View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

necessarily represent the official views of the NIAMS or NIH.

REFERENCES

Bernink JH, Peters CP, Munneke M *et al.* (2013) Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat Immunol* 14:221–9

Chang Y-J, Kim H, Albacker L *et al.* (2011) Innate lymphoid cells mediate influenza-induced airway hyperreactivity independent of adaptive immunity. *Nat Immunol* 12:631–8

Coccia M, Harrison OJ, Schiering C *et al.* (2012) IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells. *J Exp Med* 209:1595–609

Gudjonsson JE, Thorarinsson AM, Sigurgeirsson B *et al.* (2003) Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study. *Br J Dermatol* 149:530–4

Hedrick MN, Lonsdorf AS, Shirakawa AK *et al.* (2009) CCR6 is required for IL-23-induced psoriasis-like inflammation in mice. *J Clin Invest* 119:2317–29

Kim H, Lee H, Chang Y-J *et al.* (2014) IL-17 producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. *Nat Med* 20:54–61

Neill DR, Wong SH, Bellosi A *et al.* (2010) Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 464:1367–70

Pantelyushin S, Haak S, Ingold B *et al.* (2012) Rorgammat+ innate lymphocytes and gammadelta T cells initiate psoriasiform plaque formation in mice. *J Clin Invest* 122:2252–6

Powell N, Walker AW, Stolarczyk E *et al.* (2012) The transcription factor T-bet regulates intestinal inflammation mediated by interleukin-7 receptor+ innate lymphoid cells. *Immunity* 37:674–84

Salimi M, Barlow JL, Saunders SP *et al.* (2013) A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med* 210:2939–50

Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H *et al.* (2013) The association of sore throat and psoriasis might be explained by histologically distinctive tonsils and increased expression of skin-homing molecules by tonsil T cells. *Clin Exp Immunol* 174:139–51

Teunissen M, Munneke M, Bernink J *et al.* (2014) Composition of innate lymphoid cell subsets in the human skin: Enrichment of NCR+ ILC3 in lesional skin and blood of psoriasis patients. *J Invest Dermatol* 134:2351–60

Villanova F, Flutter B, Tosi I *et al.* (2014) Characterization of innate lymphoid cells in human skin and blood demonstrates increase of NKp44+ ILC3 in psoriasis. *J Invest Dermatol* 134:984–91

Zaba LC, Suarez-Farinas M, Fuentes-Duculan J *et al.* (2009) Effective treatment of psoriasis with etanercept is linked to suppression of IL-17 signaling, not immediate response TNF genes. *J Allergy Clin Immunol* 124:1022–30

See related article on pg 2371

Gene Regulation at a Distance: Higher-Order Chromatin Folding and the Coordinated Control of Gene Transcription at the Epidermal Differentiation Complex Locus

Michael Y. Fessing[1]

Chromatin structure and spatial interactions between proximal and distal gene regulatory elements, including gene core promoters and enhancers, are important in the control of gene transcription. In this issue, Oh *et al.* characterized an AP-1-dependent enhancer at the epidermal differentiation complex locus that establishes spatial interactions with numerous gene promoter regions at that locus.

Journal of Investigative Dermatology (2014) **134,** 2307–2310. doi:10.1038/jid.2014.247

[1]*Centre for Skin Sciences and School of Medical Sciences, School of Life Sciences, University of Bradford, Bradford, UK*

Correspondence: Michael Y. Fessing, Centre for Skin Sciences and School of Medical Sciences, School of Life Sciences, University of Bradford, Bradford BD7 1DP, UK. E-mail: m.fessing@bradford.ac.uk

All somatic cells in a multicellular organism share identical DNA content, but demonstrate vastly different phenotypes, as is required for tissue and organ development and homeostasis. Such differences are based on tissue-specific programs of gene expression established in multi-potent progenitor cells and their differentiating progenies. In mammals, gene expression programs are controlled at multiple levels, including gene transcription. Transcription of protein-coding and the majority of protein noncoding genes is regulated by binding of and interactions between numerous regulatory proteins, RNA polymerase II, and proximal and distal gene *cis*-regulatory elements. The best studied type of functional interaction between *cis*-regulatory elements is spatial contact between the gene core promoter and enhancer regions, which may be separated by hundreds of kilobase pairs, or they could even be located on different chromosomes (Bulger and Groudine, 2011; Harmston and Lenhard, 2013). Because DNA is organized in a nuclear–protein complex called chromatin, chromatin structural remodeling is required to control protein binding to the regulatory regions and to establish contact between the distal and proximal gene regulatory elements (Rando and Chang, 2009).

