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Heat stress: A risk factor for skin carcinogenesis

This thesis is presented for the degree of

Doctor of Philosophy

Leslie Debra Lou S. Calapre

Edith Cowan University
Faculty of Computing, Health and Science
School of Medical Science

2015

The declaration page
is not included in this version of the thesis

ABSTRACT

BACKGROUND: The incidence of skin cancer in Australia has increased rapidly in the last few decades. Ultraviolet radiation (UV) is a major risk factor for skin carcinogenesis. UV, particularly the UVB spectrum, causes formation of cyclobutane pyrimidine dimers (CPD) in cellular DNA. Persistent and incorrectly repaired CPDs lead to DNA mutations and consequently, formation of cutaneous lesions. Interestingly, recent epidemiological studies have shown a significant increase in skin cancer incidence in geographical locations with high environmental temperatures. Thus, heat stress may potentiate the effects of UV exposure and act as an additional risk factor for skin cancer. Previous studies in mice have shown that repeated and concurrent exposure to UVB and heat stress, increases the rate and incidence of cutaneous tumour formation relative to UVB alone. However, the effects of UVB *plus* heat on human epidermal cells have yet to be determined. Furthermore, the exact mechanisms responsible for the observed effects of heat stress need to be characterised in skin keratinocytes to increase knowledge of its risk in skin cancer.

Heat stress induces upregulation of heat shock proteins (HSPs), particularly HSP72 and HSP90 which are known to affect the activity of the p53 protein. Furthermore, heat stress has been linked with increased Sirtuin1 (SIRT1) protein activity. SIRT1 is an important histone deacetylase that helps maintain chromosomal integrity but can also induce post-translational modifications of the p53 protein. By mediating deacetylation of the p53 protein, SIRT1 can diminish the ability of p53 to bind to its downstream gene targets. The p53 protein is an integral mediator of the cellular stress response in skin cells, particularly keratinocytes. Thus, impairment of p53 transcription factor functions could compromise the ability of epidermal cells to mount an appropriate response to DNA damage. Moreover, loss of p53 function may induce survival of cells harbouring DNA lesions.

We hypothesise, therefore, that exposure to UVB *plus* heat induces survival of DNA damaged keratinocytes and that these cells escape apoptosis surveillance as a result of heat-mediated alteration to the p53 signalling pathway. Thus, exposure to heat stress could exacerbate the carcinogenic effects of UV and increase the risk of skin tumour formation in humans.

AIMS: In this study, we aimed to determine whether repeated exposure to UVB followed immediately by heat stress (39°C) has a more damaging effect on human keratinocytes than UVB alone. In particular, we assessed the effects on DNA damage, apoptosis, cell cycle and DNA repair. Furthermore, we aimed to unravel the mechanism through which heat mediates the survival of UVB DNA-damaged keratinocytes, focusing on the effects on the p53 signalling pathway.

MATERIALS AND METHODOLOGY: Primary adult human epidermal keratinocytes (NHEK) and *ex vivo* punch biopsies of normal human skin called NativeSkin® (Genoskin, France), were used as experimental models for this study. A UV cabinet fitted with a TL-UVB Narrowband lamp (Philips, GERMANY), with a spectral output of 290 -315 nm, was used to administer UVB irradiation at a dose of 1 KJ/m². Heat stress involved culture in a normal CO₂ incubator, with temperature maintained at 39°C for three hours. The temperature used in the experiments was based on previous measurements of skin surface temperature of open cut miners, who are prone to intense heat stress, in the Pilbara region of Western Australia. For UVB *plus* heat exposures, cells and skin models were sequentially exposed to 1 KJ/m² of UVB, (at room temperature), followed immediately by 3 hours incubation at 39°C once per day, for four consecutive days. Unexposed skin models and NHEK, maintained at 37°C, were used as experimental controls. Cell proliferation, apoptosis and whole genome expression profiles were analysed at four hours post day 4 exposure, to understand earlier events, and at 2 days post-exposure, to assess persistent outcomes of these exposures.

Treated primary NHEK cells were counted in a Vi-Cell™ Viability Analyser and the level of apoptosis for exposed primary cells was determined using Annexin V/Propidium Iodide apoptosis assay at 4 hours and 2 days post exposure. To determine the presence of DNA damage, total and active p53 protein, as well as total and active SIRT protein, in the skin models and primary NHEKs, immunohistochemistry and/or immunocytochemistry was performed. Skin FFPE and primary NHEKs were incubated with antibodies to thymine dimers (CPD, DNA damage) and p53 (total), acetylated p53-382 (active), SIRT1 (total) or SIRT1-p (active) antibodies.

