter are embedded within a CpG-island on chromosome 14, we hypothesized hypermethylation events at *MIR203*-promoter, which can potentially suppress miR-203 expression in the metastatic cohort. Analysis of TCGA methylation data showed that promoter-associated CpGs, as well as the proximal

CpGs, are indeed differentially hypermethylated in the metastatic tumors. This finding was further corroborated with our 5-aza experiments followed by qPCR and sequencing-based quantification of promoter methylation in three different melanoma cell lines. Interestingly, bisulfite sequencing highlighted variable baseline methylation across all the cell lines used for this study. For instance, Sk-Mel-28, which has the highest miR-203 expression among the three cell lines, showed the lowest baseline methylation for the CpGs we analyzed.

In this study, we have identified SLUG, an EMT-promoting transcription factor belonging to the Snail family, as a direct target of miR-203 in melanoma. MiR-203 overexpression and SLUG loss-of-function had a similar effect on melanoma cell motility. Moreover, SLUG restoration managed to reverse the miR-203 mediated inhibitory effect on cell motility. This finding is consistent with the general observation about SLUG being a promoter of EMT and upregulated in various types of cancer (Assani and Zhou 2019:2; Pérez-Mancera et al. 2005). Similar to our results, Shirley et al. found that SLUG overexpression in primary melanocytes and melanoma cells leads to increased cell motility (Shirley et al. 2012). Additionally, our data showed a reduced abundance of SLUG protein in miR-203-overexpressing tumor cells, in both primary tumors and metastasis. Taken together, our result places SLUG and miR-203 in a regulatory axis, which regulates cell motility and metastasis in cutaneous melanoma.

4.3 A comprehensive analysis of coding and non-coding transcriptomic changes in cSCC

Rationale

The objective of this study was to establish a robust catalogue of differentially expressed protein coding and non-coding transcripts in cSCC. Although a number of articles have reported detailed characterization of genetic and transcriptomic changes of protein coding genes in cSCC, only a few studies, using array-based profiling approach, have explored the expression of lncRNAs and circRNAs in a limited number of samples (Chitsazzadeh et al. 2016; Sand, Falk G Bechara, et al. 2016; Sand, Falk G. Bechara, et al. 2016). With a larger sample size, increased sequencing depth, and stringent statistical filter, we aimed to generate a comprehensive and reliable dataset, which could be a basis for future investigations.

Main findings

Identification of differentially expressed protein coding genes, including essential transcriptional regulators of cSCC pathogenesis

For the whole transcriptome analysis by RNA-seq (100 bp, paired end), we used a discovery cohort of 7 healthy skin and 9 cSCC tumors, and identified 5,352 differentially expressed (FCH > 1.5, FDR < 0.05) protein coding genes (3,419 up- and 1,933 downregulated) in cSCC (**Paper III**, Figure I). In the validation cohort, we also included AK samples to get an indication about the dynamic expression of the selected transcripts in a precancerous stage. Three of the deregulated transcripts (MMP1 and IFN-YR2 upregulated, and NOTCH2 downregulated in cSCC) were successfully validated in an expanded cohort (healthy skin n = 11, AK n = 8, and cSCC n = 28*)* using NanoString assay (**Paper III**, Figure 3). While MMP1 and IFN-YR2 did not show any altered expression, NOTCH2 was sigficantly downregulated in the AK samples.

GO analysis for the upregulated protein coding genes were significantly enriched for terms such as “Cellular response to interferon-gamma”, “extracellular matrix organization”, and “negative regulation of programmed cell death”. In contrast, terms such as “establishment of skin barrier”, and “regulation of water loss via skin” were enriched for the downregulated genes. Besides, EGFR, KRAS, mTOR/AKT, MEK and p53-gene signatures were significantly enriched from the oncogenic gene sets (Molecular Signature database) (**Paper III**, Figure 2A, B).

To better assess the altered transcriptional network in cSCC, we aimed to identify the potential upstream regulators of the differentially expressed genes. Enrichment analysis conducted using MetaCore's TF module identified significant enrichment of 519 TFs whose target genes are overrepresented (*Figure 22A*) in our dataset. ETS1, p63, p53, KLF4, and SP1 were some of the enriched TFs, which have been previously functionally implicated in epidermal homeostasis and cSCC tumorigenesis (Benjamin and Ananthaswamy 2007; X. M. Li et al. 2019:4; Moses et al. 2019; Nagarajan et al. 2010; Nakamura et al. 2007). Out of the 519 enriched TFs, 105 were differentially regulated (64 up- and 41 downregulated) at mRNA level (**Paper III**, Figure 2C, D). Several TFs (e.g, FOXP3, USF1, EGR3, and IRF1) with previously unreported function in cSCC were found to be both enriched in the MetaCore analysis and differentially expressed at transcript level.

Identification of differentially expressed lncRNAs; highlights on oncogenic and skin-enriched lncRNAs

Consistent with the literature, we observed an overall lower abundance of lncRNA transcripts (Cabili et al., 2011) (*Figure 20A*). Differential expression analysis identified 908 annotated lncRNAs (319 upregulated and 589 downregulated), among which the majority were antisense or intergenic (*Figure 20B, C*). SNHG12, CASC9, LUCAT1, and PVT1 were some of the upregulated lncRNAs, which were previously reported for their pro-tumorigenic functions in various cancer types. Contrastingly, TINCR, a lncRNA with differentiation promoting and tumor-suppressive function in epithelial cancers, was found to be significantly downregulated in our cSCC cohort. Using an expanded validation cohort, we successfully validated two potentially oncogenic lncRNAs (SNHG3 and MIR31HG), identified to have increased expression in the RNA-seq cohort. In the validation cohort, SNHG3 was significantly upregulated already in the precancerous AK samples, and MIR31HG was only upregulated in the primary cSCC (*Figure 20D*). SCEL-AS, a previously uncharacterized skin-enriched lncRNA was also validated in the expanded cSCC cohort and found to be downregulated already in the AK samples (*Figure 20D*).

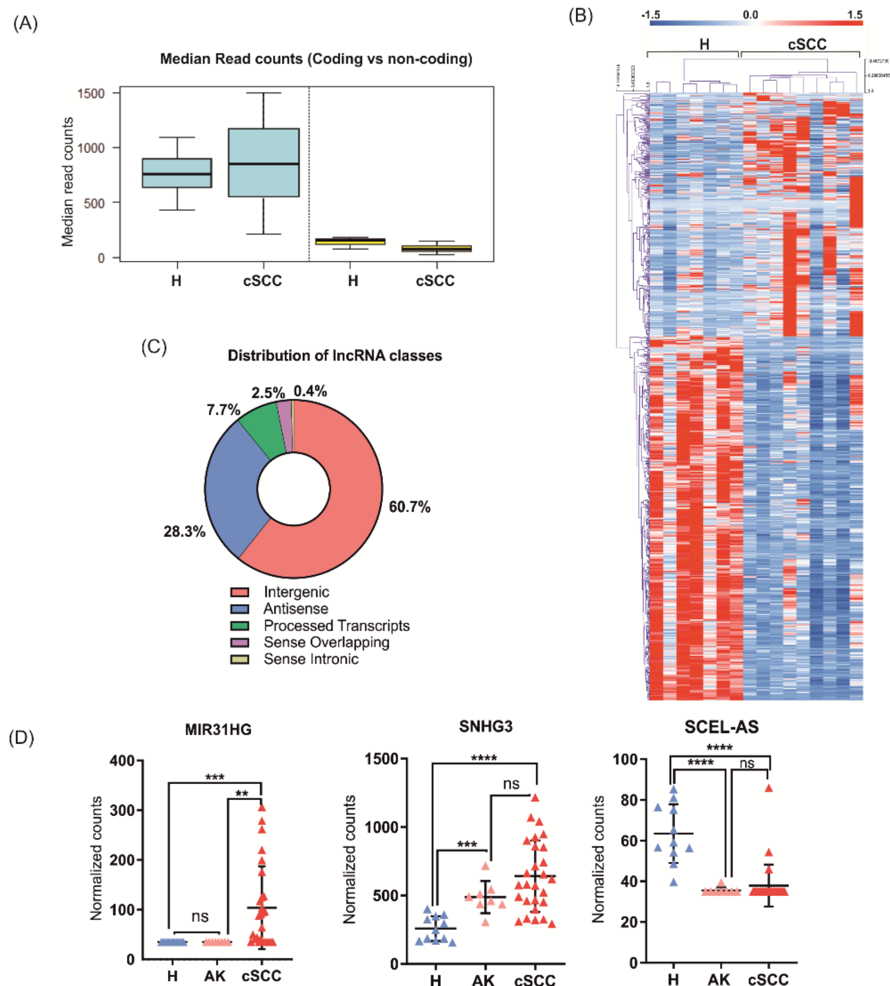

Figure 20: *(A) Median read count distribution of coding vs non-coding transcripts in healthy and cSCC samples. (B) Hierarchical clustering all differentially expressed lncRNAs in cSCC (FDR < 0.05 and FCH > 1.5). (C) Pie-chart showing the percentage of different classes of lncRNAs identified in the expression analysis. (D) Validation of selected lncRNAs (MIR31HG, SNGH3 and SCEL-AS) by NanoString nCounter assay. Mann-Whitney U test **P < 0.01, ***P < 0.001, ****P < 0.0001.*

LncRNAs, often expressed in a tissue-specific manner, play an important role in tissue development and homeostasis. These lncRNAs are also more likely to be deregulated during malignant transformation. Therefore, to gain insight into the tissue-specific expression signature, we calculated a tissue enrichment score (T) for the deregulated lncRNAs using the RNA-seq data from 27 tissues (including adult skin) available through GTEx (Genotype-Tissue expression) database. We performed a similar analysis for all the deregulated protein coding genes and found that the lncRNAs fare better in terms of skin-specificity (Ts >0.9) as seen from the comparison between the two groups for the frequency distribution of their tissue enrichment scores (T) (**Paper III**, Supplementary Figure S2). A snapshot of the top 50 differentially

expressed lncRNAs with skin-enriched expression revealed that 47 of them were downregulated in cSCC (*Figure 21*).

To identify the potential upstream regulators of the deregulated lncRNAs, we performed an enrichment analysis for overrepresented transcription factor motifs (**Paper III**, Supplementary Figure S5), which revealed that CREB1, ATF1, and NRF1 were significantly enriched for the upregulated lncRNAs, while SP1/SP4 was enriched for the downregulated lncRNAs.

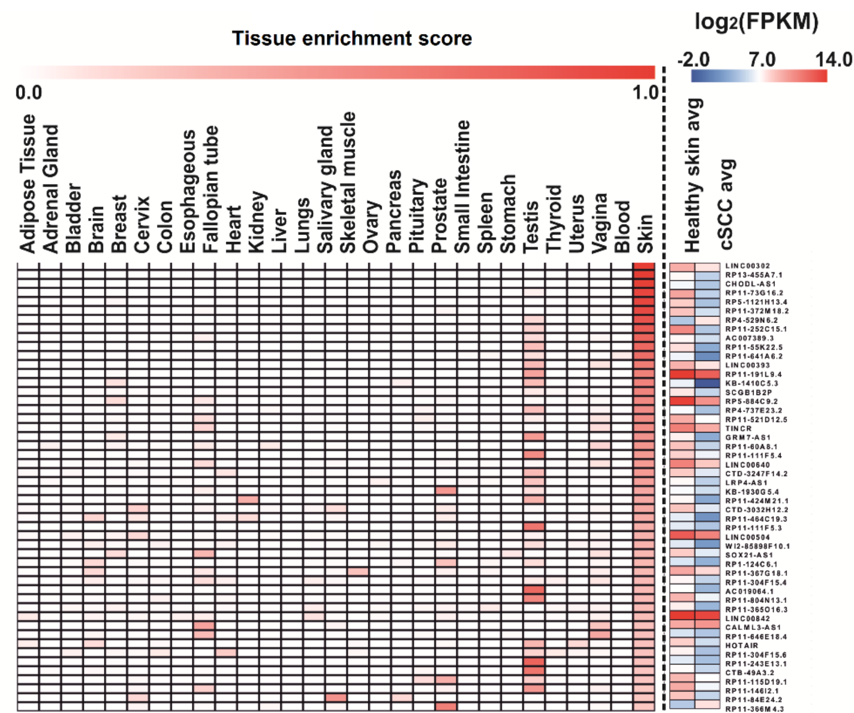

***Figure 21**: Heatmap showing the enrichment (T) of top 50 skin enriched lncRNAs in different tissue types in GTEx and their average expression in healthy skin vs cSCC cohort.*

Identification of differentially expressed circRNAs in cSCC

Using find_circ and CIRCexplorer pipelines and a stringent cutoff of at least five backspliced junction-spanning reads, we identified 197 and 139 high confidence circRNAs in healthy skin and cSCC samples. Besides the annotated circRNAs, our analysis also identified six novel circular transcripts, which were not present in the circ_base annotation catalogue. Based on the name of their respective host gene, we have denoted them as circ_IFFO2, circ_PLIN4, circ_DMKN, circ_METRNL, circ_KRT1 and circ_POF1B. The majority of the detected circRNAs have lower expression in the cSCC samples, which was later reflected in the

expression analysis. Interestingly, two of the novel circRNAs (circ_*IFFO2* and circ_*POF1B*) were particularly abundant (expression > 1 RPM) in normal skin.

From the differential expression analysis of high-confidence circRNAs obtained through two different pipelines, 55 were (2 up- and 53 downregulated) found to have significantly altered expression ($P < 0.05$) in cSCC (*Figure 22A*). To understand if the differentially expressed circRNAs have any correlation with the expression of their linear host genes, we plotted their fold change (RPM in cSCC/ RPM in Healthy skin) against circular to linear (CTL) ratios (**Paper III**, Supplementary Figure S8), and found that most of them were expressed independently of their host genes. Using NanoString assay, we validated three of our differentially expressed circRNAs. Among them, CDR1as, one of the most widely studied circRNA, and circ_IFFO2, a novel circular transcript detected in our analysis, was significantly downregulated both in AK and cSCC samples. Circ_TNFRSF21, another novel circRNA identified in our analysis, was validated to be upregulated in cSCC without any alteration in the AK samples (*Figure 22B*). We also verified the circularity of the novel circRNA (circ_METRNL, circ_IFFO2, circ_POF1B) candidates in a PCR based method using divergent primers that selectively amplify the circular transcripts. Not only had we got the amplicons with the correct size but also confirmed the correct sequence of the backspliced junctions by Sanger sequencing (*Figure 22C*).

Discussion

In conclusion, using deep-sequencing-based expression analysis, we have generated a comprehensive list of differentially expressed coding and non-coding transcripts, providing many uncharacterized targets for potential therapeutic interventions in cSCC. Of note, a large number of previously unreported mRNAs were detected to be differentially expressed in our analysis, presumably due to increased RNA-seq depth. In agreement with the previous findings, differentially expressed protein coding genes contained the signatures for extracellular matrix disassembly, increased cell proliferation, expansion of progenitor population, decreased apoptosis, altered epidermal differentiation, and heightened immune response (**Paper III**, Supplementary figure S1) (Hameetman et al. 2013; Ratushny et al. 2012). Of note, several pro-angiogenic chemokines related to cytokine-mediated signaling pathway were overexpressed in the cSCC samples, which have not appeared so prominently in the previous profiling studies (Chitsazzadeh et al. 2016; Hameetman et al. 2013; Keeley, Mehrad, and Strieter 2010).

Consistent with the earlier reports, we identified aberrant MAPK/ERK, mTOR/AKT, NF-kB, and p53-mediated DNA damage response pathways, which further proved the validity of our cohort and the dataset (Liberzon et al. 2015). Our upstream regulator analysis revealed, in addition to previously studied TFs in cSCC, a set of regulators with hitherto uncharacterized functions in epidermal homeostasis or cSCC development.

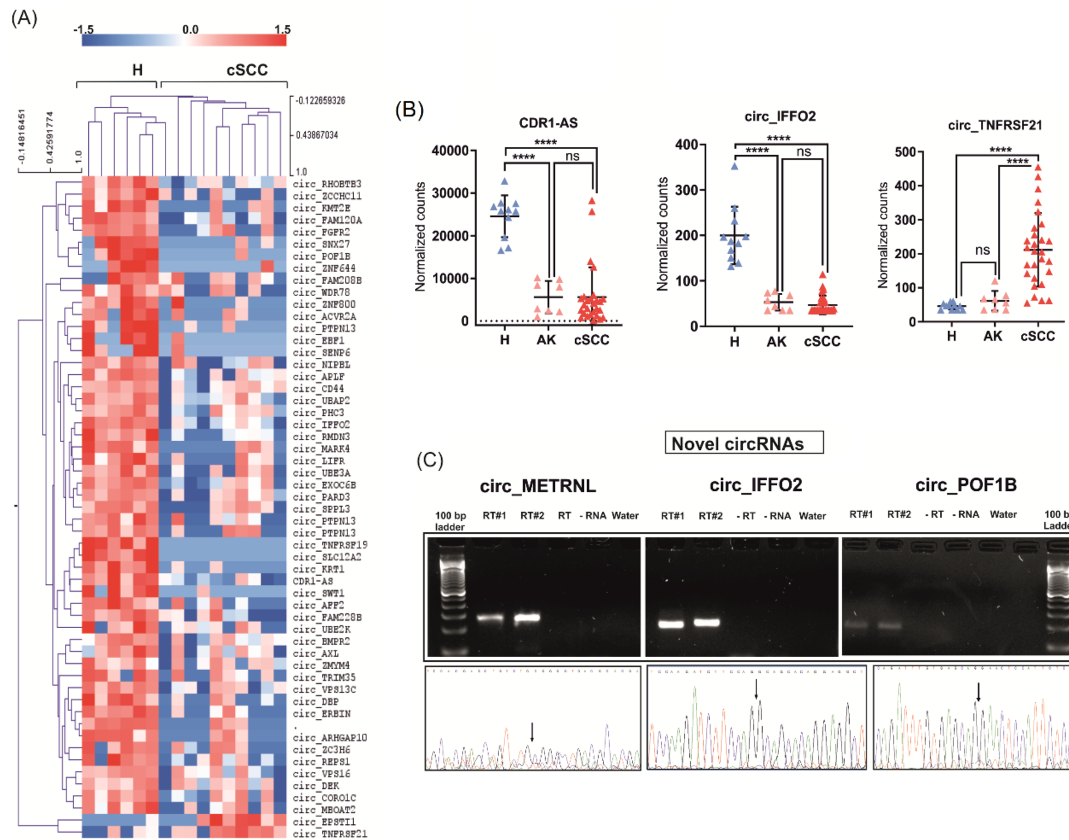

Figure 22: *(A) Hierarchical clustering of differentially expressed circRNAs in cSCC (B) Validation of selected differentially expressed circRNAs by NanoString nCounter assay in a larger cohort. Mann-Whitney U test ****P < 0.0001. (C) PCR validation of novel circRNAs (circ_METRNL, circ_IFFO2 and circ_POF1B). Agarose gel image showed the expected size of PCR product (present only in the reverse-transcribed samples). PCR products were verified by Sanger sequencing. Arrows point at the backspliced junctions.*

One of the key findings from this study was the identification of differentially expressed lncRNAs, which included several upregulated lncRNAs (MIR31HG, PVT1, LINC00152 etc.) with broad oncogenic functions. MIR31HG, one of the most upregulated lncRNAs in our dataset, has been reported to be upregulated by BRAF activation, suppresses CDKN2A epigenetically, and, promotes tumor cell growth and viability by suppressing senescence (Montes et al. 2015:31). Another prominent oncogenic lncRNA PVT1, found to be induced in

numerous solid tumors, was also upregulated in our cSCC cohort. It correlates with the disease severity, promotes tumor cell proliferation, viability, and contributes to the development of drug resistance (Derderian et al. 2019:1). LINC00152 (alternatively CYTOR) has been reported to be upregulated in colorectal cancer and contributes to cancer progression by activating NF-κB pathway (X. Wang et al. 2018). Several of these upregulated lncRNAs (e.g. LINC00346, PVT1, SNHG12, ZFAS1 and TUG1) were also found to be highly abundant in primary and metastatic cSCC cell lines, making them exciting targets for functional characterization (Piipponen et al. 2016). While comparing with the previous lncRNA profiling study in cSCC with smaller sample size (n = 3), we identified 124 commonly altered (51 up- and 73 downregulated) lncRNAs, potentially highlighting a set of frequently deregulated lncRNAs in a highly heterogeneous malignancy like cSCC (Sand, Falk G Bechara, et al. 2016). Needless to say, the majority of these lncRNAs are yet to be characterized in the context of cSCC.

The skin enriched lncRNAs (e.g. RP13-455A7.1, RP11-73G16.2, TINCR, CHODL-AS) identified in our analysis, are equally interesting for further characterization. Given that cSCC is a keratinocyte-derived malignancy, it would be intriguing to check their expression in primary keratinocytes, explore their potential role in epidermal homeostasis, and finally understand their dysregulation in premalignant AKs and primary cSCC tumors.

Due to the usage of total RNA for RNA-seq, we could simultaneously obtain expression data for circRNAs and their host genes. In addition to the previously annotated circRNAs, this unbiased approach helped us to detect six novel circRNA candidates, out of which three could be validated. In general, we observed a downregulation of circRNA expression in the cSCC samples, possibly due to an overall dysregulation of their biogenesis. While looking further for possible explanations in our expression data, we found that ADAR, which has been reported to suppress backsplicing events and circRNA biosynthesis, was sigficantly upregulated in our cSCC cohort (Shi et al. 2017). In contrast, ESRP1 and MBNL1, two of the positive regulators of circRNA biogenesis, were downregulated (Li et al. 2018). However, such a systemic change in circRNA biogenesis is yet to be put into the context of cSCC development.

Among the differentially expressed circRNAs, a number of them (e.g. CDR1as, circ_UBAP2, circ_SNX27) with previously reported oncogenic functions, demand further attention because all of them were downregulated in our cSCC cohort (Huang et al. 2017:100338; H. Zhang et al. 2017; Zhao et al. 2019). Interestingly, we observed that three circRNAs (circ_MBOAT2*,* circ_PTPN13 and circ_ACVR2A*)* previously found to be upregulated during epidermal

differentiation, were also sigficantly downregulated in cSCC (Kristensen et al. 2018). Therefore, they appear to be potential candidates for in depth characterizations in the context of epidermal homeostasis and cSCC progression.