Proper higher-order chromatin folding in the three-dimensional (3D) nuclear space is important in establishing functional interactions between the promoters and enhancers involved in controlling cell-type–specific gene transcription for numerous genes, including Shh, as well as the genes that constitute the β-globin, α-globin, and Hox gene loci (Bulger and Groudine, 2011; Gibcus and Dekker, 2013). Genome-wide association studies have demonstrated that many single-nucleotide polymorphisms identified in the intergenic regions are associated with human diseases, suggesting that such defects might perturb normal gene expression programs by affecting distal gene *cis*-regulatory elements (Maurano *et al.*, 2012).

Functionally related and coregulated genes often form multi-gene loci in mammalian genomes. Genes involved in execution of keratinocyte-specific gene expression programs are clustered in at least three evolutionally conserved

Clinical Implications

- Many genetic defects associated with human disease are located in noncoding regions, and they involve gene enhancers and other distal gene regulatory elements.
- Characterization of the 923 enhancer and its target genes at the epidermal differentiation complex locus will provide new tools to understand genetic defects associated with skin disease.
- Combining new high-throughput methodological approaches to study correlations between genetic variations, gene expression programmes, and genome-wide chromatin structural states in defined populations of the cutaneous epithelial cells will provide new insight into the etiologies of skin disease.

regions, including the epidermal differentiation complex (EDC) and Keratin type I and type II loci. However, molecular mechanisms involved in coordinated gene regulation at these loci remain largely unknown. In this issue, Guzman-Strong and her team report on the characterization of the epidermis-specific enhancer 923 within the EDC locus (Oh *et al.*, 2014). They show that this enhancer has tissue-specific activity in transgenic mice and that it is active in both proliferating and differentiating murine keratinocytes in culture. Using chromatin conformation capture (3C) technology they demonstrate that this enhancer forms spatial interactions with the promoter regions of many genes within the EDC in primary keratinocyte cultures and that some, but not all, of these contacts depend on culture conditions (proliferative versus differentiating). Finally, their data reveal that binding of the AP-1 transcription factor to the enhancer region is important for enhancer element activity and establishment of some, but not all, spatial contacts with gene promoters.

Regulation of gene transcription by enhancers

Most regulatory information required for spatial and temporal control of gene transcription programs in metazoans is located outside core promoters within distal gene regulatory elements. Among these regulatory elements, the best studied are enhancers, which are DNA regions enriched in binding of transcription factors and chromatin remodelers that can increase transcription rates of target genes in a manner that is independent from distance to and orientation of the targeted promoter.

Several relatively common genetic and epigenetic features of enhancers allow their identification in the genome. They are frequently located in the noncoding elements that are highly conserved across different mammalian species (Bulger and Groudine, 2011; Harmston and Lenhard, 2013). This feature was used initially to identify the 923 enhancer characterized by Guzman-Strong's team (Oh *et al.*, 2014). Functional enhancers are usually located in the DNase I hypersensitive regions of chromatin because of the binding of multiple nonhistone proteins. In paused and active enhancers, there is a high level of mono-methylated lysine 4 in the histone H3 tail (H3K4me1), whereas active enhancers also show high levels of acetylated lysine 27 in the histone H3 (H3K27ac) (Bulger and Groudine, 2011; Harmston and Lenhard, 2013). Oh *et al.* (2014) demonstrated clearly that the 923 enhancer is active in epidermal keratinocytes, both *in vitro* and *in vivo*, and that this activity depends on the transcription factor AP-1.

One of the major questions in enhancer biology concerns the mechanisms that underlie the interaction between enhancers with target genes, and this highlights the importance of higher-order chromatin folding and spatial organization of genes and enhancers within the nucleus for the control of gene transcription.