To measure apoptosis in skin, an anti-pan-cytokeratin marker was used to label keratinocytes and active-caspase-3 antibodies were used to identify apoptotic cells. To determine the expression of p53-downstream target genes at 4 hours, quantitative RT-PCR was performed using TaqMan probes for *BAX*, *Survivin (BIRC5)*, *ERCC1* and *XPC* genes, with Human *18S*

gene as the endogenous reference gene. Relative quantification of the expression levels of each transcript in each sample were calculated using the Delta-Delta CT method relative to untreated controls. A whole genome expression analysis was performed at 2 days post-exposure using the Human HT-12 Expression v4 BeadChip (Illumina, USA). The Ingenuity Pathway Analysis (IPA) (Qiagen, USA) software was used to annotate the effects of altered gene expression on cell function and upstream signalling pathways. Two-way ANOVA was used to analyse differences across treatment groups, while parametric unpaired t-tests were used to detect differences between specific treatment groups in all experimental categories, i.e. proliferation, apoptosis and gene expression, with p-values <0.05 considered significant.

RESULTS:

Outcome 1 –Using *ex vivo* skin models and NHEKs, we show for the first time that UVB *plus* heat treated keratinocytes exhibit DNA damage, as observed after UVB treatment alone. However, apoptosis was significantly reduced, possibly as a result of inactivation of the p53-mediated stress response, in DNA damaged cells of UVB *plus* heat treated samples. Furthermore, whole genome expression and IPA upstream analysis showed that heat induces SIRT1 activation, which was confirmed via immunohistochemistry assays. Heat-induced SIRT1 expression was linked to a decrease in acetylated p53 and consequently, downregulation of p53-regulated pro-apoptotic and DNA damage repair genes. These results suggest that p53-mediated cell cycle arrest and apoptosis, known to be induced by UVB, are ablated with the addition of heat, leading to survival of DNA damaged cells after UVB *plus* heat treatment.

Outcome 2 – We further confirmed that SIRT1 activation did not inhibit the transcription of the p53 protein but mediated deacetylation of p53, resulting in significant deregulation of expression of p53 downstream gene targets and decreased keratinocyte apoptosis in UVB *plus* heat treated samples. Importantly, chemical inhibition of SIRT1 by Ex-527, a known chemical inhibitor of SIRT1, in UVB *plus* heat exposed keratinocytes, resulted in re-activation of the p53 signalling pathway and increased apoptosis of DNA damaged keratinocytes. This clearly demonstrated the role of heat-mediated SIRT1 activation in the survival of DNA damaged keratinocytes after exposure to UVB *plus* heat.

CONCLUSION: In this study, we showed that the efficiency of cellular stress response to UVB-induced DNA damage is diminished in the presence of heat and, for the first time, provide a molecular mechanism that explains these effects. With the novel use of an *ex vivo* human skin model, this study showed that heat stress prevents human keratinocytes, damaged by UV irradiation, from undergoing apoptosis and/or necrosis. We found UV *plus* heat exposure mediates SIRT1 activation which has been found to induce deacetylation of p53 and, consequently, the inactivation of the p53 signalling pathway. SIRT1 inhibition precluded the downregulation of p53 signalling by UV *plus* heat exposure, restoring apoptosis levels to those observed in UVB-only exposures. Thus, we demonstrated that SIRT1 activation is the main molecular mechanism driving UVB *plus* heat-induced survival of DNA damaged keratinocytes.

Overall, the results of this study suggest that by allowing the survival of DNA damaged keratinocytes, via induction of SIRT1 activation, heat stress can exacerbate the carcinogenic effects of UVB radiation. Exposure to heat stress, in addition to UV, could therefore increase the accumulation of mutations in keratinocytes, possibly leading to the transformation of normal cells into pre-cancerous cells. Further research is warranted to determine the role of UVB *plus* heat in skin cancer pathogenesis. Such knowledge could be utilised in public health campaigns to decrease risk, particularly for people exposed to combinations of these environmental hazards in workplaces such as in the mining, construction and petroleum industries.

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LIST OF PUBLICATIONS:

1) **Heat Stress: A Risk Factor for Skin Carcinogenesis**

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2) **Heat-Mediated Evasion of Apoptosis in UVB-Damaged Keratinocytes**

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Submitted and currently under re-review: **Experimental Dermatology**, August 2015

3) **SIRT1 Activation Mediates the Heat-Induced Survival of UVB Damaged Keratinocytes**

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STATEMENT OF CONTRIBUTION OF OTHERS:

Leslie Calapre conducted the experiments, analysis of results and wrote the papers included in this thesis. Dr Pascal Descargues, Dr Sandrine Kurdykowski and Dr Anthony David from Genoskin provided the skin models for all experiments presented in this thesis. Dr David Raven of Saint John of God Hospital Pathology Department provided help with processing of tissues. Miss Christie Chee of Clinipath provided advice and assistance with tissue embedding and sectioning. Professor Prue Hart from the Telethon Institute contributed to the study design and critique of this research project. Dr Joe Mates and A/Prof Jacques Oosthuizen provided the skin surface measurements. AGRF assisted with the analysis of the whole genome expression results included in publication 2 (Chapter 3).