4.4 Characterization of lncRNA ELDAR as a critical regulator of late epidermal differentiation

Rationale

Disruption of the differentiation program is one of the key features in various epidermal disorders, including cutaneous squamous cell carcinoma (Ratushny et al. 2012; Scott et al. 1988). In our previous whole transcriptome profiling study (**Paper III**), we identified a set of skin-enriched lncRNAs with differential expression in the cSCC patients, wherein ELDAR was one of the most downregulated lncRNAs (Das Mahapatra et al. 2020). Since *ELDAR* locus is located within the epidermal differentiation complex (EDC) on human chromosome 1q.21; we hypothesized a regulatory role for ELDAR in epidermal differentiation, and set out to characterize it.

Main findings

ELDAR is a skin specific lncRNA, specifically induced during late differentiation

Using an RNA-seq dataset from a time-course of differentiation in primary keratinocytes (day 0, 2, 4 and 7), we found a striking increase in ELDAR expression during the late stages (at 4 and 7 days after calcium induction) of differentiation. Interestingly, the expression dynamics of ELDAR was mirrored by FLG and LOR; two other crucial structural proteins expressed during late differentiation (Kouwenhoven et al. 2015) (**Paper IV,** Figure 1A, B). The RNA-seq data was further validated in our *in vitro* differentiation assay using primary human keratinocytes. qPCR based analysis showed that compared to undifferentiated keratinocytes, ELDAR is induced approximately 56 fold and 1900 fold at day 4 and day 6 of calcium-induced differentiation (*Figure 23A, B*). Confirming our findings in **paper III,** qPCR analysis of 22 human organs/tissues demonstrated that ELDAR is a skin-specific transcript. Among different skin-constituent cell types (keratinocytes, fibroblasts, melanocytes and immune cells), it is highly expressed only in the terminally differentiated keratinocytes (*Figure 23C*, *D)*. Consistent with its induction during late stage of differentiation, RNAscope *in-situ* hybridization (ISH) disclosed that ELDAR transcript is localized at the upper granular layer of the healthy epidermis (*Figure 23E*).

Rapid amplification of cDNA ends (RACE) analysis suggested that *ELDAR* locus produces a single 589 nt long isoform (**Paper IV,** Figure 2A). Next, using bioinformatics tool and exogenous expression of the predicted ORFs, which failed to produce any peptide in primary keratinocytes, we concluded that ELDAR is a bona fide lncRNA (**Paper IV,** Figure 2B, E). Finally, cellular fractionation-coupled with qPCR and RNAscope ISH-based detection revealed that ELDAR is predominantly localized to the cytoplasm (**Paper IV,** Figure 2F, G).

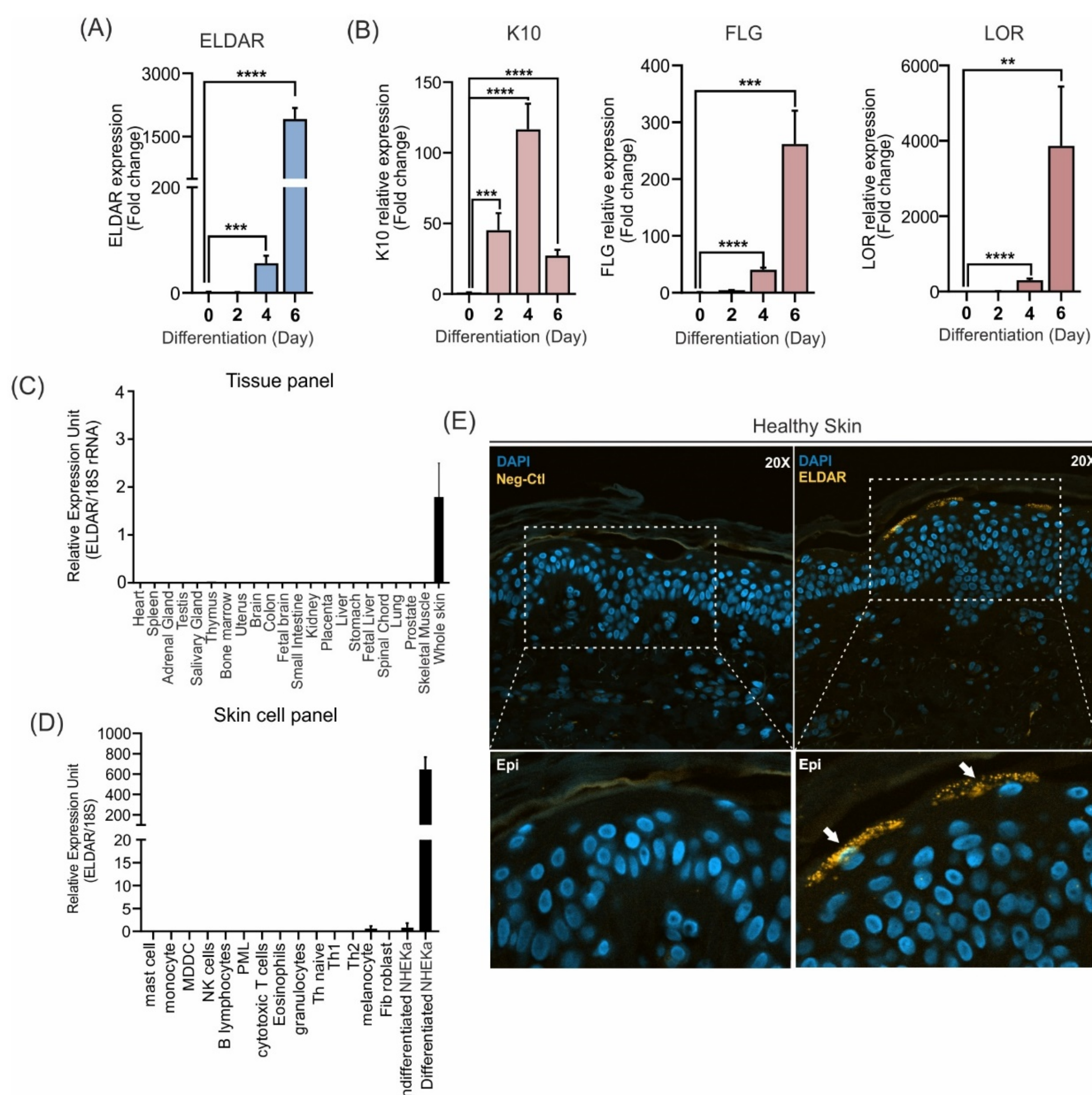

***Figure 23:** qPCR analysis to assess the expression of (A) ELDAR and (B) the early (K10) and the late (FLG, LOR) differentiation markers during calcium-induced differentiation of primary keratinocytes (n = 3). (C) qPCR analysis of ELDAR expression in a panel of 22 human tissues. (D) qPCR analysis of ELDAR expression in a panel of 16 cell types from healthy skin. (E) Single-molecule in situ hybridization analysis with negative control and ELDAR-specific probes in healthy human skin (n = 6). White arrows used to point at the location of the signal. Scale bar = 50 µm. Student's t-test (two-tailed). **P < 0.01, ***P < 0.001, ****P < 0.0001.*

Transcriptional activation of ELDAR locus induces premature differentiation

In order to assess the role of ELDAR in keratinocyte differentiation, we utilized the CRISPR-Cas transcriptional activation (CRISPRa) system to induce its expression in primary keratinocytes. Interestingly, without a stimulus for differentiation, *ELDAR* activation in undifferentiated keratinocytes managed to sigficantly induce the expression of late differentiation genes LOR and LCE2D (**Paper IV,** Figure 3B, C). However, a more comprehensive effect was observed when *ELDAR* activation was followed by calcium chloride treatment for inducing differentiation. This experimental setup demonstrated that *ELDAR*-activation has a positive regulatory effect on late differentiation markers (FLG, LOR, LCE2D, and LCE3A), without affecting the early differentiation gene K10 (*Figure 24A, B, C*). Transcriptome profiling of keratinocytes transfected with CRISPRa plasmids and induced for differentiation further substantiated this observation and affirmed a distinctive upregulation of numerous genes for structural proteins and enzymes for terminal differentiation and barrier formation (**Paper IV,** Supplementary Figure 3).

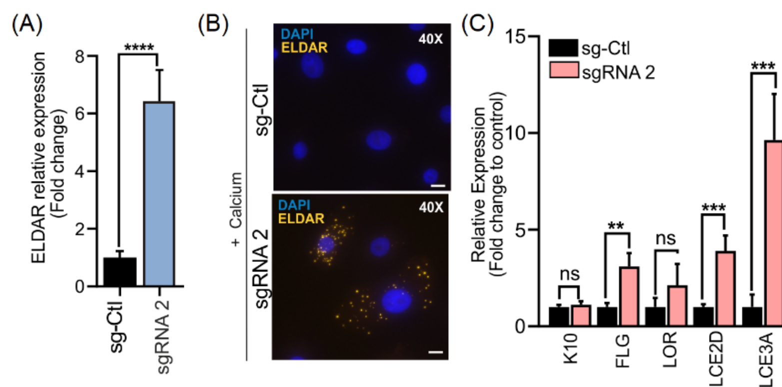

Figure 24: *(A) qPCR-based expression analysis of ELDAR upon transcriptional activation by CRISPRa followed by calcium-induced differentiation (day 4) in primary keratinocytes (B) Singe-molecule in situ hybridization of ELDAR in primary keratinocytes upon CRISPRa. Scale bar = 10 µm. (C) qPCR analysis of the early differentiation gene (K10) and the late differentiation genes (FLG, LOR, LCE2D, and LCE3A). Fold change was calculated relative to negative control sgRNA treated keratinocytes (n = 3). Student's t-test (two-tailed). ** P < 0.01, *** P < 0.001*

ELDAR knockdown impairs terminal differentiation in human organotypic skin

After finding that activation of *ELDAR* locus can induce premature differentiation in primary keratinocytes, we set out to uncouple the effect of the transcript from the transcriptional event at this locus. Therefore, we performed an siPool-mediated knockdown of ELDAR in primary

keratinocytes, which were used to build a stratified organotypic skin. In agreement with the CRISPRa result, ELDAR-depletion in the organotypic cultures resulted in significant reduction of late differentiation genes at mRNA (LOR, LCE2D, and LCE3A) and at protein level (LOR and FLG), compared to the control siPool treated tissues *(Figure 25A, B, C)*. Once again, the knockdown did not alter the expression of early differentiation genes such as K1 and K10 at protein and mRNA level (**Paper IV**, Figure 4D, Supplementary figure 4). In terms of phenotypic effect, ELDAR-depleted organotypic epidermis had a markedly thinner cornified layer, as seen from the transmission electron microscopy (TEM) analysis (**Paper IV,** Figure 4G).

A whole transcriptome analysis of ELDAR-depleted organotypic tissues comprehensively highlighted a stage-specific effect on epidermal differentiation. Apart from the structural proteins (FLG, FLG2, LOR, SPRRs, LCEs), several other enzymes essential for cornified envelope formation and desquamation, such as crosslinking enzymes (TGM3 and TGM5), lipoxygenases (ALOX12B and ALOXE3) and proteases (KLKs and CTSs) were also significantly downregulated (Eckhart et al. 2013; Ishitsuka et al. 2016) (**Paper IV,** Figure 4J, Supplementary table 3). ELDAR-regulated genes were found to be significantly enriched for gene ontology terms such as “skin development”, “keratinocyte differentiation” and “peptide crosslinking” (**Paper IV,** Figure 4I).

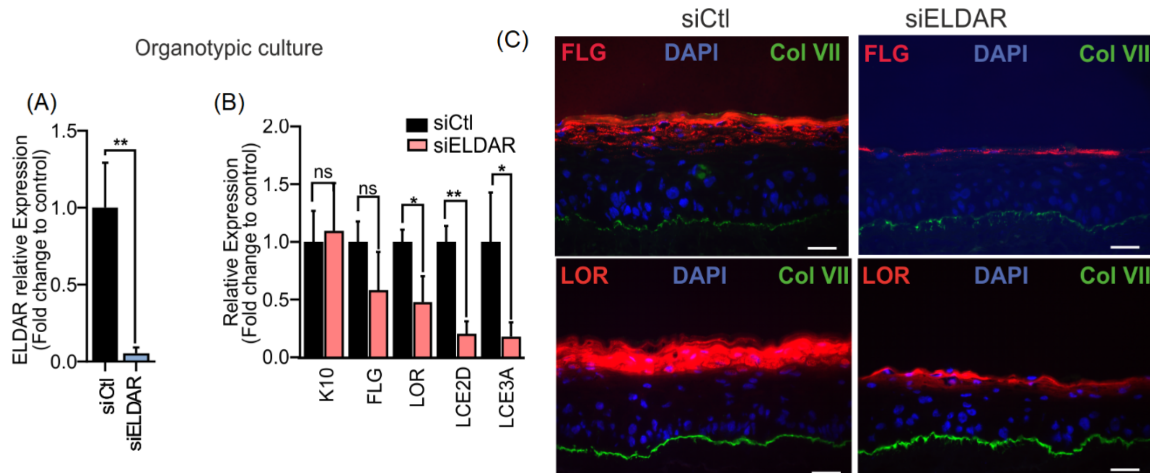

Figure 25: *qPCR analysis of (A) ELDAR expression in 3D organotypic cultures (day 4) made of keratinocytes nucleofected with control (siCtl) or ELDAR targeting siPool (siELDAR) and expression of (B) the early (K10) and late differentiation genes (FLG, LOR, LCE2D and LCE3A) (n = 3). (C) Immunofluorescence analysis of FLG and LOR (red) in ELDAR-depleted organotypic cultures. Basement membrane was stained for Col VII (green) and nucleus was stained with DAPI (blue) (n = 3). Scale bar = 50 µm. Student's t-test (two-tailed). *P < 0.05, ** P < 0.01.*

Transcription factor Yin Yang 1 (YY1) represses ELDAR expression in progenitor keratinocytes

In order identify the upstream regulators of ELDAR expression in the progenitor keratinocytes, we extracted 1 kb sequence (putative promoter) upstream of the TSS and used it in PROMO, an online TF-predication tool that identifies the cognate motifs associated with the eukaryotic TFs. This analysis identified YY1 as one of top transcription factors with multiple binding sites within ELDAR promoter. ChIP-seq data retrieved from ENCODE (anti-YY1 in K562 cell line) database also showed a distinct peak for YY1 in this region (**Paper IV,** Figure 5A**).** Finally, our ChIP-qPCR data in progenitor keratinocytes showed a similar YY1-occupancy at ELDAR promoter, suggesting that YY1 can regulate ELDAR in primary keratinocytes (**Paper IV,** Figure 5B, C**).** A second line of evidence about a potentially repressive regulatory relationship between YY1 and ELDAR came from their opposite expression pattern during keratinocyte differentiation. Our qPCR data suggested that YY1 is significantly downregulated during the late time points (day 4 and day 6) of keratinocyte differentiation. To assess the functional implication, at first, we tested the effect of YY1 depletion in undifferentiated keratinocytes, where it failed to induce ELDAR expression significantly (**Paper IV,** Supplementary figure 6). However, when coupled with calcium-induced differentiation, YY1 knockdown not only upregulated ELDAR (~6 fold) but also increased the expression of FLG and LOR, two genes which are previously shown to be regulated by ELDAR during terminal differentiation (*Figure 26A, B, C*).

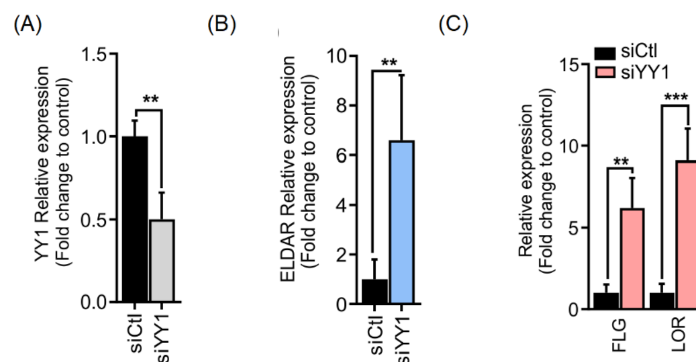

Figure 26: *qPCR analysis of (A) YY1 expression in primary keratinocytes transfected with siRNA followed by calcium-induced differentiation (day 4). (B) Expression of ELDAR and (C) ELDAR regulated late differentiation genes (FLG and LOR) (n = 3). Student's t-test (two-tailed). **P < 0.01, ***P < 0.001.*

Discussion

In this study, we have delineated the functional implications of a previously uncharacterized skin-specific lncRNA in epidermal differentiation. ELDAR is strikingly induced during the late stage of keratinocyte differentiation, and localized primarily to the cytoplasm. CRISPR-mediated activation of *ELDAR* locus led to a precocious expression of several genes associated with terminal differentiation program. Similarly, knockdown of the ELDAR transcript in organotypic-skin-equivalent tissues resulted in significant downregulation of the terminal differentiation-associated genes, without altering the early differentiation markers. Among the late markers, FLG, FLG2, LOR, SPRR proteins are key structural components of the cornified envelope (Candi, Schmidt, and Melino 2005b). A large number of genes from the LCE cluster which encodes for cornified envelop precursors were markedly downregulated. Several peptide cross-linking enzymes, which are crucial for the formation of the cornified envelope, were also downregulated. ELDAR knockdown caused a significant decrease in corneodesmosin (CDSN), which is an essential component of corneodesmosomes (cornified envelope specific junctional complex) (Kitajima 2015). Parallel to the transcriptomic changes, ELDAR-depleted epidermal tissues developed a markedly thinner cornified layer. Considering that cornification is a fundamental process for acquiring a functional epidermal barrier, it will be crucial to assess the effect of ELDAR loss directly on the barrier integrity (outside in and inside out) by dye diffusion assay and measurement of transepidermal water loss (TWEL).

Although we have explored the functional ramifications of ELDAR through gain and loss-of-function studies, the mode of its function currently remains unexplored. Since it is primarily localized to the cytoplasm, we can assume various post-transcriptional regulatory mechanisms. For instance, it can provide increased stability to a selected set of pro-differentiation mRNAs via interacting with RBPs like STAU1 or polyadenylate-binding protein PABPC1 (Kretz et al. 2013b; Webster et al. 2018). As the ELDAR-regulated genes show altered expression already at the RNA level; it is unlikely that this lncRNA specifically increases their translational output. In this scenario, poly-A interacting RBPs could be particularly interesting as they also promote the formation of translation pre-initiation complex during 5'-cap mediated translation (Benoit et al. 2005; Sachs and Davis 1989). A number of studies report that lncRNAs can alter protein half-life by modulating the interaction between the ubiquitin-proteasome complex and its substrate (Ma et al. 2019). This can be can also be applicable for ELDAR mediated regulation of pro-differentiation proteins. Further *in silico* and *in vitro* studies will be required to shed light on the various functional interacting partners of ELDAR.

YY1 is a zinc-finger containing TF that is shown to regulate cell proliferation, differentiation, and tissue development (Alvarez-Salas, Benitez-Hess, and Dipaolo 2005:1; Gregoire et al. 2017:1; Lu et al. 2018). By ChIP-qPCR and loss-of-function study, we show that transcription factor YY1 binds to the promoter of ELDAR and represses its expression in progenitor keratinocytes. Previous studies in primary murine keratinocytes and 3D culture using HaCaT cells show that YY1 has an inhibitory effect on epidermal differentiation (Taguchi et al. 2011; Xu et al. 2004). Other than validating the dynamic YY1 expression in differentiating primary human keratinocytes, our data propose that ELDAR acts as an additional player, which contributes to the YY1-mediated inhibitory effect on epidermal differentiation. Although we show that YY1 depletion in differentiated keratinocytes induces ELDAR expression, it is unclear whether YY1 is the stand-alone factor here. Given that calcium induction can activate a number of pro-differentiation signaling pathways and their downstream transcription factors, it is possible that not merely the removal of YY1 from its promoter, ELDAR expression also requires the presence of additional transcriptional activators (Ng et al. 2000; Toufighi et al. 2015). Interestingly, YY1 is an important polycomb-group protein that can establish repressive chromatin marks (Wilkinson, Park, and Atchison 2006). In addition to that, YY1 can also interact with CCCTC-binding factor (CTCF) and establish stable chromatin architecture to regulate enhancer-promoter interactions (Weintraub et al. 2017). Therefore, it will be compelling to evaluate the effect of exogenous overexpression of wild type or a mutant YY1 lacking the recruitment of polycomb (REPO) domain on ELDAR expression. Similarly, it will be intriguing to see if CTCF knockdown has an additive effect on ELDAR expression.

Since altered epidermal differentiation and barrier formation are essential hallmarks of a diverse group of cutaneous disorders (e.g. cSCC, psoriasis, atopic dermatitis, and ichthyosis); it will be intriguing to assess the change in ELDAR expression and its epidermal distribution in those conditions. In this regard, our initial assessment shows that ELDAR expression is significantly downregulated in primary cSCCs and patient derived cSCC cell lines, compared to normal skin and primary keratinocytes respectively. However, much work lies ahead to understand if ELDAR gain-of-function can suppress cSCC development, potentially by re-differentiating the tumor cells.

5 CONCLUDING REMARKS

The delicate interplay between various signaling pathways, transcription factors, epigenetic modulators, and non-coding RNAs is the key to maintaining a functional epidermis. Disruption to this process through deregulation of any component of this multilayered organization can lead to various diseases, including skin malignancies. The central aim of this thesis is to identify and functionally characterize the dynamically expressed non-coding RNAs in epidermal differentiation, and malignancies arising from different cell types (keratinocytes and melanocytes) within the epidermis. One of the recurring highlights in our studies' outcome was the nexus between non-coding RNAs and transcription factors in regulating gene networks, which ultimately manifests in the alteration of various cancer/metastasis-associated hallmarks. We show that the expression of non-coding RNAs is often tightly regulated by transcription factors and epigenetic regulators in both homeostatic and disease conditions. Finally, using suitable disease models, we have demonstrated that some of the ncRNAs could be useful targets for therapeutic interventions in skin cancers.