Higher-order chromatin folding, 3D genome organization, and control of gene expression

Progress in the analysis of higher-order chromatin folding and 3D genome organization has been achieved using two approaches. The first is confocal microscopy after 3D fluorescence *in situ* hybridization or gene loci labeling in live cells using transgenic fluorescent chimeric proteins that contain specific DNA-binding domains (Cremer and Cremer, 2010). The second is 3C technology and its modifications, based on the restriction digestion of chromatin cross-linked using formaldehyde, followed by ligation at very low chromatin concentrations to allow the formation of intramolecular, but not intermolecular, products (Dekker *et al.*, 2013). The original 3C technology provides information about spatial interaction between two selected genomic regions, often referred to as a ''one versus one'' approach. The high-throughput modifications of this technology include circular chromatin conformation capture (4C) technology, allowing analysis of spatial interaction of a single genomic site with all other regions of the genome (''one versus all'' approach), chromatin conformation capture carbon copy (5C) technology, allowing analysis of spatial interactions between a set of selected genomic regions and another set of the selected regions (''many versus many'' approach), and Hi-C technology, providing information about interactions between all genomic regions (''all versus all'' approach) (de Wit and de Laat, 2012). These studies have revealed that chromosomes are organized into chromosome territories in the interphase nucleus and that many genes are not distributed randomly within the 3D nuclear space.

Accumulating evidence suggests that signaling/transcription factor–mediated and epigenetic gene regulatory mechanisms are intimately connected (Botchkarev *et al.*, 2012). In the developing epidermis, transcription factor p63 and ATP-dependent chromatin remodeler Brg1 are required for EDC locus relocation from the nuclear periphery toward the nuclear interior in epidermal progenitor cells (Mardaryev *et al.*, 2014). Furthermore, the global

genome organizer Satb1 serves as a direct p63 target that controls the establishment of proper conformations of the EDC central domain (Fessing *et al.*, 2011). These data demonstrate that higher-order chromatin remodeling is important for the control of gene expression at this locus.

Results generated by Oh *et al.* (2014) further support this paradigm. They demonstrate that the 923 enhancer forms specific spatial contacts with the subset of genes in the EDC in mouse primary keratinocytes cultured under proliferative and differentiating conditions. These contacts depend partially on the culturing conditions and binding of the AP-1 transcription factor to the enhancer. This finding provides the first clue about mechanisms that underlie the formation of enhancer–promoter interactions in the EDC.

However, the mechanisms of establishing spatial chromatin contacts between proximal and distal gene regulatory elements remain poorly understood (Gibcus and Dekker, 2013). The GATA-1 transcription factor and FOG-1 cofactor are clearly involved in establishing contacts between the locus control region and the gene promoters at the β-globin gene locus (Vakoc *et al.*, 2005). However, studies in mouse embryonic stem and neuronal progenitor cells using high-throughput 5C technology demonstrated that the interacting chromatin regions are not enriched with the binding of the several analyzed transcription factors alone or in different combinations (Phillips-Cremins *et al.*, 2013). Instead, the interacting regions are enriched in binding of the chromatin architectural proteins CTCF and cohesin for the long-range constitutive interactions shared by embryonic stem cells and neuronal progenitors. In contrast, in embryonic stem cells, specific enhancer–promoter interactions are enriched by combination of the mediator complex and cohesin binding or cohesin binding alone. These data suggest that combination of chromatin architectural proteins and the regulatory mediator complex might have an important role in 3D chromatin interactions.

Recent studies suggest that Satb1 is an additional architectural protein involved in interactions in the EDC in keratinocytes (Fessing *et al.*, 2011). Oh *et al.* (2014) demonstrate that the transcription factor AP-1 also has a role in this process. Most likely, mechanisms controlling higher-order chromatin folding might involve a combination of chromatin architectural proteins and transcription factors bound at interacting regions.

Application of the 3C (''one versus one'') technology used by Oh *et al.* (2014) is an important step toward analyses of global higher-order chromatin folding at the EDC locus at high resolution by employing high-throughput 4C or 5C technologies. The comprehensive genome-wide interactome in different populations of epidermal and hair follicle cells could be analyzed using Hi-C (''all versus all'') technology; however, the resolution of this technology is limited by the cost of high-throughput sequencing (Dekker *et al.*, 2013). Ultimately, high-throughput modifications of 3C technology will help answer many important questions about the control of higher-order chromatin folding at the EDC and other genomic regions in different populations of the skin epithelial cells and how folding controls gene transcription programs by defining spatial interactions between proximal and distal gene regulatory regions.