Heat Stress: A Risk Factor for Skin Carcinogenesis

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GENERAL INTRODUCTION

Non-melanoma skin cancers (NMSCs), particularly basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are the most common cutaneous cancers in Caucasian populations around the world (Xiang et al., 2014). In Australia, NMSCs account for 75% of all cancers and are considered a great health and economic burden (Jou et al., 2010). BCC is defined as a malignant tumour of follicular germinative cells or trichloroblasts (Barascu et al., 2012; Hsieh et al., 2014). It accounts for approximately 80% of all diagnosed skin cancers and is considered to be the most common malignant tumour (Epstein, 2008; Saran, 2010). BCC incidence is on the rise, and many experts suggest that it will continue to increase in coming years (Basset-Seguin et al., 2015; Bath-Hextall et al., 2007). BCC is known to have a very low metastatic potential and mortality arising from this tumour type is approximately 0.1% (Lear et al., 2005; Roewert-Huber et al., 2007). BCC can be highly invasive, however, and can often cause extensive tissue destruction. BCC largely affects the elderly, although, the incidence of cutaneous BBCs has been increasing in younger adults in recent years (Bath-Hextall et al., 2007; Greinert, 2009). BCC is generally more common in males than in females, with a ratio of approximately 2:1 (Barascu et al., 2012).

The incidence of Squamous Cell Carcinoma (SCC) also appears to be on the rise (Levin et al., 2005; Nickoloff et al., 2002). SCC accounts for 20% of all cutaneous cancers; it is the second most common skin cancer after BCC (Kim et al., 2012; Sridhar et al., 2012). The incidence of SCC varies in different countries, but the US and Australia have the highest reported incidence, this being 20% of all skin cancer cases (Zhang et al., 2012). This cancer is two to three times more common in men than in women, and mostly affects adults over sixty years of age, though in recent years, the incidence of SCC in people below fifty years of age has increased (Monroe et al., 2011; Sabunciyan et al., 2015). Unlike BCC, which has a low metastatic dissemination of less than 0.1%, SCC is known to be more aggressive, and the incidence of SCC metastasis, which normally arises after one to two years of diagnosis, is reported to be between 5 and 20 percent (Smoller, 2006).

SCC, like every other skin cancer, is common in Caucasians (Gomez et al., 2015; Wei et al., 2015). In the USA, people of Irish or Scottish ancestry have the highest prevalence (Brantsch et al., 2008; Schwarz, 2005). In people of African and Asian descent, SCC is relatively rare but it is the most common form of skin cancer in these groups (Monroe et al., 2011; Rao et al., 2013). SCC in dark skin people carries a higher mortality rate, and this is attributed to delayed diagnosis as tumours are more likely to occur in sun-protected areas such as the scalp and sites of previous injury and scarring (Monroe et al., 2011)

Environmental stressors are considered the most common risk factors for skin cancer formation (DeFedericis et al., 2006; Lucas et al., 2006; Rosso et al., 2008). In particular, exposure to UV radiation is associated with formation of 80% of all skin tumours (Ananthaswamy and Pierceall, 1990; Lee et al., 2012; Xia et al., 2006). UV induces DNA damage, which often leads to formation of mutations in key regulators of cell apoptosis, survival and proliferation signalling pathways, which can lead to cancer initiation (Ananthaswamy and Pierceall, 1990; Brash et al., 1996; Courdavault et al., 2005; Grujil et al., 2001; Narayanan et al., 2010). However, given the effect of high temperature on cell viability and chromosome stability (Akerfelt et al., 2007; Takahashi et al., 2004), heat stress could be another risk factor for skin cancer. However, the exact molecular effects of repeated exposure of epidermal cells to high temperatures and UV radiation, which is what is observed in the environment, needs to be clarified.

GENERAL INTRODUCTION

CHAPTER 1: Environmental Stresses and their Role in Skin Carcinogenesis

1.1 Normal Skin Physiology

Keratinocytes and the Epidermal-Melanin Unit

The skin is the largest organ in the body and offers a protective barrier against environmental insults. The skin barrier is primarily maintained by the epidermis, which is further separated into different layers comprising the basal, granular and cornified layers (Haggarty, 2015). These epidermal layers are identified by differences in the morphology of keratinocytes, which are the most abundant cell type in the skin (Agthong et al., 2012).