In **Paper I,** we have identified an inverse correlation between miR-203 expression and differentiation grades of cSCC, with a significant downregulation in the poorly differentiated tumors. Functionally, miR-203 inhibited the cell cycle by arresting cSCC cells at G1 phase. We identified oncogene c-MYC as a direct target of miR-203 in cSCC. C-MYC restoration rescued the cell cycle inhibitory effect of miR-203 in cSCC cell lines. Additionally, miR-203 affected key oncogenic hallmarks such as self-renewal, cell motility, and angiogenesis of cSCC cells. In line with the *in vitro* effects, miR-203 overexpression led to a significant reduction of tumor volume and weight in a xenograft assay. Further studies will be necessary for an in-depth assessment of miR-203/c-MYC axis as a therapeutic target in cSCC.

In **Paper II,** using TCGA small-RNA-seq data of primary and metastatic melanoma, we have reported that miR-203 is the most downregulated miRNA in the metastatic tumors. The protective clinical relevance of miR-203 was identified when we found that higher miR-203 abundance confers longer overall survival to patients with metastatic melanoma. Using methylation data from TCGA, and our *in vitro* demethylation assay, we demonstrated that promoter hypermethylation could be partly responsible for miR-203 deregulation in metastatic melanoma. Functional assays using melanoma cell lines showed that miR-203 inhibits many key hallmarks of tumor progression and metastasis, such as self-renewability, angiogenesis, and cell motility. EMT-promoting transcription factor SLUG was identified as a direct target of

miR-203, and its downregulation partially explained the inhibitory effect of miR-203 on melanoma cell motility. In addition to suppressing tumor growth *in vivo*, miR-203 showed a strong anti-metastatic function by inhibiting dissemination of cancer cells to local lymph nodes and the lungs. In summary, our study has explored a prognostic and therapeutic potential of miR-203 in the context of metastatic melanoma. In this regard, it will be interesting to assess the changes in miR-203 abundance in different mutational and clinical subtypes of melanoma. Further studies using patient-derived-xenograft models of melanoma could provide a better assessment of its therapeutic potential.

In **Paper III,** we aimed to construct a robust catalogue of differentially expressed coding and non-coding transcripts in cSCC, with a particular aim to improve upon previously published lncRNA and circRNA profilings in cSCC. This is also the first study identifying both protein coding and long non-coding transcripts (linear and circular) from the same samples. Potentially, due to higher sequencing depth in comparison with the previous studies focusing on the coding transcripts, we have identified many previously unreported genes including transcription factors in cSCC. Apart from several uncharacterized lncRNAs, our expression analysis identified multiple lncRNAs with reported oncogenic functions, as well as several skin-enriched lncRNAs. Using circRNA identifying pipelines and differential expression analysis, we discovered both novel and annoted circRNAs with altered expression in cSCC. In addition, we successfully validated three representative transcripts from each group in an expanded cohort, including precancerous AKs, which gave further insight into the dynamic changes of these transcripts during cSCC progression. Nevertheless, a future study with a cohort made of AK, cSCC *in* situ, and different grades of cSCC will get us closer to understanding the dynamic changes in the non-coding transcriptome during cSCC progression. In conclusion, our study has generated a robust and comprehensive list of coding and non-coding transcripts, which will be useful in identifying novel regulators of cSCC pathogenesis in future.

In **Paper IV**, we have characterized ELDAR, a skin-specific lncRNA located at the EDC on human chromosome 1q.21. ELDAR is induced strikingly at the late stages of keratinocyte differentiation and located at the granular layer of the epidermis. Gain and loss-of-function studies in differentiating keratinocytes or organotypic skin equivalent tissues unveiled that ELDAR positively regulates various essential components (i.e. structural proteins, proteases, crosslinking enzymes) of terminal differentiation and cornification program. ELDAR-depleted epidermal tissues also developed markedly thinner cornified layer. All these observations raise a relevant question about the potential role of ELDAR in barrier acquisition, which could be

addressed using different barrier integrity assays. Future works should also focus on dissecting the mode of function and interacting partners of this cytoplasmic lncRNA. We have identified transcription factor YY1 as a negative regulator of ELDAR in progenitor keratinocytes. Given that YY1 often acts as a cofactor, it will be equally interesting to identify other YY1-associated transcription factors at the *ELDAR* promoter and understand their united role in regulating this lncRNA locus. Finally, this study opens up further questions about the deregulation and functional implications of ELDAR in various epidermal disorders (such as cancer, psoriasis, atopic dermatitis, etc.) where terminal differentiation program is impaired/altered.

6 ACKNOWLEDGMENTS

First, a big thanks to all the patients and healthy volunteers who contributed to the studies presented in this thesis.

I am equally grateful to the doctors and nurses, particularly **Jan**, **Iara**, **Enikö**, **Jakob, Maria**, and **Helena**, for their kind assistance in sample collection.

Andor, thank you for giving me the opportunity to pursue my doctoral training in your group. Your knowledge and enthusiasm for skin biology, or literally anything about natural science, is quite inspirational to any budding researcher. Through a fine balance of trust, encouragement, and close supervision, you have been instrumental in shaping my growth as a scientist. This thesis would not have been possible without your constant support and guidance. **Enikö**, many thanks for your invaluable scientific inputs during the weekly meetings and brainstorming sessions, and keeping us grounded to reality when it was most necessary. Seeing you at work, handling so many different responsibilities simultaneously, it is a constant learning lesson for me.

Thanks, **Mona**, for your kindness and warm welcome upon my arrival. Your words of wisdom and encouragement have been extremely helpful in those early days. Apart from your effortless leadership quality, I remain in awe with your infinite zest for science, translational research, and life at large. **Johan** and **Leonard**, thank you both for being my co-supervisors and for providing the necessary feedback from time to time. **Alex**, not just for your mentorship, I am grateful for the useful suggestions and feedbacks about my CRISPR experiments.

Gunilla, without your help, it would not have been possible to wade through the bureaucratic difficulties in Karolinska. **Anna-Lena**, thank you for the necessary lessons about lab safety, coffee-hour stories, and hosting us in your beautiful summerhouse.

Ning, Kilian, Jakob, **Etty**, **Pernilla** and **Klas**, many thanks for all the lively discussions and useful feedback during the journal club sessions and other meetings.

A big thanks to all the fantastic collaborators.

Markus, for introducing me to the fascinating yet painful world of lncRNAs. You have remained a role model to me when it comes to doing research rigorously and nurturing a healthy degree of caution while (over)interpreting data. **Chris** and **Johannes**, for your help with the experiments and making my stay at Regensburg smooth and productive. **Lara**, I can definitely say that you are one of the coolest teachers one can find. Your positive energy is somewhat contagious. It was so effortless and enjoyable to learn the essentials of DNA methylation and pyrosequencing from you. **Claudia**, for the fruitful collaboration and your inputs for the rebuttal. **Jonas**, thank you for being endlessly helpful, and patient with my bioinformatics

naivety. **Lasse**, for bringing circRNAs in the picture and making an extremely efficient and productive collaboration.

To my Mol-Derm colleagues.

Bo, it is a pleasure to know you. Your appetite for life and Asian buffets can only be matched by your unreal capacity for multi-tasking. Big thanks for your help in many of my projects, and making the day-to-day functioning of our lab hassle-free. **Harry**, it was a brief but meaningful encounter. I remain thankful to you for all the engaging talks, gaming sessions, and our shared interest in science and society. **Ankit**, your willingness to help people is second to none, and thank you for that. Our intense discussion about topics ranging from Cricket to Indian politics sometimes made me feel that I never left India. **Lorenzo**, thank you for joining me in wrestling with the RNA-seq data, confocal sessions, and other experiments. It was always a treat to look at your data presentation, spotless office desk, and thoroughly written protocols (and frequent usage of multi-colored pencils). I wish you a great future ahead. **Ritu**, in addition to your help in the lab, I must thank you for the scientific and non-scientific discussions, and your excellent movie/series recommendations. **Mansi**, thank you for the yoga lessons, and the timely supply of food and humor to save our lives. Thanks, **Amit**, for shouldering the mammoth task of managing our lab, orderings, and endless freezer-related issues. **Maria**, it is delightful to share my enthusiasm for food and cats with you. Thank you for being my partner in crime for embarrassing Lorenzo time after time. **Alex**, thank you for being such a great company in and outside the lab. Our frequent table tennis and badminton-sessions truly brightened up those dark winter days. **Dong**, thank you for being my go-to person for all the protocol details and different expression plasmids. **Letian**, it is great to share my interest in badminton and lncRNAs with you. All the new people, **Chen**, **Longlong**, and **Chen-ying**, you guys are not only extremely smart and hardworking but also excellent team players. I cannot thank you enough for your help in the lab.

To the colleagues from the other floors and campus.

Bobby, for sharing your fabled (mis)adventures over the coffee breaks, and making those spontaneous plans for afterwork to get over bad data or lousy weather (often both). **Ton**, for all the help during the pub nights. **Natalia**, **Pooja**, **Elena**, **Jaanika**, **Iman, Elisa,** and **Irène**, for creating and maintaining such a vibrant and friendly work environment. I miss our boisterous Friday fikas, which helped a great deal in letting off the weakly steam. **William**, for your generous help with the CRISPR-experiments and our spirited conversations about football and the transfer market. **Sahl**, for all the chitchats about the struggles of PhD life and help with the CNV analysis. **Raquel,** for your pearls of wisdom about academia and constant reminders about the importance of having a healthy work-life balance. **Clara**, for always being so kind, friendly, and helpful to me. **Carolina** and **Orlando,** for your continually encouraging words and tidbits about a career in science.

To the past members of Mol-Derm.

Winnie, for your unrelenting optimism, kindness, and helping nature (and the endless supply of snacks). I look forward to all the food-adventures that we have planned, but never materialized because of our (admit it) innately lazy nature. I hope someday you will agree with me and see how pointless "a walk to remember" is. **Sandeep**, for all the hearty talks and words of encouragement, to make me run. **Manika**, for being kind and helpful, and taking away my monopoly over being the last person to join a party. I thoroughly enjoyed our conversations and your inscrutable banters. **Ivone**, for sharing my taste for acerbic humor and cats, and being so empathetic. Thank you for being in touch. **Eva,** for all the help in the lab, and sharing your amazing culinary skill from time to time. **Einar,** for all the conversations about work, non-work, Swedish culture and board games. You are truly one of the kindest souls I have ever come across.

A special thanks to all my students, **Jigishu**, **Daniel**, **Lara**, **Benedikt** and **Nica**. You guys not only contributed immensely to my work and helped me become a better teacher/person (I can only hope) but also made our corridor a lively place.

The CMM IT crew; **Timmy**, **Annika, Hugo, Daniel**, **Christer** and **Oliver.** You all are simply amazing. Many thanks for the competence and lively attitude that you bring to the CMM work environment. I also thank **KIGene** and **BEA** for their excellent support throughout these years.

Many thanks to all my friends whom I met through Cricket and badminton.

Pradeep, Harkamal, Vivek(s), Ravi, Nilesh, Deepak, Shahul, Madhu, Sulman, Akram, Sampath, Kalai, Suhas, and many more, life in Stockholm would not have been the same without you people, on and off the field. I cannot wait for this pandemic to be over so that we can start preparing for the next season.

Satya, thank you for the firsthand lesson on how to become the best paratha-maker in the world. You are indeed the best roommate one can ever wish for. My stay at your apartment was probably the most fun and enjoyable phase of my Stockholm days. I immensely enjoyed our cooking sessions and all the animated conversations about sports, politics, and life in general.

Sachin and **Swapnali**, for all the delicious food and warm company that always made me feel at home.

Vishal and **Lamia**, for introducing me to **Baji** (and more recently to Cozmo)**,** and hosting me in countless unplanned lunch/dinners/board-game sessions. You people have made my life in Stockholm immensely enjoyable.

Joydeep and **Shankhamala,** for sharing my love for Bengali cuisine and nostalgia about Kolkata.

Thank you, **Mayank**, for being such an amazing friend through many seasons.

My deepest gratitude to the IBANers.

Sam, for being there, always trying to bring the best and the most authentic self of me. **Rohit**, for exemplifying that good food, sports, and bad jokes matter as much as anything else in life. **Sagar**, for reminding me to be rooted to my friends and families, and showing me the importance of working diligently and patiently towards a goal.

Prajakta, thank you for being patient with all my idiosyncrasies and providing an unwavering source of kindness, and support.

I will remain forever indebted to my teachers in high school/college, especially **Uttam Ghosh** and **Chandralekha Duttagupta**. Your fantastic pedagogical skill and timely encouragement were instrumental in shaping up my interest in those early years.

No words will ever be enough to express the sea of gratitude I feel towards my dear **parents** and my **brother**. Without your unconditional love, support, and innumerable sacrifices, I could not have pursued this path.

7 REFERENCES

Abdallah, Florence, Lily Mijouin, and Chantal Pichon. 2017. “Skin Immune Landscape: Inside and Outside the Organism.” *Mediators of Inflammation* 2017:e5095293. Retrieved July 6, 2020 (https://www.hindawi.com/journals/mi/2017/5095293/)

Abdo, Joseph M., Nikolai A. Sopko, and Stephen M. Milner. 2020. “The Applied Anatomy of Human Skin: A Model for Regeneration.” *Wound Medicine* 28:100179.

Abhishek, Sinha, and Suresh Palamadai Krishnan. 2016. “Epidermal Differentiation Complex: A Review on Its Epigenetic Regulation and Potential Drug Targets.” *Cell Journal (Yakhteh)* 18(1):1–6.

Akbani, Rehan, Kadir C. Akdemir, B. Arman Aksoy, Monique Albert, Adrian Ally, Samirkumar B. Amin, Harindra Arachchi, Arshi Arora,... Lisa Zimmer, Erik Zmuda, and Lihua Zou. 2015. “Genomic Classification of Cutaneous Melanoma.” *Cell* 161(7):1681–96.

Alam, M., and D. Ratner. 2001. “Cutaneous Squamous-Cell Carcinoma.” *The New England Journal of Medicine* 344(13):975–83.

Allen-Petersen, Brittany L., and Rosalie C. Sears. 2019. “Mission Possible: Advances in MYC Therapeutic Targeting in Cancer.” *BioDrugs* 33(5):539–53.

Altuvia, Yael, Pablo Landgraf, Gila Lithwick, Naama Elefant, Sébastien Pfeffer, Alexei Aravin, Michael J. Brownstein, Thomas Tuschl, and Hanah Margalit. 2005. “Clustering and Conservation Patterns of Human MicroRNAs.” *Nucleic Acids Research* 33(8):2697–2706.

Alvarez-Salas, L. M., M. L. Benitez-Hess, and J. A. Dipaolo. 2005. “YY-1 and c-Jun Transcription Factors Participate in the Repression of the Human Involucrin Promoter.” *International Journal of Oncology* 26(1):259–66.

American Cancer Society. 2020. “Melanoma Skin Cancer Statistics.” *Key Statistics for Melanoma Skin Cancer*. Retrieved July 28, 2020 (https://www.cancer.org/cancer/melanoma-skin-cancer/about/key-statistics.html).

Asangani, I. A., S. a. K. Rasheed, D. A. Nikolova, J. H. Leupold, N. H. Colburn, S. Post, and H. Allgayer. 2008. “MicroRNA-21 (MiR-21) Post-Transcriptionally Downregulates Tumor Suppressor Pdcd4 and Stimulates Invasion, Intravasation and Metastasis in Colorectal Cancer.” *Oncogene* 27(15):2128–36.

Ashwal-Fluss, Reut, Markus Meyer, Nagarjuna Reddy Pamudurti, Andranik Ivanov, Osnat Bartok, Mor Hanan, Naveh Evantal, Sebastian Memczak, Nikolaus Rajewsky, and Sebastian Kadener. 2014. “CircRNA Biogenesis Competes with Pre-MRNA Splicing.” *Molecular Cell* 56(1):55–66.

Assani, Ganiou, and Yunfeng Zhou. 2019. “Effect of Modulation of Epithelial-mesenchymal Transition Regulators Snail1 and Snail2 on Cancer Cell Radiosensitivity by Targeting of the Cell Cycle, Cell Apoptosis and Cell Migration/Invasion (Review).” *Oncology Letters* 17(1):23–30.

Bachellerie, Jean-Pierre, Bernard Michot, Monique Nicoloso, Andrey Balakin, Jingwei Ni, and Maurille J. Fournier. 1995. “Antisense SnoRNAs: A Family of Nucleolar RNAs with Long Complementarities to RRNA.” *Trends in Biochemical Sciences* 20(7):261–64.

Bamford, S., E. Dawson, S. Forbes, J. Clements, R. Pettett, A. Dogan, A. Flanagan, J. Teague, P. A. Futreal, M. R. Stratton, and R. Wooster. 2004. "The COSMIC (Catalogue of Somatic Mutations in Cancer) Database and Website." *British Journal of Cancer* 91(2):355–58.

Barrett, Steven P., Peter L. Wang, and Julia Salzman. n.d. "Circular RNA Biogenesis Can Proceed through an Exon-Containing Lariat Precursor." *ELife* 4.

Bartel, David P. 2009. "MicroRNA Target Recognition and Regulatory Functions." *Cell* 136(2):215–33.

Bastian, Boris C. 2014. "THE MOLECULAR PATHOLOGY OF MELANOMA: AN INTEGRATED TAXONOMY OF MELANOCYTIC NEOPLASIA." *Annual Review of Pathology* 9:239–71.

Benaich, Nathan, Samuel Woodhouse, Stephen J. Goldie, Ajay Mishra, Sven R. Quist, and Fiona M. Watt. 2014. "Rewiring of an Epithelial Differentiation Factor, MiR-203, to Inhibit Human Squamous Cell Carcinoma Metastasis." *Cell Reports* 9(1):104–17.

Benjamin, Cara L., and Honnavara N. Ananthaswamy. 2007. "P53 and the Pathogenesis of Skin Cancer." *Toxicology and Applied Pharmacology* 224(3):241–48.

Benoit, Béatrice, Géraldine Mitou, Aymeric Chartier, Claudia Temme, Sophie Zaessinger, Elmar Wahle, Isabelle Busseau, and Martine Simonelig. 2005. "An Essential Cytoplasmic Function for the Nuclear Poly(A) Binding Protein, PABP2, in Poly(A) Tail Length Control and Early Development in Drosophila." *Developmental Cell* 9(4):511–22.

Bikle, Daniel D., Zhongjian Xie, and Chia-Ling Tu. 2012. "Calcium Regulation of Keratinocyte Differentiation." *Expert Review of Endocrinology & Metabolism* 7(4):461–72.

BLACK, A. P. B., and G. S. OGG. 2003. "The Role of P53 in the Immunobiology of Cutaneous Squamous Cell Carcinoma." *Clinical and Experimental Immunology* 132(3):379–84.

Blanpain, Cédric, and Elaine Fuchs. 2007. "P63: Revving up Epithelial Stem-Cell Potential." *Nature Cell Biology* 9(7):731–33.

Blanpain, Cédric, and Elaine Fuchs. 2009. "Epidermal Homeostasis: A Balancing Act of Stem Cells in the Skin." *Nature Reviews Molecular Cell Biology* 10(3):207–17.

Bond, Charles S., and Archa H. Fox. 2009. "Paraspeckles: Nuclear Bodies Built on Long Noncoding RNA." *Journal of Cell Biology* 186(5):637–44.

Borchert, Glen M., William Lanier, and Beverly L. Davidson. 2006. "RNA Polymerase III Transcribes Human MicroRNAs." *Nature Structural & Molecular Biology* 13(12):1097–1101.

Bracken, Cameron P., Philip A. Gregory, Natasha Kolesnikoff, Andrew G. Bert, Jun Wang, M. Frances Shannon, and Gregory J. Goodall. 2008. "A Double-Negative Feedback Loop between ZEB1-SIP1 and the MicroRNA-200 Family Regulates Epithelial-Mesenchymal Transition." *Cancer Research* 68(19):7846–54.

Brash, D. E., J. A. Rudolph, J. A. Simon, A. Lin, G. J. McKenna, H. P. Baden, A. J. Halperin, and J. Pontén. 1991. "A Role for Sunlight in Skin Cancer: UV-Induced P53 Mutations in Squamous Cell Carcinoma." *Proceedings of the National Academy of Sciences of the United States of America* 88(22):10124–28.

Brennecke, Julius, Alexander Stark, Robert B. Russell, and Stephen M. Cohen. 2005. "Principles of MicroRNA–Target Recognition." *PLOS Biology* 3(3):e85.

Cabili, Moran N., Margaret C. Dunagin, Patrick D. McClanahan, Andrew Biaesch, Olivia Padovan-Merhar, Aviv Regev, John L. Rinn, and Arjun Raj. 2015. "Localization and Abundance Analysis of Human LncRNAs at Single-Cell and Single-Molecule Resolution." *Genome Biology* 16(1):20.

Calin, George Adrian, Calin Dan Dumitru, Masayoshi Shimizu, Roberta Bichi, Simona Zupo, Evan Noch, Hansjuerg Aldler, Sashi Rattan, Michael Keating, Kanti Rai, Laura Rassenti, Thomas Kipps, Massimo Negrini, Florencia Bullrich, and Carlo M. Croce. 2002. "Frequent Deletions and Down-Regulation of Micro- RNA Genes MiR15 and MiR16 at 13q14 in Chronic Lymphocytic Leukemia." *Proceedings of the National Academy of Sciences* 99(24):15524–29.

Candi, Eleonora, Richard A. Knight, Emanuele Panatta, Artem Smirnov, and Gerry Melino. 2016. "Cornification of the Skin: A Non-Apoptotic Cell Death Mechanism." Pp. 1–10 in *eLS*. American Cancer Society.

Candi, Eleonora, Rainer Schmidt, and Gerry Melino. 2005a. "The Cornified Envelope: A Model of Cell Death in the Skin." *Nature Reviews Molecular Cell Biology* 6(4):328–40.

Cañueto, J., E. Cardeñoso, J. L. García, Á. Santos-Briz, A. Castellanos-Martín, E. Fernández-López, A. Blanco Gómez, J. Pérez-Losada, and C. Román-Curto. 2017. "Epidermal Growth Factor Receptor Expression Is Associated with Poor Outcome in Cutaneous Squamous Cell Carcinoma." *The British Journal of Dermatology* 176(5):1279–87.