Recent studies demonstrate that mammalian genomes are compartmentalized spatially at several different scales, including global segregation of active and inactive genomic domains, chromosomal territories, topologically associated domains (TADs) and subdomains (sub-TADs; Gibcus and Dekker, 2013). TADs are genomic regions from several hundred Kbs to two Mbs in size, where the spatial interactions within domains are much more frequent compared with between domains. Interestingly, the borders of more than 90% of TADs are cell-type invariant. Further studies using high-throughput 5C or Hi-C technologies will help identify the organization of the chromatin in TADs and sub-TADs at the EDC.

Finally, interesting features of spatial genome organization are interaction of a single promoter with multiple enhancers and interaction of an enhancer with multiple promoters, suggesting the presence of complex gene regulatory element networks (Gibcus and Dekker, 2013). The 923 enhancer described by Guzman-Strong's team is one of several distal gene regulatory elements controlling coordinated gene transcription at the EDC. Future studies will help identify the spatial interactions between different proximal and distant gene regulatory elements in keratinocytes and assess their roles in gene expression in skin disease.

Conclusion

Recent technological and methodological advances have advanced our knowledge of higher-order chromatin folding in the control of gene expression programs in mammals in health and disease, and they have raised many exciting questions that can now be addressed. New knowledge about 3D genome organization and its role in gene regulation in skin will lead to new understanding of fundamental biological processes, and they will form a basis for the development of novel therapeutic and diagnostic tools to combat skin disease.

CONFLICT OF INTEREST

The author states no conflict of interest.

REFERENCES

Botchkarev VA, Gdula MR, Mardaryev AN *et al.* (2012) Epigenetic regulation of gene expression in keratinocytes. *J Invest Dermatol* 132:2505–21

Bulger M, Groudine M (2011) Functional and mechanistic diversity of distal transcription enhancers. *Cell* 144:327–39

Cremer T, Cremer M (2010) Chromosome territories. *Cold Spring Harb Perspect Biol* 2:a003889

de Wit E, de Laat W (2012) A decade of 3C technologies: insights into nuclear organization. *Genes Dev* 26:11–24

Dekker J, Marti-Renom MA, Mirny LA (2013) Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. *Nat Rev Genet* 14:390–403

Fessing MY, Mardaryev AN, Gdula MR *et al.* (2011) p63 regulates Satb1 to control tissue-specific chromatin remodeling during development of the epidermis. *J Cell Biol* 194: 825–39

Gibcus JH, Dekker J (2013) The hierarchy of the 3D genome. *Mol Cell* 49:773–82

Harmston N, Lenhard B (2013) Chromatin and epigenetic features of long-range gene regulation. *Nucleic Acids Res* 41:7185–99

Mardaryev AN, Gdula MR, Yarker JL *et al.* (2014) p63 and Brg1 control developmentally regulated higher-order chromatin remodelling at the epidermal differentiation complex locus in epidermal progenitor cells. *Development* 141:101–11

Maurano MT, Humbert R, Rynes E *et al.* (2012) Systematic localization of common disease-associated variation in regulatory DNA. *Science* 337:1190–5

Oh IY, Albea DM, Goodwin ZA *et al.* (2014) Regulation of the dynamic chromatin architecture of the epidermal differentiation complex is mediated by a c-Jun/AP-1-modulated enhancer. *J Invest Dermatol* 134:2371–80

Phillips-Cremins JE, Sauria ME, Sanyal A *et al.* (2013) Architectural protein subclasses shape 3D organization of genomes during lineage commitment. *Cell* 153:1281–95

Rando OJ, Chang HY (2009) Genome-wide views of chromatin structure. *Annu Rev Biochem* 78:245–71

Vakoc CR, Letting DL, Gheldof N *et al.* (2005) Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1. *Mol Cell* 17:453–62

See related article on pg 2408

Keratinocyte Growth Regulation TRP-ed Up Over Downregulated TRPV4?