The stratum basalis, or basal layer, of the epidermis consists of stem cells which continuously undergo mitotic division to provide new keratinocytes (Leung et al., 2015; Marshall, 2015; Sexton-Oates et al., 2015). Daughter cells of basal keratinocytes then detach from the basal membrane and undergo further differentiation as they move towards the epidermal surface, over a period of approximately two weeks (Zitzmann et al., 2015). Keratinocytes then undergo terminal differentiation, lose their nuclei and adapt a flattened morphology, to become corneocytes of the stratum corneum of the epidermis (Santen et al., 2009; Verdin and Ott, 2015). The terminal differentiation process of keratinocytes in the cornified layer represents the physiological apoptosis of epidermal cells (Nowotarski et al., 2015). Imbalances in the delicate physiological cycle of keratinocyte proliferation or differentiation can significantly affect the integrity of the skin and result in the formation of cutaneous malignancies (Schraml et al., 2009; Villanueva et al., 2015).

Apart from keratinocytes, one of the most prominent cell types in the skin are the melanocytes, which are also located in the basal layer of the epidermis (Chen et al., 2015). Melanocytes are specialised cells that are responsible for the production of melanin, an important skin chromophore (Abdel-Malek et al., 2000; McCarty and Loeb, 2015; Ratajczak-Wrona et al., 2009). Normal skin physiology includes an epidermal-melanin unit, comprised of keratinocytes and melanocytes arranged at a ratio 35:1, the function of which is to produce melanin to protect keratinocytes from the harmful effects of environmental stressors (Chen et al., 2015; Costin and Hearing, 2007).

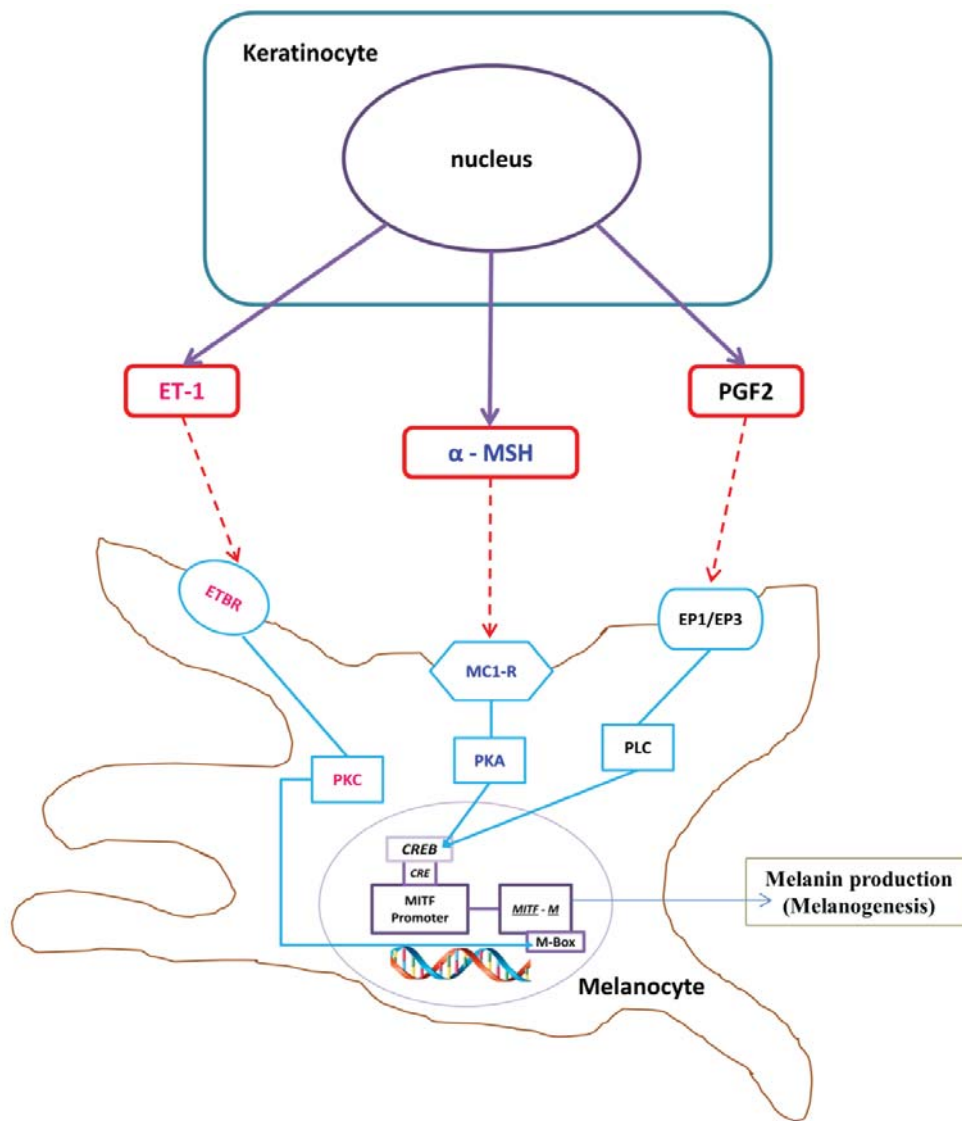


Figure 1.1: Keratinocyte-Melanocyte Complex. This figure highlights mechanisms by which keratinocyte-derived factors such as endothelins (ET) and melanocyte-stimulating hormone (MSH) (red boxes) act on human melanocyte proliferation and stimulate melanogenesis.[Adapted from: (Hirobe, 2005)]