Caporali, Simona, Adriana Amaro, Lauretta Levati, Ester Alvino, Pedro Miguel Lacal, Simona Mastroeni, Federica Ruffini, Laura Bonmassar, Gian Carlo Antonini Cappellini, Nadia Felli, Alessandra Carè, Ulrich Pfeffer, and Stefania D'Atri. 2019. "MiR-126-3p down-Regulation Contributes to Dabrafenib Acquired Resistance in Melanoma by up-Regulating ADAM9 and VEGF-A." *Journal of Experimental & Clinical Cancer Research: CR* 38(1):272.

Carninci, P., T. Kasukawa, S. Katayama, J. Gough, M. C. Frith….J. Kawai, and Y. Hayashizaki. 2005. "The Transcriptional Landscape of the Mammalian Genome." *Science* 309(5740):1559–63.

Carrieri, Claudia, Laura Cimatti, Marta Biagioli, Anne Beugnet, Silvia Zucchelli, Stefania Fedele, Elisa Pesce, Isidre Ferrer, Licio Collavin, Claudio Santoro, Alistair R. R. Forrest, Piero Carninci, Stefano Biffo, Elia Stupka, and Stefano Gustincich. 2012. "Long Non-Coding Antisense RNA Controls Uchl1 Translation through an Embedded SINEB2 Repeat." *Nature* 491(7424):454–57.

Celli, A., D. S. Mackenzie, D. S. Crumrine, C. L. Tu, M. Hupe, D. D. Bikle, P. M. Elias, and T. M. Mauro. 2011. "ER Ca2+ Depletion Activates XBP1 and Controls Terminal Differentiation in Keratinocytes and Epidermis." *The British Journal of Dermatology* 164(1):16–25.

Chahal, Harvind S., Yuan Lin, Katherine J. Ransohoff, David A. Hinds, Wenting Wu, Hong-Ji Dai, Abrar A. Qureshi, Wen-Qing Li, Peter Kraft, Jean Y. Tang, Jiali Han, and Kavita Y. Sarin. 2016. "Genome-Wide Association Study Identifies Novel Susceptibility Loci for Cutaneous Squamous Cell Carcinoma." *Nature Communications* 7(1):12048.

Chahoud, Jad, Adele Semaan, Yong Chen, Ming Cao, Alyssa G. Rieber, Peter Rady, and Stephen K. Tyring. 2016. "Association Between β-Genus Human Papillomavirus and Cutaneous Squamous Cell Carcinoma in Immunocompetent Individuals—A Meta-Analysis." *JAMA Dermatology* 152(12):1354–64.

Chang, Tsung-Cheng, Duonan Yu, Yun-Sil Lee, Erik A. Wentzel, Dan E. Arking, Kristin M. West, Chi V. Dang, Andrei Thomas-Tikhonenko, and Joshua T. Mendell. 2008. "Widespread MicroRNA Repression by Myc Contributes to Tumorigenesis." *Nature Genetics* 40(1):43–50.

Chen, Edward Y., Christopher M. Tan, Yan Kou, Qiaonan Duan, Zichen Wang, Gabriela Meirelles, Neil R. Clark, and Avi Ma'ayan. 2013. "Enrichr: Interactive and Collaborative HTML5 Gene List Enrichment Analysis Tool." *BMC Bioinformatics* 14(1):128.

Chen, Ling-Ling. 2016. "Linking Long Noncoding RNA Localization and Function." *Trends in Biochemical Sciences* 41(9):761–72.

Chendrimada, Thimmaiah P., Richard I. Gregory, Easwari Kumaraswamy, Jessica Norman, Neil Cooch, Kazuko Nishikura, and Ramin Shiekhattar. 2005. "TRBP Recruits the Dicer Complex to Ago2 for MicroRNA Processing and Gene Silencing." *Nature* 436(7051):740–44.

Chitsazzadeh, Vida, Cristian Coarfa, Jennifer A. Drummond, Tri Nguyen, Aaron Joseph, Suneel Chilukuri, Elizabeth Charpiot, Charles H. Adelmann, Grace Ching, Tran N. Nguyen, Courtney Nicholas, Valencia D. Thomas, Michael Migden, Deborah MacFarlane, Erika Thompson, Jianjun Shen, Yoko Takata, Kayla McNiece, Maxim A. Polansky, Hussein A. Abbas, Kimal Rajapakshe, Adam Gower, Avrum Spira, Kyle R. Covington, Weimin Xiao, Preethi Gunaratne, Curtis Pickering, Mitchell Frederick, Jeffrey N. Myers, Li Shen, Hui Yao, Xiaoping Su, Ronald P. Rapini, David A. Wheeler, Ernest T. Hawk, Elsa R. Flores, and Kenneth Y. Tsai. 2016. "Cross-Species Identification of Genomic Drivers of Squamous Cell Carcinoma Development across Preneoplastic Intermediates." *Nature Communications* 7(1).

Christensen, G., C. Ingvar, L. Hartman, H. Olsson, and K. Nielsen. 2019. "Sunbed Use Increases Cutaneous Squamous Cell Carcinoma Risk in Women: A Large-Scale, Prospective Study in Sweden." *Acta Dermato Venereologica* 99(10):878–83.

Cichorek, Mirosława, Małgorzata Wachulska, Aneta Stasiewicz, and Agata Tymińska. 2013. "Skin Melanocytes: Biology and Development." *Advances in Dermatology and Allergology/Postępy Dermatologii I Alergologii* 30(1):30–41.

Clayton, Elizabeth, David P. Doupé, Allon M. Klein, Douglas J. Winton, Benjamin D. Simons, and Philip H. Jones. 2007. "A Single Type of Progenitor Cell Maintains Normal Epidermis." *Nature* 446(7132):185–89.

Clemson, Christine M., John N. Hutchinson, Sergio A. Sara, Alexander W. Ensminger, Archa H. Fox, Andrew Chess, and Jeanne B. Lawrence. 2009. "An Architectural Role for a Nuclear Noncoding RNA: NEAT1 RNA Is Essential for the Structure of Paraspeckles." *Molecular Cell* 33(6):717–26.

Cocquerelle, Claude, Bénédicte Mascrez, Dominique Hétuin, and Bernard Bailleul. 1993. "Mis-Splicing Yields Circular RNA Molecules." *The FASEB Journal* 7(1):155–60.

Conn, Vanessa M., Véronique Hugouvieux, Aditya Nayak, Stephanie A. Conos, Giovanna Capovilla, Gökhan Cildir, Agnès Jourdain, Vinay Tergaonkar, Markus Schmid, Chloe Zubieta, and Simon J. Conn. 2017. "A CircRNA from SEPALLATA3 Regulates Splicing of Its Cognate MRNA through R-Loop Formation." *Nature Plants* 3:17053.

Dai, Xing, and Julia A. Segre. 2004. "Transcriptional Control of Epidermal Specification and Differentiation." *Current Opinion in Genetics & Development* 14(5):485–91.

Damsky, W. E., N. Theodosakis, and M. Bosenberg. 2014. "Melanoma Metastasis: New Concepts and Evolving Paradigms." *Oncogene* 33(19):2413–22.

Danen, E. H. J., T. J. de Vries, R. Morandini, G. G. Ghanem, D. J. Ruiter, and G. N. P. van Muijen. 1996. "E-Cadherin Expression in Human Melanoma." *Melanoma Research* 6(2):127–32.

Darvin, Pramod, Salman M. Toor, Varun Sasidharan Nair, and Eyad Elkord. 2018. “Immune Checkpoint Inhibitors: Recent Progress and Potential Biomarkers.” *Experimental & Molecular Medicine* 50(12):1–11.

Das Mahapatra, Kunal, Lorenzo Pasquali, Jonas Nørskov Søndergaard, Jan Lapins, István Balazs Nemeth, Eszter Baltás, Lajos Kemény, Bernhard Homey, Liviu-Ionut Moldovan, Jørgen Kjems, Claudia Kutter, Enikö Sonkoly, Lasse Sommer Kristensen, and Andor Pivarcsi. 2020. “A Comprehensive Analysis of Coding and Non-Coding Transcriptomic Changes in Cutaneous Squamous Cell Carcinoma.” *Scientific Reports* 10(1):3637.

Davis, Andrew John, Maksym Tsinkevich, Jason Rodencal, Hussein A. Abbas, Xiao-hua Su, Young-Jin Gi, Bin Fang, Kimal Rajapakshe, Cristian Coarfa, Preethi H. Gunaratne, John M. Koomen, Kenneth Y. Tsai, and Elsa R. Flores. 2020. “TAp63-Regulated MicroRNAs Suppress Cutaneous Squamous Cell Carcinoma through Inhibition of a Network of Cell Cycle Genes.” *Cancer Research*.

Derderian, Camille, Akintunde T. Orunmuyi, E. Oluwabunmi Olapade-Olaopa, and Olorunseun O. Ogunwobi. 2019. “PVT1 Signaling Is a Mediator of Cancer Progression.” *Frontiers in Oncology* 9.

Derrien, Thomas, Rory Johnson, Giovanni Bussotti, Andrea Tanzer, Sarah Djebali, Hagen Tilgner, Gregory Guernec, David Martin, Angelika Merkel, David G. Knowles, Julien Lagarde, Lavanya Veeravalli, Xiaoan Ruan, Yijun Ruan, Timo Lassmann, Piero Carninci, James B. Brown, Leonard Lipovich, Jose M. Gonzalez, Mark Thomas, Carrie A. Davis, Ramin Shiekhattar, Thomas R. Gingeras, Tim J. Hubbard, Cedric Notredame, Jennifer Harrow, and Roderic Guigó. 2012. “The GENCODE v7 Catalog of Human Long Noncoding RNAs: Analysis of Their Gene Structure, Evolution, and Expression.” *Genome Research* 22(9):1775–89.

Descargues, Pascal, Alok K. Sil, and Michael Karin. 2008. “IKKα, a Critical Regulator of Epidermal Differentiation and a Suppressor of Skin Cancer.” *The EMBO Journal* 27(20):2639–47.

Di Leva, Gianpiero, Michela Garofalo, and Carlo M. Croce. 2014. “MicroRNAs in Cancer.” *Annual Review of Pathology: Mechanisms of Disease* 9(1):287–314.

Djebali, Sarah, Carrie A. Davis, Angelika Merkel, Alex Dobin, Timo Lassmann,…Barbara Wold, Piero Carninci, Roderic Guigó, and Thomas R. Gingeras. 2012. “Landscape of Transcription in Human Cells.” *Nature* 489(7414):101–8.

Dlugosz, Andrzej, Glenn Merlino, and Stuart H. Yuspa. 2002. “Progress in Cutaneous Cancer Research11We Have Attempted to Adhere to Standard Nomenclature Guidelines (Http://Www.Nature.Com/Ng/Web_specials/Nomen/Nomen_guidelines.Html) through Most of the Text. Human Genes and Proteins Are Indicated in Upper Case, with Only the Gene Name Italicized (e.g., PTCH1 and PTCH1). For Mouse Homologs, Only the First Letter in Each Is Upper Case (Ptch1 and Ptch1).” *Journal of Investigative Dermatology Symposium Proceedings* 7(1):17–26.

Doench, John G., and Phillip A. Sharp. 2004. “Specificity of MicroRNA Target Selection in Translational Repression.” *Genes & Development* 18(5):504–11.

Domingues, Beatriz, José Manuel Lopes, Paula Soares, and Helena Pópulo. 2018. “Melanoma Treatment in Review.” *ImmunoTargets and Therapy* 7:35–49.

Donato, R., B. R. Cannon, G. Sorci, F. Riuzzi, K. Hsu, D. J. Weber, and C. L. Geczy. 2013. “Functions of S100 Proteins.” *Current Molecular Medicine* 13(1):24–57.

Ebert, Margaret S., and Phillip A. Sharp. 2010. “MicroRNA Sponges: Progress and Possibilities.” *RNA* 16(11):2043–50.

Ebert, Margaret S., and Phillip A. Sharp. 2012. “Roles for MicroRNAs in Conferring Robustness to Biological Processes.” *Cell* 149(3):515–24.

Eckert, Richard L., Gautam Adhikary, Christina A. Young, Ralph Jans, James F. Crish, Wen Xu, and Ellen A. Rorke. 2013. “AP1 Transcription Factors in Epidermal Differentiation and Skin Cancer.” *Journal of Skin Cancer* 2013.

Eckert, Richard L., Tatiana Efimova, Shervin R. Dashti, Sivaprakasam Balasubramanian, Anne Deucher, James F. Crish, Michael Sturniolo, and Frederic Bone. 2002. “Keratinocyte Survival, Differentiation, and Death: Many Roads Lead to Mitogen-Activated Protein Kinase.” *Journal of Investigative Dermatology Symposium Proceedings* 7(1):36–40.

Eckhart, Leopold, Saskia Lippens, Erwin Tschachler, and Wim Declercq. 2013. “Cell Death by Cornification.” *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1833(12):3471–80.

Efimova, Tatiana, Ann-Marie Broome, and Richard L. Eckert. 2003. “A Regulatory Role for P38δ MAPK in Keratinocyte Differentiation EVIDENCE FOR P38δ-ERK1/2 COMPLEX FORMATION.” *Journal of Biological Chemistry* 278(36):34277–85.

Errichelli, Lorenzo, Stefano Dini Modigliani, Pietro Laneve, Alessio Colantoni, Ivano Legnini, Davide Capauto, Alessandro Rosa, Riccardo De Santis, Rebecca Scarfò, Giovanna Peruzzi, Lei Lu, Elisa Caffarelli, Neil A. Shneider, Mariangela Morlando, and Irene Bozzoni. 2017. “FUS Affects Circular RNA Expression in Murine Embryonic Stem Cell-Derived Motor Neurons.” *Nature Communications* 8.

Falchook, Gerald S., Rom Leidner, Elizabeth Stankevich, Brian Piening, Carlo Bifulco, Israel Lowy, and Matthew G. Fury. 2016. “Responses of Metastatic Basal Cell and Cutaneous Squamous Cell Carcinomas to Anti-PD1 Monoclonal Antibody REGN2810.” *Journal for ImmunoTherapy of Cancer* 4(1):70.

Fischer, Joseph W., Veronica F. Busa, Yue Shao, and Anthony K. L. Leung. 2020. “Structure-Mediated RNA Decay by UPF1 and G3BP1.” *Molecular Cell* 78(1):70-84.e6.

Friedman, Robin C., Kyle Kai-How Farh, Christopher B. Burge, and David P. Bartel. 2009. “Most Mammalian MRNAs Are Conserved Targets of MicroRNAs.” *Genome Research* 19(1):92–105.

Fujiwara, Hironobu, Ko Tsutsui, and Ritsuko Morita. 2018. “Multi-Tasking Epidermal Stem Cells: Beyond Epidermal Maintenance.” *Development, Growth & Differentiation* 60(9):531–41.

Fuziwara, Cesar Seigi, and Edna Teruko Kimura. 2015. “Insights into Regulation of the MiR-17-92 Cluster of MiRNAs in Cancer.” *Frontiers in Medicine* 2.

Gabay, Meital, Yulin Li, and Dean W. Felsher. 2014. “MYC Activation Is a Hallmark of Cancer Initiation and Maintenance.” *Cold Spring Harbor Perspectives in Medicine* 4(6).

Galardi, Silvia, Neri Mercatelli, Maria G. Farace, and Silvia A. Ciafrè. 2011. “NF-KB and c-Jun Induce the Expression of the Oncogenic MiR-221 and MiR-222 in Prostate Carcinoma and Glioblastoma Cells.” *Nucleic Acids Research* 39(9):3892–3902.

García-Sancha, Natalia, Roberto Corchado-Cobos, Jesús Pérez-Losada, and Javier Cañueto. 2019. “MicroRNA Dysregulation in Cutaneous Squamous Cell Carcinoma.” *International Journal of Molecular Sciences* 20(9).

Gaur, Arti, David A. Jewell, Yu Liang, Dana Ridzon, Jason H. Moore, Caifu Chen, Victor R. Ambros, and Mark A. Israel. 2007. "Characterization of MicroRNA Expression Levels and Their Biological Correlates in Human Cancer Cell Lines." *Cancer Research* 67(6):2456–68.

German, Marcelo A., Manoj Pillay, Dong-Hoon Jeong, Amit Hetawal, Shujun Luo, Prakash Janardhanan, Vimal Kannan, Linda A. Rymarquis, Kan Nobuta, Rana German, Emanuele De Paoli, Cheng Lu, Gary Schroth, Blake C. Meyers, and Pamela J. Green. 2008. "Global Identification of MicroRNA–Target RNA Pairs by Parallel Analysis of RNA Ends." *Nature Biotechnology* 26(8):941–46.

Ghadially, Ruby. 2012. "25 Years of Epidermal Stem Cell Research." *Journal of Investigative Dermatology* 132(3, Part 2):797–810.

Gironella, Meritxell, Mylène Seux, Min-Jue Xie, Carla Cano, Richard Tomasini, Julien Gommeaux, Stephane Garcia, Jonathan Nowak, Man Lung Yeung, Kuan-Teh Jeang, Amandine Chaix, Ladan Fazli, Yoshiharu Motoo, Qing Wang, Palma Rocchi, Antonio Russo, Martin Gleave, Jean-Charles Dagorn, Juan L. Iovanna, Alice Carrier, Marie-Josèphe Pébusque, and Nelson J. Dusetti. 2007. "Tumor Protein 53-Induced Nuclear Protein 1 Expression Is Repressed by MiR-155, and Its Restoration Inhibits Pancreatic Tumor Development." *Proceedings of the National Academy of Sciences* 104(41):16170–75.

Glaich, Ohad, Shivang Parikh, Rachel E. Bell, Keren Mekahel, Maya Donyo, Yodfat Leader, Ronna Shayevitch, Danna Sheinboim, Sivan Yannai, Dror Hollander, Ze'ev Melamed, Galit Lev-Maor, Gil Ast, and Carmit Levy. 2019. "DNA Methylation Directs MicroRNA Biogenesis in Mammalian Cells." *Nature Communications* 10(1):5657.

Goldstein, Alisa M., Maria Martinez, Margaret A. Tucker, and Florence Demenais. 2000. "Gene-Covariate Interaction between Dysplastic Nevi and the CDKN2A Gene in American Melanoma-Prone Families." *Cancer Epidemiology and Prevention Biomarkers* 9(9):889–94.

Gomez, German G., Jill Wykosky, Ciro Zanca, Frank B. Furnari, and Webster K. Cavenee. 2013. "Therapeutic Resistance in Cancer: MicroRNA Regulation of EGFR Signaling Networks." *Cancer Biology & Medicine* 10(4):192–205.

Gong, Chenguang, and Lynne E. Maquat. 2011. "LncRNAs Transactivate STAU1-Mediated MRNA Decay by Duplexing with 3' UTRs via Alu Elements." *Nature* 470(7333):284–88.

Goodwin, Zane A., and Cristina de Guzman Strong. 2016. "Recent Positive Selection in Genes of the Mammalian Epidermal Differentiation Complex Locus." *Frontiers in Genetics* 7:227.

Gregoire, Serge, Guang Li, Anthony C. Sturzu, Robert J. Schwartz, and Sean M. Wu. 2017. "YY1 Expression Is Sufficient for the Maintenance of Cardiac Progenitor Cell State." *STEM CELLS* 35(8):1913–23.

Gregory, Philip A., Andrew G. Bert, Emily L. Paterson, Simon C. Barry, Anna Tsykin, Gelareh Farshid, Mathew A. Vadas, Yeesim Khew-Goodall, and Gregory J. Goodall. 2008. "The MiR-200 Family and MiR-205 Regulate Epithelial to Mesenchymal Transition by Targeting ZEB1 and SIP1." *Nature Cell Biology* 10(5):593–601.

Grywalska, Ewelina, Marcin Pasiarski, Stanisław Góźdź, and Jacek Roliński. 2018. "Immune-Checkpoint Inhibitors for Combating T-Cell Dysfunction in Cancer." *OncoTargets and Therapy* 11:6505–24.

Gugasyan, Raffi, Anne Voss, George Varigos, Tim Thomas, Raelene J. Grumont, Pritinder Kaur, George Grigoriadis, and Steve Gerondakis. 2004. "The Transcription Factors C-Rel and RelA

Control Epidermal Development and Homeostasis in Embryonic and Adult Skin via Distinct Mechanisms.” *Molecular and Cellular Biology* 24(13):5733–45.

Guo, Huili, Nicholas T. Ingolia, Jonathan S. Weissman, and David P. Bartel. 2010. “Mammalian MicroRNAs Predominantly Act to Decrease Target MRNA Levels.” *Nature* 466(7308):835–40.

Gupta, Rajnish A., Nilay Shah, Kevin C. Wang, Jeewon Kim, Hugo M. Horlings, David J. Wong, Miao-Chih Tsai, Tiffany Hung, Pedram Argani, John L. Rinn, Yulei Wang, Pius Brzoska, Benjamin Kong, Rui Li, Robert B. West, Marc J. van de Vijver, Saraswati Sukumar, and Howard Y. Chang. 2010. “Long Non-Coding RNA HOTAIR Reprograms Chromatin State to Promote Cancer Metastasis.” *Nature* 464(7291):1071–76.

Hacisuleyman, Ezgi, Loyal A. Goff, Cole Trapnell, Adam Williams, Jorge Henao-Mejia, Lei Sun, Patrick McClanahan, David G. Hendrickson, Martin Sauvageau, David R. Kelley, Michael Morse, Jesse Engreitz, Eric S. Lander, Mitch Guttman, Harvey F. Lodish, Richard Flavell, Arjun Raj, and John L. Rinn. 2014. “Topological Organization of Multichromosomal Regions by the Long Intergenic Noncoding RNA Firre.” *Nature Structural & Molecular Biology* 21(2):198–206.

Haflidadóttir, Benedikta S., Kristín Bergsteinsdóttir, Christian Praetorius, and Eiríkur Steingrímsson. 2010. “MiR-148 Regulates Mitf in Melanoma Cells.” *PLOS ONE* 5(7):e11574.

Hameetman, Liesbeth, Suzan Commandeur, Jan Nico Bouwes Bavinck, Hermina C. Wisgerhof, Frank R. de Gruijl, Rein Willemze, Leon Mullenders, Cornelis P. Tensen, and Harry Vrieling. 2013. “Molecular Profiling of Cutaneous Squamous Cell Carcinomas and Actinic Keratoses from Organ Transplant Recipients.” *BMC Cancer* 13:58.