Wolfgang Liedtke[1], Jennifer Y. Zhang[2], Russell P. Hall III[2] and Martin Steinhoff[3]

This commentary on an exciting new study (Fusi *et al.*, 2014) puts the finding of TRPV4 downregulation in several nonmelanoma skin cancers into context. The original paper point toward possible use of TRPV4 as dermatopathologic marker, also toward the possibility that downregulated TRPV4 can affect biological properties of the cancer, by enhancing, but also regulating tumor growth. As calcium-permeable TRPV4 has recently been identified as UVB-receptor in skin keratinocytes, where it regulates skin tissue injury and pain after UVB overexposure, it is discussed whether TRPV4 downregulation can also be found in other non-UVB-exposed cancers.

Journal of Investigative Dermatology (2014) **134,** 2310–2312. doi:10.1038/jid.2014.250

In this issue of *JID*, an exciting new study by Daniela Massi's and Romina Nassini's groups at the University of Florence, Italy, shows that keratinocytes from precancerous lesions and from malignant skin cancers exhibit considerable downregulation of TRPV4 ion channels (Fusi *et al.*, 2014). Because sweat glands and endothelial cells function as endogenous positive controls, their finding suggests that TRPV4 immunolabeling could be a novel tissue marker in several skin cancers and could improve diagnostic accuracy in dermatopathology. However, the approach's specificity needs to be confirmed by examining additional controls such as proliferative noninflammatory lesions (e.g., seborrheic keratosis and verruca vulgaris), as well as healthy skin proximal to cancer. Such a broad study would also allow investigators to correlate clinical features of a cancer with the extent of TRPV4 downregulation.

Relevance for other epithelial tumors with TRPV4 expression in the epithelium before tumorigenesis

The question arises whether TRPV4 downregulation is restricted to skin cancer, whereas other cancers, in which TRPV4 shows physiological epithelial expression, might not share this regulation. If this were so, then epidermis-specific TRPV4 downregulation in neoplastic (or hyperproliferative) epithelia might be associated with UVB exposure. Skin is often UVB exposed, but intra-oral, esophageal, and colonic epithelia—all of which express TRPV4—are not. In other words, does TRPV4 downregulation in epithelia selectively affect UVB carcinogenesis? Moreover, is TRPV4 downregulation in skin cancer ultimately caused by UVB exposure? Chronically UVB-damaged epidermis will be relevant, where initial studies have demonstrated TRPV4 upregulation (Moore *et al.*, 2013).

Does downregulation of the UV receptor, TRPV4, protect UVB-exposed keratinocytes against calcium overexposure—or is it only an epiphenomenon?

We can now consider whether in keratinocytes UVB exposure and downregulation of expression of a channel that is activated by UVB—TRPV4 (Moore *et al.*, 2013)—are linked in an autoregulatory feedback loop. TRPV4 downregulation would protect the cell against an overabundance of calcium, and, in the context of UVB exposure, against (detrimental) consequences of UVB-mediated inflammation (including neurogenic inflammation) and tissue injury. However, because keratinocytes' TRPV4 expression normally facilitates calcium influx, which is also known to sustain the layered architecture of the epidermis by regulating keratinocyte proliferation and differentiation (Yuspa *et al.*, 1989), TRPV4 downregulation may represent cause, effect, or coincidence. First, TRPV4 downregulation might enable dedifferentiation and uncontrolled growth of keratinocytes, thus leading to a malignant phenotype. Alternatively, it may be a regulatory response of malignantly transformed cells. We can entertain the concept that TRPV4 downregulation is a counter-regulatory mechanism of the malignantly transformed cell because the stratified architecture of cancerous epithelia has been lost, and calcium influx would fuel growth, migration, and metastasis of (pre) cancerous cells. Finally, it may be an epiphenomenon without critical rele-

[1]*Departments of Neurology, Neurobiology and Anesthesiology, Duke University, Durham, North Carolina, USA;* [2]*Department of Dermatology, Duke University, Durham, North Carolina, USA and* [3]*University College of Dublin, Charles Institute of Translational Dermatology, Dublin, Ireland*

Correspondence: Wolfgang Liedtke, Departments of Neurology, Neurobiology and Anesthesiology, Duke University, Durham, North Carolina 27710, USA. E-mail: wolfgang@neuro.duke.edu