Keratinocytes located in the epithelial layer of the stratum corneum can initiate melanin production within melanocytes by secreting growth factors, such as endothelins and melanocyte-stimulating hormone (MSH), which act in a paracrine manner to stimulate melanocyte function (Costin and Hearing, 2007; Hirobe, 2005). Melanocytes and keratinocytes interact with each other through cell-to-cell signalling to induce melanin production in melanocytes (**Figure 1.1**).

Melanin has an important role in the physiology, pathology and toxicology of the skin, eyes and brain (Hu, 2008). This pigment acts as a filter by absorbing near-infrared, visible light and UV photons (Gidanian et al., 2008). It also functions as a free radical scavenger, capturing oxygen and hydroxyl radicals and preventing them from causing oxidative stress (Soumyanath et al., 2006). Melanin is also known to have an affinity for metals and toxic substances and has a sun protection factor (SPF) value of 2-3 (Maddodi and Selaturi, 2008). It is also known to be able to transform light energy into heat and distribute it evenly between the capillary vessels and hair follicles (Pons and Quintanilla, 2006).

There are two types of melanin produced by mammalian melanocytes - eumelanin and pheomelanin (Tody et al., 1991). Eumelanin is brownish-black in colour, while pheomelanin appears as a reddish-yellow pigment (Pons and Quintanilla, 2006). Synthesis of both involves the enzyme, tyrosinase (Chen et al., 2009). This enzyme converts tyrosine to dopaquinone, which when further oxidised, gives rise to eumelanin (Tody et al., 1991; Williams and Ouhtit, 2005). Pheomelanin is also formed from further oxidation of dopaquinone, but requires the presence of sulphhydryl groups from the amino acid cysteine (Pons and Quintanilla, 2006; Tody et al., 1991). Eumelanin and pheomelanin are found in human skin and hair in almost equal amounts, though a relatively greater concentration of pheomelanin is found in people with red hair and those with a 'Celtic type' skin phenotype (Tody et al., 1991).

Skin pigmentation varies for each individual, due to the difference in the amount of melanin produced and distributed to neighbouring keratinocytes (Markovic et al., 2007). Lighter skin produces less melanin (Maddodi and Selaturi, 2008), while a darker skin tone produces more melanin (Yamaguchi et al., 2008). The distribution of melanosomes and the production of melanin is the main reason why people with darker skin tone are more efficient at absorbing UV radiation (Markovic et al., 2007).

While the mechanisms that regulate skin pigmentation are not completely understood, extensive data shows that UV-induced DNA damage and/or its repair produce initiating signals that induce melanogenesis (Lee et al., 2013; Mullard, 2015). In response to UV, melanin is transferred to keratinocytes and repackaged as granules in keratinocytes where it accumulates above the nuclei to protect them from the mutagenic effects of UV radiation (Costin and Hearing, 2007; Gorman et al., 2007).

If melanin is produced and distributed correctly, keratinocytes are protected from the harmful effects of UV, even as the cell is undergoing cell division (Seripa et al., 2015; Tallmadge et al., 2015; Wilken et al., 2015). However, if the melanin production and distribution is significantly reduced, or if alterations in the signalling pathways of the keratinocyte-melanocyte complex arise, leading to diminished melanin production, then it is possible for UV and other environmental stresses to cause the DNA mutations that precede skin cancers (Coelho et al., 2009).

While melanin is extremely important in protecting skin from damage caused by chemicals and radiation, it can itself be an indirect cause of damage (Yamaguchi et al., 2008). Chronic exposure to chemicals and radiation can result in changes to melanin properties, such that it fails to bind toxic substances, resulting in cell damage (Hu, 2008). Furthermore, after consistent and long term exposure to various environmental hazards and stresses, melanin accumulates free radicals, which have the potential to disrupt the synthesis of major proteins and RNA and cause dermal cell damage (Hu, 2008; Hube and Francastel, 2015; Tody et al., 1991).