Hansen, Thomas B., Trine I. Jensen, Bettina H. Clausen, Jesper B. Bramsen, Bente Finsen, Christian K. Damgaard, and Jørgen Kjems. 2013. “Natural RNA Circles Function as Efficient MicroRNA Sponges.” *Nature* 495(7441):384–88.

Hansen, Thomas B., Erik D. Wiklund, Jesper B. Bramsen, Sune B. Villadsen, Aaron L. Statham, Susan J. Clark, and Jørgen Kjems. 2011. “MiRNA-Dependent Gene Silencing Involving Ago2-Mediated Cleavage of a Circular Antisense RNA.” *The EMBO Journal* 30(21):4414–22.

Harrow, Jennifer, Adam Frankish, Jose M. Gonzalez, Electra Tapanari, Mark Diekhans, Felix Kokocinski, Bronwen L. Aken, Daniel Barrell, Amonida Zadissa, Stephen Searle, If Barnes, Alexandra Bignell, Veronika Boychenko, Toby Hunt, Mike Kay, Gaurab Mukherjee, Jeena Rajan, Gloria Despacio-Reyes, Gary Saunders, Charles Steward, Rachel Harte, Michael Lin, Cédric Howald, Andrea Tanzer, Thomas Derrien, Jacqueline Chrast, Nathalie Walters, Suganthi Balasubramanian, Baikang Pei, Michael Tress, Jose Manuel Rodriguez, Iakes Ezkurdia, Jeltje van Baren, Michael Brent, David Haussler, Manolis Kellis, Alfonso Valencia, Alexandre Reymond, Mark Gerstein, Roderic Guigó, and Tim J. Hubbard. 2012. “GENCODE: The Reference Human Genome Annotation for The ENCODE Project.” *Genome Research* 22(9):1760–74.

Hayward, Nicholas K., James S. Wilmott, Nicola Waddell, Peter A. Johansson, Matthew A. Field, Katia Nones,...Núria López-Bigas, John V. Pearson, John F. Thompson, Richard A. Scolyer, and Graham J. Mann. 2017. “Whole-Genome Landscapes of Major Melanoma Subtypes.” *Nature* 545(7653):175–80.

Hazawa, M., D. C. Lin, H. Handral, L. Xu, Y. Chen, Y. Y. Jiang, A. Mayakonda, L. W. Ding, X. Meng, A. Sharma, S. Samuel, M. M. Movahednia, R. W. Wong, H. Yang, C. Tong, and H. P. Koeffler. 2017. “ZNF750 Is a Lineage-Specific Tumour Suppressor in Squamous Cell Carcinoma.” *Oncogene* 36(16):2243–54.

He, Lin, Xingyue He, Scott W. Lowe, and Gregory J. Hannon. 2007. "MicroRNAs Join the P53 Network — Another Piece in the Tumour-Suppression Puzzle." *Nature Reviews Cancer* 7(11):819–22.

Heo, Inha, Chirlmin Joo, Young-Kook Kim, Minju Ha, Mi-Jeong Yoon, Jun Cho, Kyu-Hyeon Yeom, Jinju Han, and V. Narry Kim. 2009. "TUT4 in Concert with Lin28 Suppresses MicroRNA Biogenesis through Pre-MicroRNA Uridylation." *Cell* 138(4):696–708.

Hermeking, H. 2010. "The MiR-34 Family in Cancer and Apoptosis." *Cell Death & Differentiation* 17(2):193–99.

Herrera-Solorio, Abril Marcela, Leonel Armas-López, Oscar Arrieta, Joaquín Zúñiga, Patricia Piña-Sánchez, and Federico Ávila-Moreno. 2017. "Histone Code and Long Non-Coding RNAs (LncRNAs) Aberrations in Lung Cancer: Implications in the Therapy Response." *Clinical Epigenetics* 9(1):98.

Hess, Jochen, Peter Angel, and Marina Schorpp-Kistner. 2004. "AP-1 Subunits: Quarrel and Harmony among Siblings." *Journal of Cell Science* 117(25):5965–73.

Hodis, Eran, Ian R. Watson, Gregory V. Kryukov, Stefan T. Arold, Marcin Imielinski, Jean-Philippe Theurillat, Elizabeth Nickerson, Daniel Auclair, Liren Li, Chelsea Place, Daniel DiCara, Alex H. Ramos, Michael S. Lawrence, Kristian Cibulskis, Andrey Sivachenko, Douglas Voet, Gordon Saksena, Nicolas Stransky, Robert C. Onofrio, Wendy Winckler, Kristin Ardlie, Nikhil Wagle, Jennifer Wargo, Kelly Chong, Donald L. Morton, Katherine Stemke-Hale, Guo Chen, Michael Noble, Matthew Meyerson, John E. Ladbury, Michael A. Davies, Jeffrey E. Gershenwald, Stephan N. Wagner, Dave S. B. Hoon, Dirk Schadendorf, Eric S. Lander, Stacey B. Gabriel, Gad Getz, Levi A. Garraway, and Lynda Chin. 2012. "A Landscape of Driver Mutations in Melanoma." *Cell* 150(2):251–63.

Hofbauer, Günther F. L., Jan Nico Bouwes Bavinck, and Sylvie Euvrard. 2010. "Organ Transplantation and Skin Cancer: Basic Problems and New Perspectives." *Experimental Dermatology* 19(6):473–82.

Hoffjan, S., and S. Stemmler. 2007. "On the Role of the Epidermal Differentiation Complex in Ichthyosis Vulgaris, Atopic Dermatitis and Psoriasis." *The British Journal of Dermatology* 157(3):441–49.

Houwing, Saskia, Leonie M. Kamminga, Eugene Berezikov, Daniela Cronembold, Angélique Girard, Hans van den Elst, Dmitri V. Filippov, Heiko Blaser, Erez Raz, Cecilia B. Moens, Ronald H. A. Plasterk, Gregory J. Hannon, Bruce W. Draper, and René F. Ketting. 2007. "A Role for Piwi and PiRNAs in Germ Cell Maintenance and Transposon Silencing in Zebrafish." *Cell* 129(1):69–82.

Huang, Chuan, Dongming Liang, Deirdre C. Tatomer, and Jeremy E. Wilusz. 2018. "A Length-Dependent Evolutionarily Conserved Pathway Controls Nuclear Export of Circular RNAs." *Genes & Development* 32(9–10):639–44.

Huang, Xiu-Yan, Zi-Li Huang, Yong-Hua Xu, Qi Zheng, Zi Chen, Wei Song, Jian Zhou, Zhao-You Tang, and Xin-Yu Huang. 2017. "Comprehensive Circular RNA Profiling Reveals the Regulatory Role of the CircRNA-100338/MiR-141-3p Pathway in Hepatitis B-Related Hepatocellular Carcinoma." *Scientific Reports* 7.

Hutvagner, Gyorgy, and Martin J. Simard. 2008. "Argonaute Proteins: Key Players in RNA Silencing." *Nature Reviews Molecular Cell Biology* 9(1):22–32.

Ingolia, Nicholas T., Gloria A. Brar, Noam Stern-Ginossar, Michael S. Harris, Gaëlle J. S. Talhouarne, Sarah E. Jackson, Mark R. Wills, and Jonathan S. Weissman. 2014. “Ribosome Profiling Reveals Pervasive Translation Outside of Annotated Protein-Coding Genes.” *Cell Reports* 8(5):1365–79.

Ishitsuka, Yosuke, Aaron J. Huebner, Robert H. Rice, Peter J. Koch, Vladislav V. Speransky, Alasdair C. Steven, and Dennis R. Roop. 2016. “Lce1 Family Members Are Nrf2-Target Genes That Are Induced to Compensate for the Loss of Loricrin.” *Journal of Investigative Dermatology* 136(8):1656–63.

Jaubert, Jean, Jun Cheng, and Julia A. Segre. 2003. “Ectopic Expression of Kruppel like Factor 4 (Klf4) Accelerates Formation of the Epidermal Permeability Barrier.” *Development* 130(12):2767–77.

Ji, Zhe, Ruisheng Song, Aviv Regev, and Kevin Struhl. 2015. “Many LncRNAs, 5’UTRs, and Pseudogenes Are Translated and Some Are Likely to Express Functional Proteins” edited by N. Sonenberg. *ELife* 4:e08890.

Jiang, Weidong, Honnavara N. Ananthaswamy, H. Konrad Muller, and Margaret L. Kripke. 1999. “P53 Protects against Skin Cancer Induction by UV-B Radiation.” *Oncogene* 18(29):4247–53.

Johnson, Steven M., Helge Grosshans, Jaclyn Shingara, Mike Byrom, Rich Jarvis, Angie Cheng, Emmanuel Labourier, Kristy L. Reinert, David Brown, and Frank J. Slack. 2005. “RAS Is Regulated by the Let-7 MicroRNA Family.” *Cell* 120(5):635–47.

Johnsson, Per, Leonard Lipovich, Dan Grandér, and Kevin V. Morris. 2014. “Evolutionary Conservation of Long Noncoding RNAs; Sequence, Structure, Function.” *Biochimica et Biophysica Acta* 1840(3):1063–71.

Jonason, A. S., S. Kunala, G. J. Price, R. J. Restifo, H. M. Spinelli, J. A. Persing, D. J. Leffell, R. E. Tarone, and D. E. Brash. 1996. “Frequent Clones of P53-Mutated Keratinocytes in Normal Human Skin.” *Proceedings of the National Academy of Sciences* 93(24):14025–29.

Jones, Philip, and Benjamin D. Simons. 2008. “OPINION Epidermal Homeostasis: Do Committed Progenitors Work While Stem Cells Sleep?” *Nature Reviews Molecular Cell Biology* 9(1):82–88.

Kalinin, Andrey, Lyuben N. Marekov, and Peter M. Steinert. 2001. “Assembly of the Epidermal Cornified Cell Envelope.” *Journal of Cell Science* 114(17):3069–70.

Kapranov, Philipp, Jill Cheng, Sujit Dike, David A. Nix, Radharani Duttagupta, Aarron T. Willingham, Peter F. Stadler, Jana Hertel, Jörg Hackermüller, Ivo L. Hofacker, Ian Bell, Evelyn Cheung, Jorg Drenkow, Erica Dumais, Sandeep Patel, Gregg Helt, Madhavan Ganesh, Srinka Ghosh, Antonio Piccolboni, Victor Sementchenko, Hari Tammana, and Thomas R. Gingeras. 2007. “RNA Maps Reveal New RNA Classes and a Possible Function for Pervasive Transcription.” *Science (New York, N.Y.)* 316(5830):1484–88.

Keeley, Ellen C., Borna Mehrad, and Robert M. Strieter. 2010. “CXC Chemokines in Cancer Angiogenesis and Metastases.” *Advances in Cancer Research* 106:91–111.

Keklikoglou, I., C. Koerner, C. Schmidt, J. D. Zhang, D. Heckmann, A. Shavinskaya, H. Allgayer, B. Gückel, T. Fehm, A. Schneeweiss, Ö. Sahin, S. Wiemann, and U. Tschulena. 2012. “MicroRNA-520/373 Family Functions as a Tumor Suppressor in Estrogen Receptor Negative Breast Cancer by Targeting NF-KB and TGF-β Signaling Pathways.” *Oncogene* 31(37):4150–63.

Kim, D. J., J. M. Angel, S. Sano, and J. DiGiovanni. 2009. "Constitutive Activation and Targeted Disruption of Signal Transducer and Activator of Transcription 3 (Stat3) in Mouse Epidermis Reveal Its Critical Role in UVB-Induced Skin Carcinogenesis." *Oncogene* 28(7):950–60.

Kitajima, Yasuo. 2015. "Implications of Normal and Disordered Remodeling Dynamics of Corneodesmosomes in Stratum Corneum." *Dermatologica Sinica* 33(2):58–63.

Klein, Rachel Herndon, and Bogi Andersen. 2015. "Dynamic Networking for Epidermal Differentiation." *Developmental Cell* 32(6):661–62.

Klein, Rachel Herndon, Ziguang Lin, Amelia Soto Hopkin, William Gordon, Lam C. Tsoi, Yun Liang, Johann E. Gudjonsson, and Bogi Andersen. 2017. "GRHL3 Binding and Enhancers Rearrange as Epidermal Keratinocytes Transition between Functional States." *PLOS Genetics* 13(4):e1006745.

Kobielak, Agnieszka, and Elaine Fuchs. 2006. "Links between α-Catenin, NF-KB, and Squamous Cell Carcinoma in Skin." *Proceedings of the National Academy of Sciences* 103(7):2322–27.

Koch, Peter J., Pierre A. de Viragh, Elisabeth Scharer, Donnie Bundman, Mary Ann Longley, Jackie Bickenbach, Yasuhiro Kawachi, Yasushi Suga, Zhijian Zhou, Marcel Huber, Daniel Hohl, Tonja Kartasova, Michal Jarnik, Alasdair C. Steven, and Dennis R. Roop. 2000. "Lessons from Loricrin-Deficient MiceCompensatory Mechanisms Maintaining Skin Barrier Function in the Absence of a Major Cornified Envelope Protein." *Journal of Cell Biology* 151(2):389–400.

Kolev, Vihren, Anna Mandinova, Juan Guinea-Viniegra, Bing Hu, Karine Lefort, Chiara Lambertini, Victor Neel, Reinhard Dummer, Erwin F. Wagner, and G. Paolo Dotto. 2008. "EGFR Signaling as a Negative Regulator of Notch1 Gene Expression : A Differentiation/Apoptosis Control Mechanism for Proliferating Keratinocytes and Cancer Cells." *Nature Cell Biology* 10(8):902–11.

Konermann, Silvana, Mark D. Brigham, Alexandro E. Trevino, Julia Joung, Omar O. Abudayyeh, Clea Barcena, Patrick D. Hsu, Naomi Habib, Jonathan S. Gootenberg, Hiroshi Nishimasu, Osamu Nureki, and Feng Zhang. 2015. "Genome-Scale Transcriptional Activation by an Engineered CRISPR-Cas9 Complex." *Nature* 517(7536):583–88.

Kouwenhoven, Evelyn N., Martin Oti, Hanna Niehues, Simon J. van Heeringen, Joost Schalkwijk, Hendrik G. Stunnenberg, Hans van Bokhoven, and Huiqing Zhou. 2015. "Transcription Factor P63 Bookmarks and Regulates Dynamic Enhancers during Epidermal Differentiation." *EMBO Reports* 16(7):863–78.

Kramer, Marianne C., Dongming Liang, Deirdre C. Tatomer, Beth Gold, Zachary M. March, Sara Cherry, and Jeremy E. Wilusz. 2015. "Combinatorial Control of Drosophila Circular RNA Expression by Intronic Repeats, HnRNPs, and SR Proteins." *Genes & Development* 29(20):2168–82.

Krauthammer, Michael, Yong Kong, Byung Hak Ha, Perry Evans, Antonella Bacchiocchi, James P. McCusker, Elaine Cheng, Matthew J. Davis, Gerald Goh, Murim Choi, Stephan Ariyan, Deepak Narayan, Ken Dutton-Regester, Ana Capatana, Edna C. Holman, Marcus Bosenberg, Mario Sznol, Harriet M. Kluger, Douglas E. Brash, David F. Stern, Miguel A. Materin, Roger S. Lo, Shrikant Mane, Shuangge Ma, Kenneth K. Kidd, Nicholas K. Hayward, Richard P. Lifton, Joseph Schlessinger, Titus J. Boggon, and Ruth Halaban. 2012. "Exome Sequencing Identifies Recurrent Somatic RAC1 Mutations in Melanoma." *Nature Genetics* 44(9):1006–14.

Kretz, Markus, Zurab Siprashvili, Ci Chu, Dan E. Webster, Ashley Zehnder, Kun Qu, Carolyn S. Lee, Ross J. Flockhart, Abigail F. Groff, Jennifer Chow, Danielle Johnston, Grace E. Kim, Robert C. Spitale, Ryan A. Flynn, Grace X. Y. Zheng, Subhadra Aiyer, Arjun Raj, John L. Rinn, Howard

Y. Chang, and Paul A. Khavari. 2013a. "Control of Somatic Tissue Differentiation by the Long Non-Coding RNA TINCR." *Nature* 493(7431):231–35.

Kretz, Markus, Dan E. Webster, Ross J. Flockhart, Carolyn S. Lee, Ashley Zehnder, Vanessa Lopez-Pajares, Kun Qu, Grace X. Y. Zheng, Jennifer Chow, Grace E. Kim, John L. Rinn, Howard Y. Chang, Zurab Siprashvili, and Paul A. Khavari. 2012. "Suppression of Progenitor Differentiation Requires the Long Noncoding RNA ANCR." *Genes & Development* 26(4):338–43.

Kristensen, Lasse S., Maria S. Andersen, Lotte V. W. Stagsted, Karoline K. Ebbesen, Thomas B. Hansen, and Jørgen Kjems. 2019. "The Biogenesis, Biology and Characterization of Circular RNAs." *Nature Reviews Genetics* 20(11):675–91.

Kristensen, Lasse Sommer, Trine Line Hauge Okholm, Morten Trillingsgaard Venø, and Jørgen Kjems. 2018. "Circular RNAs Are Abundantly Expressed and Upregulated during Human Epidermal Stem Cell Differentiation." *RNA Biology* 15(2):280–91.

Kruger, Kelly, Paula J. Grabowski, Arthur J. Zaug, Julie Sands, Daniel E. Gottschling, and Thomas R. Cech. 1982. "Self-Splicing RNA: Autoexcision and Autocyclization of the Ribosomal RNA Intervening Sequence of Tetrahymena." *Cell* 31(1):147–57.

Kung, Johnny T. Y., David Colognori, and Jeannie T. Lee. 2013. "Long Noncoding RNAs: Past, Present, and Future." *Genetics* 193(3):651–69.

Kwa, Raymond E., Kirk Campana, and Ronald L. Moy. 1992. "Biology of Cutaneous Squamous Cell Carcinoma." *Journal of the American Academy of Dermatology* 26(1):1–26.

Kwong, Lawrence N., and Michael A. Davies. 2013. "Navigating the Therapeutic Complexity of PI3K Pathway Inhibition in Melanoma." *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research* 19(19).

Kypriotou, Magdalini, Marcel Huber, and Daniel Hohl. 2012. "The Human Epidermal Differentiation Complex: Cornified Envelope Precursors, S100 Proteins and the 'Fused Genes' Family." *Experimental Dermatology* 21(9):643–49.

Lai, Fan, Ulf A. Orom, Matteo Cesaroni, Malte Beringer, Dylan J. Taatjes, Gerd A. Blobel, and Ramin Shiekhattar. 2013. "Activating RNAs Associate with Mediator to Enhance Chromatin Architecture and Transcription." *Nature* 494(7438):497–501.

Lander, Eric S., Lauren M. Linton, Bruce Birren,…Aristides Patrinos, Michael J. Morgan, International Human Genome Sequencing Consortium, Center for Genome Research: Whitehead Institute for Biomedical Research, The Sanger Centre:, Washington University Genome Sequencing Center, US DOE Joint Genome Institute:, Baylor College of Medicine Human Genome Sequencing Center:, RIKEN Genomic Sciences Center:, Genoscope and CNRS UMR-8030:, Institute of Molecular Biotechnology: Department of Genome Analysis, GTC Sequencing Center:, Beijing Genomics Institute/Human Genome Center:, The Institute for Systems Biology: Multimegabase Sequencing Center, Stanford Genome Technology Center:, University of Oklahoma's Advanced Center for Genome Technology:, Max Planck Institute for Molecular Genetics:, Lita Annenberg Hazen Genome Center: Cold Spring Harbor Laboratory, GBF—German Research Centre for Biotechnology:, also includes individuals listed under other headings): *Genome Analysis Group (listed in alphabetical order, US National Institutes of Health: Scientific management: National Human Genome Research Institute, Stanford Human Genome Center:, University of Washington Genome Center:, Keio University School of Medicine: Department of Molecular Biology, University of Texas Southwestern Medical Center at Dallas:, US Department of Energy: Office of Science, and The Wellcome Trust: 2001. "Initial Sequencing and Analysis of the Human Genome." *Nature* 409(6822):860–921.

Lasda, Erika, and Roy Parker. 2016. “Circular RNAs Co-Precipitate with Extracellular Vesicles: A Possible Mechanism for CircRNA Clearance.” *PLOS ONE* 11(2):e0148407.

Lawrence, Michael S., Petar Stojanov, Paz Polak, Gregory V. Kryukov, Kristian Cibulskis, Andrey Sivachenko, Scott L. Carter, Chip Stewart, Craig H. Mermel, Steven A. Roberts, Adam Kiezun, Peter S. Hammerman, Aaron McKenna, Yotam Drier, Lihua Zou, Alex H. Ramos, Trevor J. Pugh, Nicolas Stransky, Elena Helman, Jaegil Kim, Carrie Sougnez, Lauren Ambrogio, Elizabeth Nickerson, Erica Shefler, Maria L. Cortés, Daniel Auclair, Gordon Saksena, Douglas Voet, Michael Noble, Daniel DiCara, Pei Lin, Lee Lichtenstein, David I. Heiman, Timothy Fennell, Marcin Imielinski, Bryan Hernandez, Eran Hodis, Sylvan Baca, Austin M. Dulak, Jens Lohr, Dan-Avi Landau, Catherine J. Wu, Jorge Melendez-Zajgla, Alfredo Hidalgo-Miranda, Amnon Koren, Steven A. McCarroll, Jaume Mora, Brian Crompton, Robert Onofrio, Melissa Parkin, Wendy Winckler, Kristin Ardlie, Stacey B. Gabriel, Charles W. M. Roberts, Jaclyn A. Biegel, Kimberly Stegmaier, Adam J. Bass, Levi A. Garraway, Matthew Meyerson, Todd R. Golub, Dmitry A. Gordenin, Shamil Sunyaev, Eric S. Lander, and Gad Getz. 2013. “Mutational Heterogeneity in Cancer and the Search for New Cancer-Associated Genes.” *Nature* 499(7457):214–18.

Lee, Carolyn S., Angela Mah, Cody J. Aros, Vanessa Lopez-Pajares, Aparna Bhaduri, Dan E. Webster, Markus Kretz, and Paul A. Khavari. 2018. “Cancer-Associated Long Noncoding RNA SMRT-2 Controls Epidermal Differentiation.” *Journal of Investigative Dermatology* 138(6):1445–49.