1.2 UV Radiation and Skin Cancers

Environmental stresses and their role in skin cancer pathogenesis have been studied extensively, to gain a better understanding of the molecular mechanisms underpinning the tumourigenic process and to identify treatment or protection strategies. The factor identified as the main cause of most skin cancers is UV radiation, which induces specific mutations in skin cell DNA (Ikehata and Ono, 2011; Miller, 1985).

Solar radiation is a spectrum of electromagnetic radiation that includes ultraviolet rays, infrared and visible light (Maddodi and Selaturi, 2008; Setlow et al., 1993; Sinha and Hader, 2002). UV radiation is considered a potent carcinogen, and it can enter the earth's atmosphere and cause mutations in various mammalian cells including epidermal cells (Brenner et al., 2009). The association between UV radiation and skin cancer was first established in the late 60's and was confirmed by Fears et al. (1977). Fears and colleagues showed that the incidence of non-melanoma skin cancers is far higher in areas where people are exposed to excessive sunlight. This led them to conclude that solar radiation is one of the main causes and suggested that formation of skin cancers is most likely related to annual rates of UV exposure.

Retrospective epidemiologic data gathered by various researchers over many years supports the claims made by Fears and colleagues (1997). One particular study conducted by Williams and Ouhtit (2005), also showed a clear link between skin cancer risk and UV exposure. Their systematic review showed clearly that exposure to sunlight both in childhood and adulthood contributes greatly to the risk. This result was mirrored by a study conducted in 2004 (Solomon et al., 2004), where the researchers also found that lifetime UV exposure leads to an increased likelihood of skin cancer.

The epidemiological link between skin cancer and UV exposure provides only half of the evidence of the important role of this particular environmental stress in skin carcinogenesis. The discovery of UV signature gene mutations that are present in UV-induced BCC and SCC also confirmed the role of UV in skin carcinogenesis (Ananthaswamy and Pierceall, 1990; Andreassi, 2011; Miller, 1985). These UV signature mutations initiate tumourigenesis when found in genes involved in signalling cascades that control cell proliferation and apoptosis (Markovitsi et al., 2010).

UVA Radiation

Ultraviolet radiation is comprised of type A (UVA), which makes up 90% of all solar radiation and has wavelengths between 315-400 nm, and type B, which has long wavelengths of 290-320 nm (Weigmann et al., 2010). These relatively long wavelengths allow UV rays to penetrate the epidermis where they inflict cellular damage, including morphological and genetic alterations to keratinocytes and melanocytes (Courdavault et al., 2005). In particular, UVA is known to penetrate the deeper layers of the epidermis and induce significant damage to the cells located in the stratum basale.

While UVA is capable of inducing DNA damage via thymine dimerisation (Ikehata and Ono, 2011), the basis for UVA mutagenesis and subsequent carcinogenesis lies predominantly in production of reactive oxygen species (ROS). ROS can covalently bind or directly oxidise proteins and can cause reversible or irreversible modifications including protein-protein cross linking, carbonylation, formation of adducts with lipid peroxidation products, and nitration (Perluigi et al., 2010; Vidal et al., 2014). These modifications often result in changes in stability, as well as functional and structural changes in proteins, which can lead to loss of protein function, protein aggregation and degradation, in turn causing DNA missense mutations. ROS are also capable of inducing DNA strand breaks and distorting nucleotide bases (Dai et al., 2012a).

UVB Radiation

Considered the most-cancer inducing spectrum, UVB induces formation of multiple thymine dimer photoproducts, which can distort the DNA and affect its stability. UVB is implicated as the main cause of skin cancers, particularly keratinocyte-derived carcinomas. Several studies have shown that UVB, can induce histological and morphological alterations in epidermal cells (Delgado-Cruzata et al., 2014; Hillman et al., 2015).

Previous studies in mice have shown that UVB induces significant changes to skin histology, including decreased epidermal thickness, increased epidermal differentiation and significantly reduced numbers of proliferating cells in UVB-treated skin (Luo et al., 2007; Riyahi et al., 2015; Saksouk et al., 2014). Furthermore, irradiation with solar and/or narrowband UVB strongly alters keratinocyte morphology, inducing cytoplasmic vacuolisation and disintegration of nuclear and cellular membranes (Kebir et al., 2014; Reich et al., 2007). Irregular distribution and bleb-like protrusions of the cell surface, rearrangement of the cytoskeleton and thinning or redistribution of microfilaments to the cell periphery, are also considered some of the most prominent narrowband UVB-induced morphological alterations in keratinocytes (Reich et al., 2007; Riyahi et al., 2015).