Lee, R. C., R. L. Feinbaum, and V. Ambros. 1993. “The C. Elegans Heterochronic Gene Lin-4 Encodes Small RNAs with Antisense Complementarity to Lin-14.” *Cell* 75(5):843–54.

Lee, Yoontae, Chiyoung Ahn, Jinju Han, Hyounjeong Choi, Jaekwang Kim, Jeongbin Yim, Junho Lee, Patrick Provost, Olof Rådmark, Sunyoung Kim, and V. Narry Kim. 2003. “The Nuclear RNase III Drosha Initiates MicroRNA Processing.” *Nature* 425(6956):415–19.

Lee, Yoontae, Minju Kim, Jinju Han, Kyu-Hyun Yeom, Sanghyuk Lee, Sung Hee Baek, and V. Narry Kim. 2004. “MicroRNA Genes Are Transcribed by RNA Polymerase II.” *The EMBO Journal* 23(20):4051–60.

Lefort, Karine, Yang Brooks, Paola Ostano, Muriel Cario-André, Valérie Calpini, Juan Guinea-Viniegra, Andrea Albinger-Hegyi, Wolfram Hoetzenecker, Ingrid Kolfschoten, Erwin F. Wagner, Sabine Werner, and Gian Paolo Dotto. 2013. “A MiR-34a-SIRT6 Axis in the Squamous Cell Differentiation Network.” *The EMBO Journal* 32(16):2248–63.

Lefort, Karine, Anna Mandinova, Paola Ostano, Vihren Kolev, Valerie Calpini, Ingrid Kolfschoten, Vikram Devgan, Jocelyn Lieb, Wassim Raffoul, Daniel Hohl, Victor Neel, Jonathan Garlick, Giovanna Chiorino, and G. Paolo Dotto. 2007. “Notch1 Is a P53 Target Gene Involved in Human Keratinocyte Tumor Suppression through Negative Regulation of ROCK1/2 and MRCKα Kinases.” *Genes & Development* 21(5):562–77.

Lena, A. M., R. Shalom-Feuerstein, P. Rivetti di Val Cervo, D. Aberdam, R. A. Knight, G. Melino, and E. Candi. 2008. “MiR-203 Represses ‘stemness’ by Repressing DeltaNp63.” *Cell Death and Differentiation* 15(7):1187–95.

Leonardi, Giulia C., Luca Falzone, Rossella Salemi, Antonino Zanghì, Demetrios A. Spandidos, James A. McCubrey, Saverio Candido, and Massimo Libra. 2018. “Cutaneous Melanoma: From Pathogenesis to Therapy (Review).” *International Journal of Oncology* 52(4):1071–80.

Levy, Carmit, Mehdi Khaled, Kathleen C. Robinson, Rosa A. Veguilla, Po-Hao Chen, Satoru Yokoyama, Eiichi Makino, Jun Lu, Lionel Larue, Friedrich Beermann, Lynda Chin, Marcus

Bosenberg, Jun S. Song, and David E. Fisher. 2010. "Lineage-Specific Transcriptional Regulation of DICER by MITF in Melanocytes." *Cell* 141(6):994–1005.

Lewis, Benjamin P., Christopher B. Burge, and David P. Bartel. 2005. "Conserved Seed Pairing, Often Flanked by Adenosines, Indicates That Thousands of Human Genes Are MicroRNA Targets." *Cell* 120(1):15–20.

Li, Chun-xiao, Hua-guo Li, Lin-ting Huang, Yu-wei Kong, Fu-ying Chen, Jian-yin Liang, Hong Yu, and Zhi-rong Yao. 2017. "H19 LncRNA Regulates Keratinocyte Differentiation by Targeting MiR-130b-3p." *Cell Death & Disease* 8(11):e3174–e3174.

Li, Jianchang, Xiuming Liu, Chaopeng Li, and Wenqi Wang. 2019. "MiR-224-5p Inhibits Proliferation, Migration, and Invasion by Targeting PIK3R3/AKT3 in Uveal Melanoma." *Journal of Cellular Biochemistry* 120(8):12412–21.

Li, Mengyang, Wei Ding, Teng Sun, Muhammad A. Tariq, Tao Xu, Peifeng Li, and Jianxun Wang. 2018. "Biogenesis of Circular RNAs and Their Roles in Cardiovascular Development and Pathology." *The FEBS Journal* 285(2):220–32.

Li, Xue Mei, Soo Jung Kim, Dong-Kyun Hong, Kyoung Eun Jung, Chong Won Choi, Young-Joon Seo, Jeung-Hoon Lee, Young Lee, and Chang-Deok Kim. 2019. "KLF4 Suppresses the Tumor Activity of Cutaneous Squamous Cell Carcinoma (SCC) Cells via the Regulation of SMAD Signaling and SOX2 Expression." *Biochemical and Biophysical Research Communications* 516(4):1110–15.

Li, Yvonne Y., Glenn J. Hanna, Alvaro C. Laga, Robert I. Haddad, Jochen H. Lorch, and Peter S. Hammerman. 2015. "Genomic Analysis of Metastatic Cutaneous Squamous Cell Carcinoma." *Clinical Cancer Research* 21(6):1447–56.

Liao, D. Joshua, Archana Thakur, Jack Wu, Hector Biliran, and Fazlul H. Sarkar. 2007. "Perspectives on C-Myc, Cyclin D1, and Their Interaction in Cancer Formation, Progression, and Response to Chemotherapy." *Critical Reviews in Oncogenesis* 13(2):93–158.

Liberzon, Arthur, Chet Birger, Helga Thorvaldsdóttir, Mahmoud Ghandi, Jill P. Mesirov, and Pablo Tamayo. 2015. "The Molecular Signatures Database Hallmark Gene Set Collection." *Cell Systems* 1(6):417–25.

Lim, Xinhong, Si Hui Tan, Winston Lian Chye Koh, Rosanna Man Wah Chau, Kelley S. Yan, Calvin J. Kuo, Renée van Amerongen, Allon Moshe Klein, and Roel Nusse. 2013. "Interfollicular Epidermal Stem Cells Self-Renew via Autocrine Wnt Signaling." *Science* 342(6163):1226–30.

Lippens, S., G. Denecker, P. Ovaere, P. Vandenabeele, and W. Declercq. 2005. "Death Penalty for Keratinocytes: Apoptosis versus Cornification." *Cell Death & Differentiation* 12(2):1497–1508.

Liu, Shujing, Michael T. Tetzlaff, Rutao Cui, and Xiaowei Xu. 2012. "MiR-200c Inhibits Melanoma Progression and Drug Resistance through down-Regulation of BMI-1." *The American Journal of Pathology* 181(5):1823–35.

Liu, Xuefeng, Dan Li, Weimin Zhang, Mingzhou Guo, and Qimin Zhan. 2012. "Long Non-Coding RNA *Gadd7* Interacts with TDP-43 and Regulates *Cdk6* MRNA Decay: LncRNA *Gadd7* Regulation of *Cdk6*." *The EMBO Journal* 31(23):4415–27.

Lodrini, Marco, Ina Oehme, Christina Schroeder, Till Milde, Marie C. Schier, Annette Kopp-Schneider, Johannes H. Schulte, Matthias Fischer, Katleen De Preter, Filip Pattyn, Mirco Castoldi, Martina U. Muckenthaler, Andreas E. Kulozik, Frank Westermann, Olaf Witt, and Hedwig E.

Deubzer. 2013. “MYCN and HDAC2 Cooperate to Repress MiR-183 Signaling in Neuroblastoma.” *Nucleic Acids Research* 41(12):6018–33.

Longobardi, Stephen J., Brian Sullivan, and E. Hani Mansour. 2011. “Invasive Cutaneous Squamous Cell Carcinoma Presenting as a Nonhealing Radiation Wound: A Case Report.” *Journal of the American Podiatric Medical Association* 101(4):360–62.

Lopez-Pajares, Vanessa, Kun Qu, Jiajing Zhang, Dan E. Webster, Brook C. Barajas, Zurab Siprashvili, Brian J. Zarnegar, Lisa D. Boxer, Eon J. Rios, Shiying Tao, Markus Kretz, and Paul A. Khavari. 2015. “A LncRNA-MAF:MAFB Transcription Factor Network Regulates Epidermal Differentiation.” *Developmental Cell* 32(6):693–706.

Lopez-Pajares, Vanessa, Karen Yan, Brian J. Zarnegar, Katherine L. Jameson, and Paul A. Khavari. 2013. “Genetic Pathways in Disorders of Epidermal Differentiation.” *Trends in Genetics : TIG* 29(1):31–40.

Lu, Zhanping, Courtney C. Hong, Guangyao Kong, Anna L. F. V. Assumpção, Irene M. Ong, Emery H. Bresnick, Jing Zhang, and Xuan Pan. 2018. “Polycomb Group Protein YY1 Is an Essential Regulator of Hematopoietic Stem Cell Quiescence.” *Cell Reports* 22(6):1545–59.

Luebker, Stephen A., and Scott A. Koepsell. 2019. “Diverse Mechanisms of BRAF Inhibitor Resistance in Melanoma Identified in Clinical and Preclinical Studies.” *Frontiers in Oncology* 9.

Luke, Jason J., and Gary K. Schwartz. 2013. “Chemotherapy in the Management of Advanced Cutaneous Malignant Melanoma.” *Clinics in Dermatology* 31(3):290–97.

Ma, Xuda, Yamei Dang, Xiaowen Shao, Xuechun Chen, Fei Wu, and Yongmei Li. 2019. “Ubiquitination and Long Non-Coding RNAs Regulate Actin Cytoskeleton Regulators in Cancer Progression.” *International Journal of Molecular Sciences* 20(12).

Marchese, Francesco P., Ivan Raimondi, and Maite Huarte. 2017. “The Multidimensional Mechanisms of Long Noncoding RNA Function.” *Genome Biology* 18(1):206.

Marenholz, Ingo, Maja Zirra, David F. Fischer, Claude Backendorf, Andreas Ziegler, and Dietmar Mischke. 2001. “Identification of Human Epidermal Differentiation Complex (EDC)-Encoded Genes by Subtractive Hybridization of Entire YACs to a Gridded Keratinocyte CDNA Library.” *Genome Research* 11(3):341–55.

Marks, R. 1995. “An Overview of Skin Cancers. Incidence and Causation.” *Cancer* 75(2 Suppl):607–12.

Martianov, Igor, Aroul Ramadass, Ana Serra Barros, Natalie Chow, and Alexandre Akoulitchev. 2007. “Repression of the Human Dihydrofolate Reductase Gene by a Non-Coding Interfering Transcript.” *Nature* 445(7128):666–70.

Martincorena, Iñigo, Amit Roshan, Moritz Gerstung, Peter Ellis, Peter Van Loo, Stuart McLaren, David C. Wedge, Anthony Fullam, Ludmil B. Alexandrov, Jose M. Tubio, Lucy Stebbings, Andrew Menzies, Sara Widaa, Michael R. Stratton, Philip H. Jones, and Peter J. Campbell. 2015. “High Burden and Pervasive Positive Selection of Somatic Mutations in Normal Human Skin.” *Science* 348(6237):880–86.

Mas-Ponte, David, Joana Carlevaro-Fita, Emilio Palumbo, Toni Hermoso Pulido, Roderic Guigo, and Rory Johnson. 2017. “LncATLAS Database for Subcellular Localization of Long Noncoding RNAs.” *RNA* 23(7):1080–87.

Mastroianni, Justin, Natalie Stickel, Hana Andrlova, Kathrin Hanke, Wolfgang Melchinger, Sandra Duquesne, Dominik Schmidt, Martina Falk, Geoffroy Andrieux, Dietmar Pfeifer, Heide Dierbach, Annette Schmitt-Graeff, Frank Meiss, Melanie Boerries, and Robert Zeiser. 2019. "MiR-146a Controls Immune Response in the Melanoma Microenvironment." *Cancer Research* 79(1):183–95.

Maubec, Eve, Peter Petrow, Isabelle Scheer-Senyarich, Pierre Duvillard, Ludovic Lacroix, Julien Gelly, Agnès Certain, Xavier Duval, Béatrice Crickx, Valérie Buffard, Nicole Basset-Seguin, Pierre Saez, Anne-Bénédicte Duval-Modeste, Henri Adamski, Sandrine Mansard, Florent Grange, Anne Dompmartin, Sandrine Faivre, France Mentré, and Marie-Françoise Avril. 2011. "Phase II Study of Cetuximab As First-Line Single-Drug Therapy in Patients With Unresectable Squamous Cell Carcinoma of the Skin." *Journal of Clinical Oncology* 29(25):3419–26.

Maverakis, Emanual, Lynn A. Cornelius, Glen M. Bowen, Tiffany Phan, Falin B. Patel, Sarah Fitzmaurice, Young He, Barbara Burrall, Christopher Duong, April M. Kloxin, Hawa Sultani, Reason Wilken, Steve R. Martinez, and Forum Patel. 2015. "Metastatic Melanoma - a Review of Current and Future Treatment Options." *Acta Dermato-Venereologica* 95(5):516–24.

Memczak, Sebastian, Marvin Jens, Antigoni Elefsinioti, Francesca Torti, Janna Krueger, Agnieszka Rybak, Luisa Maier, Sebastian D. Mackowiak, Lea H. Gregersen, Mathias Munschauer, Alexander Loewer, Ulrike Ziebold, Markus Landthaler, Christine Kocks, Ferdinand le Noble, and Nikolaus Rajewsky. 2013. "Circular RNAs Are a Large Class of Animal RNAs with Regulatory Potency." *Nature* 495(7441):333–38.

Menon, Gopinathan K., Sang Eun Lee, and Seung-Hun Lee. 2018. "An Overview of Epidermal Lamellar Bodies: Novel Roles in Biological Adaptations and Secondary Barriers." *Journal of Dermatological Science* 92(1):10–17.

Mercer, Tim R., Marcel E. Dinger, Susan M. Sunkin, Mark F. Mehler, and John S. Mattick. 2008. "Specific Expression of Long Noncoding RNAs in the Mouse Brain." *Proceedings of the National Academy of Sciences* 105(2):716–21.

Miao, Hui, Linlin Wang, Haomiao Zhan, Jiangshan Dai, Yanbo Chang, Fan Wu, Tao Liu, Zhongyu Liu, Chunfang Gao, Ling Li, and Xu Song. 2019. "A Long Noncoding RNA Distributed in Both Nucleus and Cytoplasm Operates in the PYCARD-Regulated Apoptosis by Coordinating the Epigenetic and Translational Regulation." *PLoS Genetics* 15(5).

Miller, Arlo J., and Martin C. Mihm. 2006. "Melanoma." *New England Journal of Medicine* 355(1):51–65.

Mills, Alea A., Binhai Zheng, Xiao-Jing Wang, Hannes Vogel, Dennis R. Roop, and Allan Bradley. 1999. "P63 Is a P53 Homologue Required for Limb and Epidermal Morphogenesis." *Nature* 398(6729):708–13.

Miyai, Masashi, Michito Hamada, Takashi Moriguchi, Junichiro Hiruma, Akiyo Kamitani-Kawamoto, Hajime Watanabe, Mariko Hara-Chikuma, Kenzo Takahashi, Satoru Takahashi, and Kohsuke Kataoka. 2016. "Transcription Factor MafB Coordinates Epidermal Keratinocyte Differentiation." *Journal of Investigative Dermatology* 136(9):1848–57.

Mizrahi, A., A. Barzilai, D. Gur-Wahnon, I. Z. Ben-Dov, S. Glassberg, T. Meningher, E. Elharar, M. Masalha, J. Jacob-Hirsch, H. Tabibian-Keissar, I. Barshack, J. Roszik, R. Leibowitz-Amit, Y. Sidi, and D. Avni. 2018. "Alterations of MicroRNAs throughout the Malignant Evolution of Cutaneous Squamous Cell Carcinoma: The Role of MiR-497 in Epithelial to Mesenchymal Transition of Keratinocytes." *Oncogene* 37(2):218–30.

Montaudié, Henri, Julien Viotti, Patrick Combemale, Caroline Dutriaux, Nicolas Dupin, Caroline Robert, Laurent Mortier, Régis Kaphan, Anne-Bénédicte Duval-Modeste, Stéphane Dalle, Julie De Quatrebarbes, Andrea Stefan, Florence Brunet-Possenti, Maria Kogay, Alexandra Picard-Gauci, Gilles Poissonnet, and Frédéric Peyrade. 2020. "Cetuximab Is Efficient and Safe in Patients with Advanced Cutaneous Squamous Cell Carcinoma: A Retrospective, Multicentre Study." *Oncotarget* 11(4):378–85.

Montes, Marta, Morten M. Nielsen, Giulia Maglieri, Anders Jacobsen, Jonas Højfeldt, Shuchi Agrawal-Singh, Klaus Hansen, Kristian Helin, Harmen J. G. van de Werken, Jakob S. Pedersen, and Anders H. Lund. 2015. "The LncRNA MIR31HG Regulates P16 INK4A Expression to Modulate Senescence." *Nature Communications* 6(1):1–15.

Moriyama, Mariko, André-Dante Durham, Hiroyuki Moriyama, Kiyotaka Hasegawa, Shin-Ichi Nishikawa, Freddy Radtke, and Masatake Osawa. 2008. "Multiple Roles of Notch Signaling in the Regulation of Epidermal Development." *Developmental Cell* 14(4):594–604.

Morlando, Mariangela, Monica Ballarino, and Alessandro Fatica. 2015. "Long Non-Coding RNAs: New Players in Hematopoiesis and Leukemia." *Frontiers in Medicine* 2.

Mort, Richard L., Ian J. Jackson, and E. Elizabeth Patton. 2015. "The Melanocyte Lineage in Development and Disease." *Development* 142(4):620–32.

Moses, Michael A., Andrea L. George, Nozomi Sakakibara, Kanwal Mahmood, Roshini M. Ponnamperuma, Kathryn E. King, and Wendy C. Weinberg. 2019. "Molecular Mechanisms of P63-Mediated Squamous Cancer Pathogenesis." *International Journal of Molecular Sciences* 20(14).

Mulrane, Laoighse, Sharon F. McGee, William M. Gallagher, and Darran P. O'Connor. 2013. "MiRNA Dysregulation in Breast Cancer." *Cancer Research* 73(22):6554–62.

My, Hsu, Meier Fe, Nesbit M, Hsu Jy, Van Belle P, Elder De, and Herlyn M. 2000. "E-Cadherin Expression in Melanoma Cells Restores Keratinocyte-Mediated Growth Control and down-Regulates Expression of Invasion-Related Adhesion Receptors." *The American Journal of Pathology* 156(5):1515–25.

Nagarajan, P., S. S. Chin, D. Wang, S. Liu, S. Sinha, and L. A. Garrett-Sinha. 2010. "Ets1 Blocks Terminal Differentiation of Keratinocytes and Induces Expression of Matrix Metalloproteases and Innate Immune Mediators." *Journal of Cell Science* 123(20):3566–75.

Nakamura, Yasuhiro, Yasuhiro Kawachi, Xuezhu Xu, Hideko Sakurai, Yoshiyuki Ishii, Takenori Takahashi, and Fujio Otsuka. 2007. "The Combination of Ubiquitous Transcription Factors AP-1 and Sp1 Directs Keratinocyte-Specific and Differentiation-Specific Gene Expression in Vitro." *Experimental Dermatology* 16(2):143–50.

Nakazawa, H., D. English, P. L. Randell, K. Nakazawa, N. Martel, B. K. Armstrong, and H. Yamasaki. 1994. "UV and Skin Cancer: Specific P53 Gene Mutation in Normal Skin as a Biologically Relevant Exposure Measurement." *Proceedings of the National Academy of Sciences of the United States of America* 91(1):360–64.

Natsuga, Ken. 2014. "Epidermal Barriers." *Cold Spring Harbor Perspectives in Medicine* 4(4):a018218.

Neagu, Monica, Constantin Caruntu, Carolina Constantin, Daniel Boda, Sabina Zurac, Demetrios A. Spandidos, and Aristidis M. Tsatsakis. 2016. "Chemically Induced Skin Carcinogenesis: Updates in Experimental Models (Review)." *Oncology Reports* 35(5):2516–28.

Ng, Dean C., Simira Shafaee, David Lee, and Daniel D. Bikle. 2000. "Requirement of an AP-1 Site in the Calcium Response Region of the Involucrin Promoter." *Journal of Biological Chemistry* 275(31):24080–88.

Nguyen, Bach-Cuc, Karine Lefort, Anna Mandinova, Dario Antonini, Vikram Devgan, Giusy Della Gatta, Maranke I. Koster, Zhuo Zhang, Jian Wang, Alice Tommasi di Vignano, Jan Kitajewski, Giovanna Chiorino, Dennis R. Roop, Caterina Missero, and G. Paolo Dotto. 2006. "Cross-Regulation between Notch and P63 in Keratinocyte Commitment to Differentiation." *Genes & Development* 20(8):1028–42.

Noguchi, Shunsuke, Minami Kumazaki, Yuki Yasui, Takashi Mori, Nami Yamada, and Yukihiro Akao. 2014. "MicroRNA-203 Regulates Melanosome Transport and Tyrosinase Expression in Melanoma Cells by Targeting Kinesin Superfamily Protein 5b." *Journal of Investigative Dermatology* 134(2):461–69.

Noguchi, Shunsuke, Takashi Mori, Yusami Otsuka, Nami Yamada, Yuki Yasui, Junya Iwasaki, Minami Kumazaki, Kohji Maruo, and Yukihiro Akao. 2012. "Anti-Oncogenic MicroRNA-203 Induces Senescence by Targeting E2F3 Protein in Human Melanoma Cells." *Journal of Biological Chemistry* 287(15):11769–77.

Obayashi, Mariko, Maki Yoshida, Takaaki Tsunematsu, Ikuko Ogawa, Tomonori Sasahira, Hiroki Kuniyasu, Issei Imoto, Yoshimitsu Abiko, Dan Xu, Saori Fukunaga, Hidetoshi Tahara, Yasusei Kudo, Toshitaka Nagao, and Takashi Takata. 2016. "MicroRNA-203 Suppresses Invasion and Epithelial-Mesenchymal Transition Induction via Targeting NUAK1 in Head and Neck Cancer." *Oncotarget* 7(7):8223–39.

Oh, Inez Y., and Cristina de Guzman Strong. 2017. "The Molecular Revolution in Cutaneous Biology: EDC and Locus Control." *Journal of Investigative Dermatology* 137(5):e101–4.