Formation of major carcinogenic photoproducts, such as cyclobutane pyrimidine dimers (CPD) and pyrimidine(6-4)-pyrimidone (6-4PPs), at pyrimidine sites on the DNA of epidermal cells are known to be induced by UVB irradiation (Courdavault et al., 2005; Ikehata and Ono, 2011; Matsunaga et al., 1991). CPDs and 6-4PPs are formed as a result of a photochemical reaction between UVB and the DNA, leading to covalent linkage of adjacent thymine dimers (**Figure 1.2**), inducing significant changes in the nucleotide structure (Besarutinia et al., 2004; Yogianti et al., 2012). CPD formation generally occurs at sites of methylated cytosines, and thus an abundance of methylated cytosines in a particular DNA region increases susceptibility to UV radiation-induced damage at these sites (Aigal et al., 2015; Brenner et al., 2009). Thus, CpG islands, DNA regions containing 5-methylcytosines, are hotspots for such DNA mutations (Ikehata et al., 2003; Ikehata and Ono, 2011).

Keratinocytes undergo rigorous processes of surveillance to ensure that cells which harbour CPDs or 6-4PPs DNA damage do not proliferate (Alonso et al., 2015; Khalkhali-Ellis et al., 2014). Conserved processes of cell cycle arrest and photoproduct-repair, primarily via the nucleotide excision repair pathway (NER) and activation of DNA polymerases such as translesion DNA synthesis (TLS) polymerases, are imposed on cells with DNA lesions (Loughlin, 2015; Pathania et al., 2015; Rubin et al., 2014; Vilahur et al., 2014). These innate cellular stress responses to DNA damage help in guarding the genome against mutations that may induce unrestricted proliferation and/or evasion of apoptosis, leading to the formation of tumours.

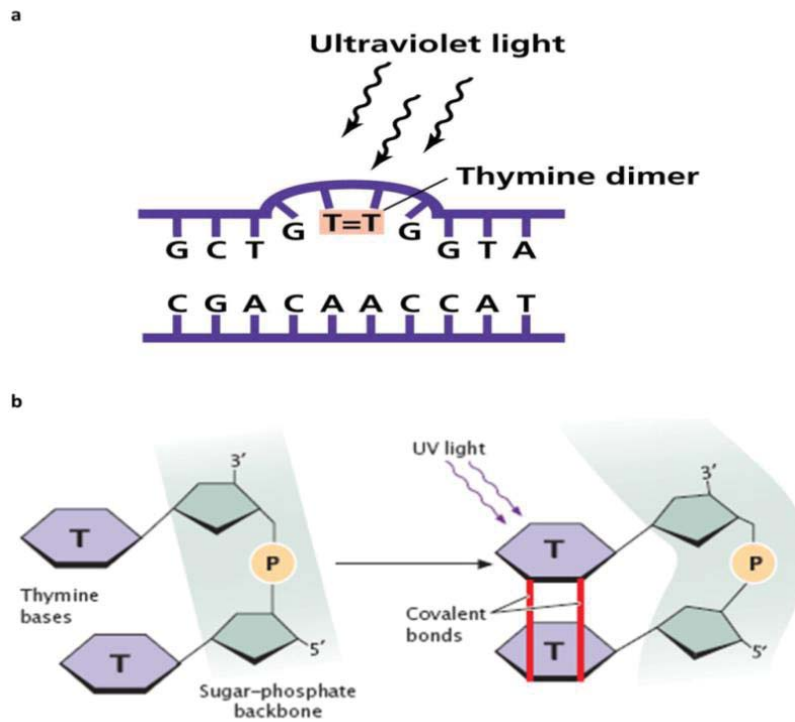


Figure 1.2: UV-Induced Thymine Dimer Formation. a) Diagrammatical representation of UV-induced thymine dimer formation in cells and distortion of the DNA helix (Pearson Publishing Inc, Benjamin Cummings). b) Covalent linkage of thymine bases as a result of UV radiation.

UV Damage and Cell Cycle Arrest

Cell cycle arrest is imposed on cells with DNA damage to provide time for correction of any DNA defects (Andersen, 2015; Baud et al., 2014). The cell cycle arrest mechanism in mammalian cells is primarily controlled by the p53 protein, a product of the *TP53* gene (Geranton and Tochiki, 2015; Kastan et al., 1991). In response to genotoxic stress, particularly UV-induced DNA damage, the levels of p53 protein increase, which determines either a transient arrest of cell cycle progression or triggers apoptosis (Brash, 2006; Chipuk and Green, 2004). The p53 protein can activate agents of the cell cycle checkpoints, controlling their activity, to primarily induce a transient arrest at a specific stage of the cell cycle (Andersen, 2015; Geranton and Tochiki, 2015; Mourad et al., 2014). There are two well-known p53-regulated checkpoints at which DNA damage is monitored (**Figure 1.3**)-, the G₁/S transition checkpoint, which prevents replication of damaged DNA, and the G₂/M transition, at which stage cell cycle is inhibited in response to persistent DNA damage and/or incompletely replicated DNA (Harris and Levine, 2005).