Okada, Chimari, Eiki Yamashita, Soo Jae Lee, Satoshi Shibata, Jun Katahira, Atsushi Nakagawa, Yoshihiro Yoneda, and Tomitake Tsukihara. 2009. "A High-Resolution Structure of the Pre-MicroRNA Nuclear Export Machinery." *Science* 326(5957):1275–79.

Ørom, Ulf Andersson, Thomas Derrien, Malte Beringer, Kiranmai Gumireddy, Alessandro Gardini, Giovanni Bussotti, Fan Lai, Matthias Zytnicki, Cedric Notredame, Qihong Huang, Roderic Guigo, and Ramin Shiekhattar. 2010. "Long Noncoding RNAs with Enhancer-like Function in Human Cells." *Cell* 143(1):46–58.

Ozsolak, Fatih, Laura L. Poling, Zhengxin Wang, Hui Liu, X. Shirley Liu, Robert G. Roeder, Xinmin Zhang, Jun S. Song, and David E. Fisher. 2008. "Chromatin Structure Analyses Identify MiRNA Promoters." *Genes & Development* 22(22):3172–83.

Panatta, Emanuele, Anna Maria Lena, Mara Mancini, Artem Smirnov, Alberto Marini, Riccardo Delli Ponti, Teresa Botta-Orfila, Gian Gaetano Tartaglia, Alessandro Mauriello, Xinna Zhang, George A. Calin, Gerry Melino, and Eleonora Candi. 2020. "Long Non-Coding RNA Uc.291 Controls Epithelial Differentiation by Interfering with the ACTL6A/BAF Complex." *EMBO Reports* 21(3):e46734.

Patel, Satyakam, Zong Fang Xi, Eun Young Seo, David McGaughey, and Julia A. Segre. 2006. "Klf4 and Corticosteroids Activate an Overlapping Set of Transcriptional Targets to Accelerate in Utero Epidermal Barrier Acquisition." *Proceedings of the National Academy of Sciences of the United States of America* 103(49):18668–73.

Peng, Yong, and Carlo M. Croce. 2016. "The Role of MicroRNAs in Human Cancer." *Signal Transduction and Targeted Therapy* 1(1):1–9.

Pérez-Mancera, Pedro Antonio, Inés González-Herrero, María Pérez-Caro, Noelia Gutiérrez-Cianca, Teresa Flores, Alfonso Gutiérrez-Adán, Belén Pintado, Manuel Sánchez-Martín, and Isidro Sánchez-García. 2005. "SLUG in Cancer Development." *Oncogene* 24(19):3073–82.

Pichler, M., and G. A. Calin. 2015. "MicroRNAs in Cancer: From Developmental Genes in Worms to Their Clinical Application in Patients." *British Journal of Cancer* 113(4):569–73.

Pickering, Curtis R., Jane H. Zhou, J. Jack Lee, Jennifer A. Drummond, S. Andrew Peng, Rami E. Saade, Kenneth Y. Tsai, Jonathan L. Curry, Michael T. Tetzlaff, Stephen Y. Lai, Jun Yu, Donna M. Muzny, Harshavardhan Doddapaneni, Eve Shinbrot, Kyle R. Covington, Jianhua Zhang, Sahil Seth, Carlos Caulin, Gary L. Clayman, Adel K. El-Naggar, Richard A. Gibbs, Randal S. Weber, Jeffrey N. Myers, David A. Wheeler, and Mitchell J. Frederick. 2014. "Mutational Landscape of Aggressive Cutaneous Squamous Cell Carcinoma." *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research* 20(24):6582–92.

Pierceall, William E., Leonard H. Goldberg, Michael A. Tainsky, Tapas Mukhopadhyay, and Honnavara N. Ananthaswamy. 1991. "Ras Gene Mutation and Amplification in Human Nonmelanoma Skin Cancers." *Molecular Carcinogenesis* 4(3):196–202.

Piipponen, Minna, Liisa Nissinen, Mehdi Farshchian, Pilvi Riihilä, Atte Kivisaari, Markku Kallajoki, Juha Peltonen, Sirkku Peltonen, and Veli-Matti Kähäri. 2016. "Long Noncoding RNA PICSAR Promotes Growth of Cutaneous Squamous Cell Carcinoma by Regulating ERK1/2 Activity." *The Journal of Investigative Dermatology* 136(8):1701–10.

Piipponen, Minna, Liisa Nissinen, and Veli-Matti Kähäri. 2020. "Long Non-Coding RNAs in Cutaneous Biology and Keratinocyte Carcinomas." *Cellular and Molecular Life Sciences*.

Piipponen, Minna, Liisa Nissinen, Pilvi Riihilä, Mehdi Farshchian, Markku Kallajoki, Juha Peltonen, Sirkku Peltonen, and Veli-Matti Kähäri. 2020. "P53-Regulated Long Noncoding RNA PRECSIT Promotes Progression of Cutaneous Squamous Cell Carcinoma via STAT3 Signaling." *The American Journal of Pathology* 190(2):503–17.

Poliseno, Laura, Leonardo Salmena, Jiangwen Zhang, Brett Carver, William J. Haveman, and Pier Paolo Pandolfi. 2010. "A Coding-Independent Function of Gene and Pseudogene MRNAs Regulates Tumour Biology." *Nature* 465(7301):1033–38.

Pollock, Pamela M., Ursula L. Harper, Katherine S. Hansen, Laura M. Yudt, Mitchell Stark, Christiane M. Robbins, Tracy Y. Moses, Galen Hostetter, Urs Wagner, John Kakareka, Ghadi Salem, Tom Pohida, Peter Heenan, Paul Duray, Olli Kallioniemi, Nicholas K. Hayward, Jeffrey M. Trent, and Paul S. Meltzer. 2003. "High Frequency of BRAF Mutations in Nevi." *Nature Genetics* 33(1):19–20.

Polycarpou-Schwarz, Maria, Matthias Groß, Pieter Mestdagh, Johanna Schott, Stefanie E. Grund, Catherina Hildenbrand, Joachim Rom, Sebastian Aulmann, Hans-Peter Sinn, Jo Vandesompele, and Sven Diederichs. 2018. "The Cancer-Associated Microprotein CASIMO1 Controls Cell Proliferation and Interacts with Squalene Epoxidase Modulating Lipid Droplet Formation." *Oncogene* 37(34):4750–68.

Ponjavic, Jasmina, Chris P. Ponting, and Gerton Lunter. 2007. "Functionality or Transcriptional Noise? Evidence for Selection within Long Noncoding RNAs." *Genome Research* 17(5):556–65.

Proksch, Ehrhardt, Johanna M. Brandner, and Jens-Michael Jensen. 2008. "The Skin: An Indispensable Barrier." *Experimental Dermatology* 17(12):1063–72.

Que, Syril Keena T., Fiona O. Zwald, and Chrysalyne D. Schmults. 2018. “Cutaneous Squamous Cell Carcinoma: Incidence, Risk Factors, Diagnosis, and Staging.” *Journal of the American Academy of Dermatology* 78(2):237–47.

Rangarajan, Annapoorni, Claudio Talora, Ryuhei Okuyama, Michael Nicolas, Cristina Mammucari, Heysun Oh, Jon C. Aster, Sudhir Krishna, Daniel Metzger, Pierre Chambon, Lucio Miele, Michel Aguet, Freddy Radtke, and G. Paolo Dotto. 2001. “Notch Signaling Is a Direct Determinant of Keratinocyte Growth Arrest and Entry into Differentiation.” *The EMBO Journal* 20(13):3427–36.

Ratushny, Vladimir, Michael D. Gober, Ryan Hick, Todd W. Ridky, and John T. Seykora. 2012. “From Keratinocyte to Cancer: The Pathogenesis and Modeling of Cutaneous Squamous Cell Carcinoma.” *The Journal of Clinical Investigation* 122(2):464–72.

Rehman, I., M. Takata, Y. Y. Wu, and J. L. Rees. 1996. “Genetic Change in Actinic Keratoses.” *Oncogene* 12(12):2483–90.

Research, Center for Drug Evaluation and. 2019. “FDA Approves Cemiplimab-Rwlc for Metastatic or Locally Advanced Cutaneous Squamous Cell Carcinoma.” *FDA*.

Restifo, Nicholas P., Mark J. Smyth, and Alexander Snyder. 2016. “Acquired Resistance to Immunotherapy and Future Challenges.” *Nature Reviews. Cancer* 16(2):121–26.

Reyes-Uribe, Patricia, Maria Paz Adrianzen-Ruesta, Zhong Deng, Ileabett Echevarria-Vargas, Ilgen Mender, Steven Saheb, Qin Liu, Dario C. Altieri, Maureen E. Murphy, Jerry W. Shay, Paul M. Lieberman, and Jessie Villanueva. 2018. “Exploiting TERT Dependency as a Therapeutic Strategy for NRAS-Mutant Melanoma.” *Oncogene* 37(30):4058–72.

Riemondy, Kent, Xiao-jing Wang, Enrique C. Torchia, Dennis R. Roop, and Rui Yi. 2015. “MicroRNA-203 Represses Selection and Expansion of Oncogenic Hras Transformed Tumor Initiating Cells” edited by C. V. Dang. *ELife* 4:e07004.

Rigoutsos, Isidore. 2009. “New Tricks for Animal MicroRNAs: Targeting of Amino Acid Coding Regions at Conserved and Nonconserved Sites.” *Cancer Research* 69(8):3245–48.

Rinn, John L., Michael Kertesz, Jordon K. Wang, Sharon L. Squazzo, Xiao Xu, Samantha A. Brugmann, L. Henry Goodnough, Jill A. Helms, Peggy J. Farnham, Eran Segal, and Howard Y. Chang. 2007. “Functional Demarcation of Active and Silent Chromatin Domains in Human HOX Loci by Noncoding RNAs.” *Cell* 129(7):1311–23.

Robinson, Mark D., Davis J. McCarthy, and Gordon K. Smyth. 2010. “EdgeR: A Bioconductor Package for Differential Expression Analysis of Digital Gene Expression Data.” *Bioinformatics* 26(1):139–40.

Rogers, Howard W., Martin A. Weinstock, Steven R. Feldman, and Brett M. Coldiron. 2015. “Incidence Estimate of Nonmelanoma Skin Cancer (Keratinocyte Carcinomas) in the US Population, 2012.” *JAMA Dermatology* 151(10):1081–86.

Romano, Rose-Anne, Kirsten Smalley, Caitlin Magraw, Vanida Ann Serna, Takeshi Kurita, Srikala Raghavan, and Satrajit Sinha. 2012. “ΔNp63 Knockout Mice Reveal Its Indispensable Role as a Master Regulator of Epithelial Development and Differentiation.” *Development (Cambridge, England)* 139(4):772–82.

Rosenfeld, Nitzan, Ranit Aharonov, Eti Meiri, Shai Rosenwald, Yael Spector, Merav Zepeniuk, Hila Benjamin, Norberto Shabes, Sarit Tabak, Asaf Levy, Danit Lebanony, Yaron Goren, Erez Silberschein, Nurit Targan, Alex Ben-Ari, Shlomit Gilad, Netta Sion-Vardy, Ana Tobar, Meora Feinmesser, Oleg Kharenko, Ofer Nativ, Dvora Nass, Marina Perelman, Ady Yosepovich, Bruria

Shalmon, Sylvie Polak-Charcon, Eddie Fridman, Amir Avniel, Isaac Bentwich, Zvi Bentwich, Dalia Cohen, Ayelet Chajut, and Iris Barshack. 2008. "MicroRNAs Accurately Identify Cancer Tissue Origin." *Nature Biotechnology* 26(4):462–69.

Rowe, D. E., R. J. Carroll, and C. L. Day. 1992. "Prognostic Factors for Local Recurrence, Metastasis, and Survival Rates in Squamous Cell Carcinoma of the Skin, Ear, and Lip. Implications for Treatment Modality Selection." *Journal of the American Academy of Dermatology* 26(6):976–90.

Sachs, Alan B., and Ronald W. Davis. 1989. "The Poly(A) Binding Protein Is Required for Poly(A) Shortening and 60S Ribosomal Subunit-Dependent Translation Initiation." *Cell* 58(5):857–67.

Saini, Sharanjot, Shahana Majid, Soichiro Yamamura, Laura Tabatabai, Seong O. Suh, Varahram Shahryari, Yi Chen, Guoren Deng, Yuichiro Tanaka, and Rajvir Dahiya. 2011. "Regulatory Role of Mir-203 in Prostate Cancer Progression and Metastasis." *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 17(16):5287–98.

Sample, Ashley, and Yu-Ying He. 2018. "Mechanisms and Prevention of UV-Induced Melanoma." *Photodermatology, Photoimmunology & Photomedicine* 34(1):13–24.

Sand, Michael, Falk G. Bechara, Thilo Gambichler, Daniel Sand, Michael Bromba, Stephan A. Hahn, Eggert Stockfleth, and Schapoor Hessam. 2016. "Circular RNA Expression in Cutaneous Squamous Cell Carcinoma." *Journal of Dermatological Science* 83(3):210–18.

Sand, Michael, Falk G Bechara, Daniel Sand, Thilo Gambichler, Stephan A. Hahn, Michael Bromba, Eggert Stockfleth, and Schapoor Hessam. 2016. "Expression Profiles of Long Noncoding RNAs in Cutaneous Squamous Cell Carcinoma." *Epigenomics* 8(4):501–18.

Sandilands, Aileen, Calum Sutherland, Alan D. Irvine, and W. H. Irwin McLean. 2009. "Filaggrin in the Frontline: Role in Skin Barrier Function and Disease." *Journal of Cell Science* 122(9):1285–94.

Sandru, A., S. Voinea, E. Panaitescu, and A. Blidaru. 2014. "Survival Rates of Patients with Metastatic Malignant Melanoma." *Journal of Medicine and Life* 7(4):572–76.

Sarin, Kavita Y., Yuan Lin, Roxana Daneshjou, Andrey Ziyatdinov, Gudmar Thorleifsson, Adam Rubin, Luba M. Pardo, Wenting Wu, Paul A. Khavari, Andre Uitterlinden, Tamar Nijsten, Amanda E. Toland, Jon H. Olafsson, Bardur Sigurgeirsson, Kristin Thorisdottir, Eric Jorgensen, Alice S. Whittemore, Peter Kraft, Simon N. Stacey, Kari Stefansson, Maryam M. Asgari, and Jiali Han. 2020. "Genome-Wide Meta-Analysis Identifies Eight New Susceptibility Loci for Cutaneous Squamous Cell Carcinoma." *Nature Communications* 11(1):820.

Schepeler, Troels, Mahalia E. Page, and Kim B. Jensen. 2014. "Heterogeneity and Plasticity of Epidermal Stem Cells." *Development* 141(13):2559–67.

Schmitt, J., A. Seidler, T. L. Diepgen, and A. Bauer. 2011. "Occupational Ultraviolet Light Exposure Increases the Risk for the Development of Cutaneous Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis." *British Journal of Dermatology* 164(2):291–307.

Schmuth, Matthias, Joachim W. Fluhr, Debra C. Crumrine, Yoshikazu Uchida, Jean-Pierre Hachem, Martin Behne, David G. Moskowitz, Angela M. Christiano, Kenneth R. Feingold, and Peter M. Elias. 2004. "Structural and Functional Consequences of Loricrin Mutations in Human Loricrin Keratoderma (Vohwinkel Syndrome with Ichthyosis)." *Journal of Investigative Dermatology* 122(4):909–22.

Scholl, Florence A., Phillip A. Dumesic, and Paul A. Khavari. 2004. “Mek1 Alters Epidermal Growth and Differentiation.” *Cancer Research* 64(17):6035–40.

Schultz, Julia, Peter Lorenz, Gerd Gross, Saleh Ibrahim, and Manfred Kunz. 2008. “MicroRNA Let-7b Targets Important Cell Cycle Molecules in Malignant Melanoma Cells and Interferes with Anchorage-Independent Growth.” *Cell Research* 18(5):549–57.

Scott, R. E., M. S. Wilke, J. J. Wille, M. R. Pittelkow, B. M. Hsu, and J. L. Kasperbauer. 1988. “Human Squamous Carcinoma Cells Express Complex Defects in the Control of Proliferation and Differentiation.” *The American Journal of Pathology* 133(2):374–80.

Segre, J. A., C. Bauer, and E. Fuchs. 1999. “Klf4 Is a Transcription Factor Required for Establishing the Barrier Function of the Skin.” *Nature Genetics* 22(4):356–60.

Sen, George L., Lisa D. Boxer, Dan E. Webster, Rose T. Bussat, Kun Qu, Brian J. Zarnegar, Danielle Johnston, Zurab Siprashvili, and Paul A. Khavari. 2012. “ZNF750 Is a P63 Target Gene That Induces KLF4 to Drive Terminal Epidermal Differentiation.” *Developmental Cell* 22(3):669–77.

Shain, A. Hunter, and Boris C. Bastian. 2016. “From Melanocytes to Melanomas.” *Nature Reviews Cancer* 16(6):345–58.

Shain, A. Hunter, Nancy M. Joseph, Richard Yu, Jamal Benhamida, Shanshan Liu, Tarl Prow, Beth Ruben, Jeffrey North, Laura Pincus, Iwei Yeh, Robert Judson, and Boris C. Bastian. 2018. “Genomic and Transcriptomic Analysis Reveals Incremental Disruption of Key Signaling Pathways during Melanoma Evolution.” *Cancer Cell* 34(1):45-55.e4.

Shain, A. Hunter, Iwei Yeh, Ivanka Kovalyshyn, Aravindhan Sriharan, Eric Talevich, Alexander Gagnon, Reinhard Dummer, Jeffrey North, Laura Pincus, Beth Ruben, William Rickaby, Corrado D’Arrigo, Alistair Robson, and Boris C. Bastian. 2015. “The Genetic Evolution of Melanoma from Precursor Lesions.” *New England Journal of Medicine* 373(20):1926–36.

Shang, Qingfeng, Zhi Yang, Renbing Jia, and Shengfang Ge. 2019. “The Novel Roles of CircRNAs in Human Cancer.” *Molecular Cancer* 18(1):6.

SHEN, YANYUN, JINHUA XU, JIN JIN, HUI TANG, and JUN LIANG. 2014. “Cyclin D1 Expression in Bowen’s Disease and Cutaneous Squamous Cell Carcinoma.” *Molecular and Clinical Oncology* 2(4):545–48.

Sherwood, Victoria, and Irene M. Leigh. 2016. “WNT Signaling in Cutaneous Squamous Cell Carcinoma: A Future Treatment Strategy?” *Journal of Investigative Dermatology* 136(9):1760–67.

Shi, Liang, Peijian Yan, Yuelong Liang, Yin Sun, Jiliang Shen, Senjun Zhou, Hui Lin, Xiao Liang, and Xiujun Cai. 2017. “Circular RNA Expression Is Suppressed by Androgen Receptor (AR)-Regulated Adenosine Deaminase That Acts on RNA (ADAR1) in Human Hepatocellular Carcinoma.” *Cell Death & Disease* 8(11):e3171.

Shin, V. Y., J. M. Siu, I. Cheuk, E. K. O. Ng, and A. Kwong. 2015. “Circulating Cell-Free MiRNAs as Biomarker for Triple-Negative Breast Cancer.” *British Journal of Cancer* 112(11):1751–59.

Shirley, Stephanie H., Victoria R. Greene, Lyn M. Duncan, Carlos A. Torres Cabala, Elizabeth A. Grimm, and Donna F. Kusewitt. 2012. “Slug Expression during Melanoma Progression.” *The American Journal of Pathology* 180(6):2479–89.

Si, M. L., S. Zhu, H. Wu, Z. Lu, F. Wu, and Y. Y. Mo. 2007. “MiR-21 -Mediated Tumor Growth.” *Oncogene* 26(19):2799–2803.

Simpson, Cory L., Dipal M. Patel, and Kathleen J. Green. 2011. “Deconstructing the Skin: Cytoarchitectural Determinants of Epidermal Morphogenesis.” *Nature Reviews Molecular Cell Biology* 12(9):565–80.

Sonkoly, E., J. Lovén, N. Xu, F. Meisgen, T. Wei, P. Brodin, V. Jaks, M. Kasper, T. Shimokawa, M. Harada, J. Heilborn, M. A. Hedblad, A. Hippe, D. Grandér, B. Homey, P. G. Zaphiropoulos, M. Arsenian-Henriksson, M. Ståhle, and A. Pivarcsi. 2012. “MicroRNA-203 Functions as a Tumor Suppressor in Basal Cell Carcinoma.” *Oncogenesis* 1(3):e3–e3.

Sonkoly, Eniko, Zsuzsanna Bata-Csorgo, Andor Pivarcsi, Hilda Polyanka, Anna Kenderessy-Szabo, Gergely Molnar, Karoly Szentpali, Lilla Bari, Klara Megyeri, Yvette Mandi, Attila Dobozy, Lajos Kemeny, and Marta Szell. 2005. “Identification and Characterization of a Novel, Psoriasis Susceptibility-Related Noncoding RNA Gene, PRINS.” *Journal of Biological Chemistry* 280(25):24159–67.

Sonkoly, Enikö, Tianling Wei, Elizabeth Pavez Loriè, Hiroyuki Suzuki, Mitsuyasu Kato, Hans Törmä, Mona Ståhle, and Andor Pivarcsi. 2010. “Protein Kinase C-Dependent Upregulation of MiR-203 Induces the Differentiation of Human Keratinocytes.” *Journal of Investigative Dermatology* 130(1):124–34.

South, Andrew P., Karin J. Purdie, Stephen A. Watt, Sam Haldenby, Nicoline den Breems, Michelle Dimon, Sarah T. Arron, Michael J. Kluk, Jon C. Aster, Angela McHugh, Dylan J. Xue, Jasbani HS Dayal, Kim S. Robinson, SM Hasan Rizvi, Charlotte M. Proby, Catherine A. Harwood, and Irene M. Leigh. 2014. “NOTCH1 Mutations Occur Early during Cutaneous Squamous Cell Carcinogenesis.” *The Journal of Investigative Dermatology* 134(10):2630–38.

Spector, David L. 2001. “Nuclear Domains.” *Journal of Cell Science* 114(16):2891–93.

Stahlhut, Carlos, and Frank J. Slack. 2015. “Combinatorial Action of MicroRNAs Let-7 and MiR-34 Effectively Synergizes with Erlotinib to Suppress Non-Small Cell Lung Cancer Cell Proliferation.” *Cell Cycle* 14(13):2171–80.

Stark, Mitchell S., Kerenaftali Klein, Benjamin Weide, Lauren E. Haydu, Annette Pflugfelder, Yue Hang Tang, Jane M. Palmer, David C. Whiteman, Richard A. Scolyer, Graham J. Mann, John F. Thompson, Georgina V. Long, Andrew P. Barbour, H. Peter Soyer, Claus Garbe, Adrian Herington, Pamela M. Pollock, and Nicholas K. Hayward. 2015. “The Prognostic and Predictive Value of Melanoma-Related MicroRNAs Using Tissue and Serum: A MicroRNA Expression Analysis.” *EBioMedicine* 2(7):671–80.

Stenman, Caroline, Helena Gonzalez, Martin Gillstedt, Göran Dellgren, Bengt Hasséus, Erik Holmberg, Helena Rexius, Jenny Öhman, and John Paoli. 2018. “Degree of Differentiation of Cutaneous Squamous Cell Carcinoma: A Comparison between a Swedish Cohort of Organ Transplant Recipients and Immunocompetent Patients.” *Dermatology Practical & Conceptual* 8(4):330–36.

Struhl, Kevin. 2007. “Transcriptional Noise and the Fidelity of Initiation by RNA Polymerase II.” *Nature Structural & Molecular Biology* 14(2):103–5.

Sullivan, Ryan J., Jeffrey S. Weber, Sapna P. Patel, Reinhard Dummer, Matteo S. Carlino, Daniel Shao-Weng Tan, Celeste Lebbe, Salvatore Siena, Elena Élez, Lance Wollenberg, Michael Pickard, Victor Sandor, and Paolo A. Ascierto. 2020. “A Phase 1b/2 Study of the BRAF Inhibitor Encorafenib plus the MEK Inhibitor Binimetinib in Patients with BRAF V600E/K-Mutant Solid Tumors.” *Clinical Cancer Research* clincanres.3550.2019.

Sun, Qinyu, Qinyu Hao, and Kannanganattu V. Prasanth. 2018. “Nuclear Long Noncoding RNAs: Key Regulators of Gene Expression.” *Trends in Genetics* 34(2):142–57.

Suzuki, Hiromu, Reo Maruyama, Eiichiro Yamamoto, and Masahiro Kai. 2012. “DNA Methylation and MicroRNA Dysregulation in Cancer.” *Molecular Oncology* 6(6):567–78.

Syed, Zanobia A., Weihong Yin, Kendall Hughes, Jennifer N. Gill, Runhua Shi, and John L. Clifford. 2011. “HGF/c-Met/Stat3 Signaling during Skin Tumor Cell Invasion: Indications for a Positive Feedback Loop.” *BMC Cancer* 11(1):180.

Taguchi, Shijima, Yasuhiro Kawachi, Yosuke Ishitsuka, Yasuhiro Fujisawa, Junichi Furuta, Yasuhiro Nakamura, Xuezhu Xu, Dai Ikebe, Mitsuyasu Kato, and Fujio Otsuka. 2011. “Overexpression of the Transcription Factor Yin-Yang-1 Suppresses Differentiation of HaCaT Cells in Three-Dimensional Cell Culture.” *Journal of Investigative Dermatology* 131(1):37–45.

Thompson, Agnieszka K., Benjamin F. Kelley, Larry J. Prokop, M. Hassan Murad, and Christian L. Baum. 2016. “Risk Factors for Cutaneous Squamous Cell Carcinoma Outcomes: A Systematic Review and Meta-Analysis.” *JAMA Dermatology* 152(4):419–28.

Timis, Teodora Larisa, and Remus Ioan Orasan. 2018. “Understanding Psoriasis: Role of MiRNAs (Review).” *Biomedical Reports* 9(5):367–74.

Toll, A., R. Salgado, M. Yébenes, G. Martín-Ezquerra, M. Gilaberte, T. Baró, F. Solé, F. Alameda, B. Espinet, and R. M. Pujol. 2009. “MYC Gene Numerical Aberrations in Actinic Keratosis and Cutaneous Squamous Cell Carcinoma.” *British Journal of Dermatology* 161(5):1112–18.

Torarinsson, Elfar, Milena Sawera, Jakob H. Havgaard, Merete Fredholm, and Jan Gorodkin. 2006. “Thousands of Corresponding Human and Mouse Genomic Regions Unalignable in Primary Sequence Contain Common RNA Structure.” *Genome Research* 16(7):885–89.

Toufighi, Kiana, Jae-Seong Yang, Nuno Miguel Luis, Salvador Aznar Benitah, Ben Lehner, Luis Serrano, and Christina Kiel. 2015. “Dissecting the Calcium-Induced Differentiation of Human Primary Keratinocytes Stem Cells by Integrative and Structural Network Analyses.” *PLOS Computational Biology* 11(5):e1004256.

Trapnell, Cole, Adam Roberts, Loyal Goff, Geo Pertea, Daehwan Kim, David R. Kelley, Harold Pimentel, Steven L. Salzberg, John L. Rinn, and Lior Pachter. 2012. “Differential Gene and Transcript Expression Analysis of RNA-Seq Experiments with TopHat and Cufflinks.” *Nature Protocols* 7(3):562–78.

Tripathi, Vidisha, Jonathan D. Ellis, Zhen Shen, David Y. Song, Qun Pan, Andrew T. Watt, Susan M. Freier, C. Frank Bennett, Alok Sharma, Paula A. Bubulya, Benjamin J. Blencowe, Supriya G. Prasanth, and Kannanganattu V. Prasanth. 2010. “The Nuclear-Retained Noncoding RNA MALAT1 Regulates Alternative Splicing by Modulating SR Splicing Factor Phosphorylation.” *Molecular Cell* 39(6):925–38.

Truong, Amy B., and Paul A. Khavari. 2007. “Control of Keratinocyte Proliferation and Differentiation by P63.” *Cell Cycle* 6(3):295–99.

Tsai, Miao-Chih, Ohad Manor, Yue Wan, Nima Mosammaparast, Jordon K. Wang, Fei Lan, Yang Shi, Eran Segal, and Howard Y. Chang. 2010. “Long Noncoding RNA as Modular Scaffold of Histone Modification Complexes.” *Science (New York, N.Y.)* 329(5992):689–93.

Tsoi, Lam C., Matthew K. Iyer, Philip E. Stuart, William R. Swindell, Johann E. Gudjonsson, Trilokraj Tejasvi, Mrinal K. Sarkar, Bingshan Li, Jun Ding, John J. Voorhees, Hyun M. Kang, Rajan P. Nair, Arul M. Chinnaiyan, Goncalo R. Abecasis, and James T. Elder. 2015. “Analysis of Long

Non-Coding RNAs Highlights Tissue-Specific Expression Patterns and Epigenetic Profiles in Normal and Psoriatic Skin.” *Genome Biology* 16(1):24.

Tu, Chia-Ling, and Daniel D. Bikle. 2013. “Role of the Calcium-Sensing Receptor in Calcium Regulation of Epidermal Differentiation and Function.” *Best Practice & Research. Clinical Endocrinology & Metabolism* 27(3):415–27.

Tuong, Zewen K., Andrew Lewandowski, Jennifer A. Bridge, Jazmina L. G. Cruz, Miko Yamada, Duncan Lambie, Richard Lewandowski, Raymond J. Steptoe, Graham R. Leggatt, Fiona Simpson, Ian H. Frazer, H. Peter Soyer, and James W. Wells. 2019. “Cytokine/Chemokine Profiles in Squamous Cell Carcinoma Correlate with Precancerous and Cancerous Disease Stage.” *Scientific Reports* 9(1):17754.

Van Roosbroeck, Katrien, and George A. Calin. 2016. “MicroRNAs in Chronic Lymphocytic Leukemia: MiRacle or MiRage for Prognosis and Targeted Therapies?” *Seminars in Oncology* 43(2):209–14.

Varrone, Francesca, and Emilia Caputo. 2020. “The MiRNAs Role in Melanoma and in Its Resistance to Therapy.” *International Journal of Molecular Sciences* 21(3).

Wang, Aoxue, Ning Xu Landén, Florian Meisgen, Warangkana Lohcharoenkal, Mona Ståhle, Enikö Sonkoly, and Andor Pivarcsi. 2014. “MicroRNA-31 Is Overexpressed in Cutaneous Squamous Cell Carcinoma and Regulates Cell Motility and Colony Formation Ability of Tumor Cells.” *PLOS ONE* 9(7):e103206.

Wang, Dongmei, Zhaojie Zhang, Evan O’Loughlin, Li Wang, Xiying Fan, Eric C. Lai, and Rui Yi. 2013. “MicroRNA-205 Controls Neonatal Expansion of Skin Stem Cells by Modulating the PI3K Pathway.” *Nature Cell Biology* 15(10):1153–63.

Wang, Hao, Ran Peng, Junjie Wang, Zelian Qin, and Lixiang Xue. 2018. “Circulating MicroRNAs as Potential Cancer Biomarkers: The Advantage and Disadvantage.” *Clinical Epigenetics* 10(1):59.

Wang, Nan, Hongwei Liang, Yong Zhou, Chen Wang, Suyang Zhang, Yi Pan, Yanbo Wang, Xin Yan, Junfeng Zhang, Chen-Yu Zhang, Ke Zen, Donghai Li, and Xi Chen. 2014. “MiR-203 Suppresses the Proliferation and Migration and Promotes the Apoptosis of Lung Cancer Cells by Targeting SRC.” *PLOS ONE* 9(8):e105570.

Wang, Xue, Hongfei Yu, Wenjie Sun, Jianlu Kong, Lei Zhang, Jinlong Tang, Jingyu Wang, Enping Xu, Maode Lai, and Honghe Zhang. 2018. “The Long Non-Coding RNA CYTOR Drives Colorectal Cancer Progression by Interacting with NCL and Sam68.” *Molecular Cancer* 17(1):110.

Waterson, Robert H., Eric S. Lander, Richard K. Wilson, and The Chimpanzee Sequencing and Analysis Consortium. 2005. “Initial Sequence of the Chimpanzee Genome and Comparison with the Human Genome.” *Nature* 437(7055):69–87.

Watt, Fiona M., Soline Estrach, and Carrie A. Ambler. 2008. “Epidermal Notch Signalling: Differentiation, Cancer and Adhesion.” *Current Opinion in Cell Biology* 20(2):171–79.

Webster, Michael W., Ying-Hsin Chen, James A. W. Stowell, Najwa Alhusaini, Thomas Sweet, Brenton R. Graveley, Jeff Coller, and Lori A. Passmore. 2018. “MRNA Deadenylation Is Coupled to Translation Rates by the Differential Activities of Ccr4-Not Nucleases.” *Molecular Cell* 70(6):1089-1100.e8.

Wei, Tianling, Kyriakos Orfanidis, Ning Xu, Peter Janson, Mona Ståhle, Andor Pivarcsi, and Enikö Sonkoly. 2010. “The Expression of MicroRNA-203 during Human Skin Morphogenesis.” *Experimental Dermatology* 19(9):854–56.

Weintraub, Abraham S., Charles H. Li, Alicia V. Zamudio, Alla A. Sigova, Nancy M. Hannett, Daniel S. Day, Brian J. Abraham, Malkiel A. Cohen, Behnam Nabet, Dennis L. Buckley, Yang Eric Guo, Denes Hnisz, Rudolf Jaenisch, James E. Bradner, Nathanael S. Gray, and Richard A. Young. 2017. “YY1 Is a Structural Regulator of Enhancer-Promoter Loops.” *Cell* 171(7):1573-1588.e28.

Welter, Jean F., James F. Crish, Chapla Agarwal, and Richard L. Eckert. 1995a. “Fos-Related Antigen (Fra-1), JunB, and JunD Activate Human Involucrin Promoter Transcription by Binding to Proximal and Distal AP1 Sites to Mediate Phorbol Ester Effects on Promoter Activity.” *Journal of Biological Chemistry* 270(21):12614–22.

Welter, Jean F., James F. Crish, Chapla Agarwal, and Richard L. Eckert. 1995b. “Fos-Related Antigen (Fra-1), JunB, and JunD Activate Human Involucrin Promoter Transcription by Binding to Proximal and Distal AP1 Sites to Mediate Phorbol Ester Effects on Promoter Activity.” *Journal of Biological Chemistry* 270(21):12614–22.

Wiggins, Jason F., Lynnsie Ruffino, Kevin Kelnar, Michael Omotola, Lubna Patrawala, David Brown, and Andreas G. Bader. 2010. “Development of a Lung Cancer Therapeutic Based on the Tumor Suppressor MicroRNA-34.” *Cancer Research* 70(14):5923–30.

Wilkinson, Frank H., Kyoungsook Park, and Michael L. Atchison. 2006. “Polycomb Recruitment to DNA in Vivo by the YY1 REPO Domain.” *Proceedings of the National Academy of Sciences* 103(51):19296–301.

Wilusz, Jeremy E., Hongjae Sunwoo, and David L. Spector. 2009. “Long Noncoding RNAs: Functional Surprises from the RNA World.” *Genes & Development* 23(13):1494–1504.

Wouters, Jasper, Miguel Vizoso, Anna Martinez-Cardus, F. Javier Carmona, Olivier Govaere, Teresa Laguna, Jesuchristopher Joseph, Peter Dynoodt, Claudia Aura, Mona Foth, Roy Cloots, Karin van den Hurk, Balazs Balint, Ian G. Murphy, Enda W. McDermott, Kieran Sheahan, Karin Jirström, Bjorn Nodin, Girish Mallya-Udupi, Joost J. van den Oord, William M. Gallagher, and Manel Esteller. 2017. “Comprehensive DNA Methylation Study Identifies Novel Progression-Related and Prognostic Markers for Cutaneous Melanoma.” *BMC Medicine* 15(1):101.

Wu, Xiang-Song, Fang Wang, Huai-Feng Li, Yun-Ping Hu, Lin Jiang, Fei Zhang, Mao-Lan Li, Xu-An Wang, Yun-Peng Jin, Yi-Jian Zhang, Wei Lu, Wen-Guang Wu, Yi-Jun Shu, Hao Weng, Yang Cao, Run-Fa Bao, Hai-Bin Liang, Zheng Wang, Yi-Chi Zhang, Wei Gong, Lei Zheng, Shu-Han Sun, and Ying-Bin Liu. 2017. “LncRNA-PAGBC Acts as a MicroRNA Sponge and Promotes Gallbladder Tumorigenesis.” *EMBO Reports* 18(10):1837–53.

Xiang, Fan, Robyn Lucas, Simon Hales, and Rachel Neale. 2014. “Incidence of Nonmelanoma Skin Cancer in Relation to Ambient UV Radiation in White Populations, 1978-2012: Empirical Relationships.” *JAMA Dermatology* 150(10):1063–71.

Xu, Ning, Lingyun Zhang, Florian Meisgen, Masako Harada, Johan Heilborn, Bernhard Homey, Dan Grandér, Mona Ståhle, Enikö Sonkoly, and Andor Pivarcsi. 2012. “MicroRNA-125b Down-Regulates Matrix Metallopeptidase 13 and Inhibits Cutaneous Squamous Cell Carcinoma Cell Proliferation, Migration, and Invasion.” *Journal of Biological Chemistry* 287(35):29899–908.

Xu, Xuezhu, Yasuhiro Kawachi, Yasuhiro Nakamura, Hideko Sakurai, Ayako Hirota, Tomohiro Banno, Takenori Takahashi, Dennis R. Roop, and Fujio Otsuka. 2004. “Yin-Yang 1 Negatively Regulates the Differentiation-Specific Transcription of Mouse Loricrin Gene in Undifferentiated Keratinocytes.” *The Journal of Investigative Dermatology* 123(6):1120–26.

Yang, Yibing, Xinya Gao, Maolei Zhang, Sheng Yan, Chengjun Sun, Feizhe Xiao, Nunu Huang, Xuesong Yang, Kun Zhao, Huangkai Zhou, Suyun Huang, Bo Xie, and Nu Zhang. 2018. “Novel

Role of FBXW7 Circular RNA in Repressing Glioma Tumorigenesis." *JNCI: Journal of the National Cancer Institute* 110(3):304–15.

Yanofsky, Valerie R., Stephen E. Mercer, and Robert G. Phelps. 2011. "Histopathological Variants of Cutaneous Squamous Cell Carcinoma: A Review." *Journal of Skin Cancer* 2011.

Yi, Rui, Matthew N. Poy, Markus Stoffel, and Elaine Fuchs. 2008. "A Skin MicroRNA Promotes Differentiation by Repressing 'Stemness.'" *Nature* 452(7184):225–29.

Yokoi, Akira, Juntaro Matsuzaki, Yusuke Yamamoto, Yutaka Yoneoka, Kenta Takahashi, Hanako Shimizu, Takashi Uehara, Mitsuya Ishikawa, Shun-ichi Ikeda, Takumi Sonoda, Junpei Kawauchi, Satoko Takizawa, Yoshiaki Aoki, Shumpei Niida, Hiromi Sakamoto, Ken Kato, Tomoyasu Kato, and Takahiro Ochiya. 2018. "Integrated Extracellular MicroRNA Profiling for Ovarian Cancer Screening." *Nature Communications* 9(1):4319.

Yoo, Eung Jae, Nancy E. Cooke, and Stephen A. Liebhaber. 2012. "An RNA-Independent Linkage of Noncoding Transcription to Long-Range Enhancer Function." *Molecular and Cellular Biology* 32(10):2020–29.

Yoon, Je-Hyun, Kotb Abdelmohsen, Subramanya Srikantan, Xiaoling Yang, Jennifer L. Martindale, Supriyo De, Maite Huarte, Ming Zhan, Kevin G. Becker, and Myriam Gorospe. 2012. "LincRNA-P21 Suppresses Target MRNA Translation." *Molecular Cell* 47(4):648–55.

Young, Christina A., Ellen A. Rorke, Gautam Adhikary, Wen Xu, and Richard L. Eckert. 2017. "Loss of Epidermal AP1 Transcription Factor Function Reduces Filaggrin Level, Alters Chemokine Expression and Produces an Ichthyosis-Related Phenotype." *Cell Death & Disease* 8(6):e2840–e2840.

Yu, Chun-Ying, Tung-Cheng Li, Yi-Ying Wu, Chan-Hsien Yeh, Wei Chiang, Ching-Yu Chuang, and Hung-Chih Kuo. 2017. "The Circular RNA CircBIRC6 Participates in the Molecular Circuitry Controlling Human Pluripotency." *Nature Communications* 8(1):1149.

Yue, Ming, Jue Jiang, Peng Gao, Hudan Liu, and Guoliang Qing. 2017. "Oncogenic MYC Activates a Feedforward Regulatory Loop Promoting Essential Amino Acid Metabolism and Tumorigenesis." *Cell Reports* 21(13):3819–32.

Zenz, Rainer, Harald Scheuch, Paul Martin, Carsten Frank, Robert Eferl, Lukas Kenner, Maria Sibilia, and Erwin F. Wagner. 2003. "C-Jun Regulates Eyelid Closure and Skin Tumor Development through EGFR Signaling." *Developmental Cell* 4(6):879–89.

Zhang, Hao, Guangchao Wang, Chen Ding, Peng Liu, Renkai Wang, Wenbin Ding, Dake Tong, Dajiang Wu, Cheng Li, Qiang Wei, Xin Zhang, Di Li, Peizhao Liu, Haochen Cui, Hao Tang, and Fang Ji. 2017. "Increased Circular RNA UBAP2 Acts as a Sponge of MiR-143 to Promote Osteosarcoma Progression." *Oncotarget* 8(37):61687–97.

Zhang, Lin, Jia Huang, Nuo Yang, Joel Greshock, Molly S. Megraw, Antonis Giannakakis, Shun Liang, Tara L. Naylor, Andrea Barchetti, Michelle R. Ward, George Yao, Angelica Medina, Ann O'brien-Jenkins, Dionyssios Katsaros, Artemis Hatzigeorgiou, Phyllis A. Gimotty, Barbara L. Weber, and George Coukos. 2006. "MicroRNAs Exhibit High Frequency Genomic Alterations in Human Cancer." *Proceedings of the National Academy of Sciences of the United States of America* 103(24):9136–41.

Zhang, Lu, Yi Liao, and Liling Tang. 2019. "MicroRNA-34 Family: A Potential Tumor Suppressor and Therapeutic Candidate in Cancer." *Journal of Experimental & Clinical Cancer Research* 38(1):53.

Zhang, Qiao, Ajay A. Vashisht, Jason O'Rourke, Stéphane Y. Corbel, Rita Moran, Angelica Romero, Loren Miraglia, Jia Zhang, Eric Durrant, Christian Schmedt, Srinath C. Sampath, and Srihari C. Sampath. 2017. "The Microprotein Minion Controls Cell Fusion and Muscle Formation." *Nature Communications* 8.

Zhao, Jing, Bryan K. Sun, Jennifer A. Erwin, Ji-Joon Song, and Jeannie T. Lee. 2008. "Polycomb Proteins Targeted by a Short Repeat RNA to the Mouse X Chromosome." *Science* 322(5902):750–56.

Zhao, Zhao, Mei Ji, Qianqing Wang, Nannan He, and Yue Li. 2019. "Circular RNA Cdr1as Upregulates SCAI to Suppress Cisplatin Resistance in Ovarian Cancer via MiR-1270 Suppression." *Molecular Therapy - Nucleic Acids* 18:24–33.

Zhu, Xiangyu, Kejun Er, Caiying Mao, Qi Yan, Haijun Xu, Yuanbei Zhang, Jianhong Zhu, Fang Cui, Wenxia Zhao, and Hong Shi. 2013. "MiR-203 Suppresses Tumor Growth and Angiogenesis by Targeting VEGFA in Cervical Cancer." *Cellular Physiology and Biochemistry* 32(1):64–73.

Ziegler, Christian, Johannes Graf, Stefan Faderl, Jessica Schedlbauer, Nicholas Strieder, Bianca Förstl, Rainer Spang, Astrid Bruckmann, Rainer Merkl, Sonja Hombach, and Markus Kretz. 2019. "The Long Non-Coding RNA LINC00941 and SPRR5 Are Novel Regulators of Human Epidermal Homeostasis." *EMBO Reports* 20(2):e46612.