The p53 protein mediates G₁/S phase arrest via the activation of p21^{CIP1}, which inhibits the kinase activity of cyclin/cyclin-dependent kinase complexes, which are key elements in cell cycle progression (Jimenez et al., 1999; Pellegata et al., 1996; Smith et al., 2000). By inactivating these kinases, p53 prevents the phosphorylation of the G₁ cyclin/cyclin-dependent kinase substrate pRB and inhibits the transition of cells from the G₁ to the S phase of the cycle (Abbas and Dutta, 2009; Brant et al., 2014). Arrest at G₁ provides time for innate nucleotide excision DNA repair mechanisms to repair critical damage, thereby avoiding the propagation of genetic lesions to progeny cells (Yu et al., 2000). If the nucleotide excision repair is successful in removing and/or repairing the DNA lesion, the cell cycle can resume. However, if the damage is too extensive, cells will undergo apoptosis (Pellegata et al., 1996). Loss of the G₁ checkpoint also results in genomic instability, as seen by the increase in the frequency of gene amplifications in p53-defective cells (Bodega and Orlando, 2014).

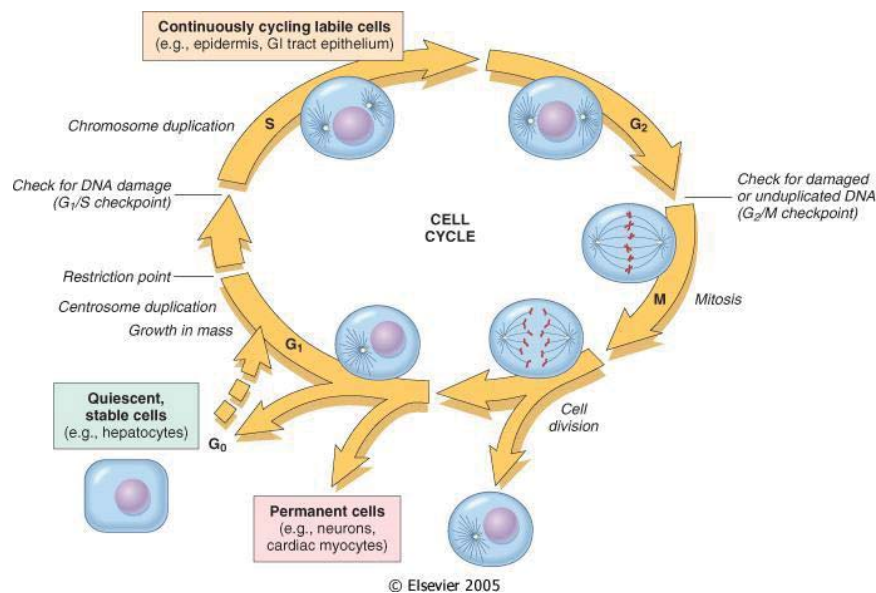


Figure 1.3: The Cell Cycle Checkpoints.

DNA Damage Repair

In all mammalian cells, the recognition and effective removal of DNA lesions is facilitated by an efficient DNA repair system. The nucleotide excision repair (NER) mechanism is responsible for the surveillance and removal of UV-induced DNA damage and other lesions that may induce distortion of the DNA helix in various cells, most particularly in keratinocytes which are constantly exposed to UV radiation (Du et al., 2015; Feng et al., 2014; Kratochwil and Meyer, 2015; Pacis et al., 2014).

Nucleotide excision repair is indispensable for the removal of UV-induced DNA lesions, as highlighted by three clinically and genetically heterogeneous human syndromes carrying defects in NER-associated genes; Xeroderma Pigmentosum (XP), Cockayne Syndrome (CS), and Trichothiodystrophy (Asada et al., 2015; Kraemer et al., 1987; Pan, 2014). People with these syndromes are all sensitive to UV exposure and, in particular, patients with Xeroderma Pigmentosum have a >1,000-fold increased susceptibility to UVB-induced skin cancers (Anink-Groenen et al., 2014; Sarasin, 1999). The nucleotide excision repair-mediated damage surveillance mechanism is subdivided into two sub-pathways (**Figure 1.4**) – Transcription Coupled - Nucleotide Excision Repair (TC-NER) and Global Genome - Nucleotide Excision Repair (GG-NER). These pathways differ primarily in the way they respond to or recognise damage (Raviram et al., 2014).

TC-NER is initiated when an elongating RNA polymerase II is stalled as a result of DNA lesions, which disrupts transcription on the template strand of active genes (Katz et al., 2014; Kolovos et al., 2014). This is followed by the recruitment and modifications to Cockayne Syndrome B (CSB) and Cockayne Syndrome A (CSA) proteins, which have DNA-binding ability (Bianchi et al., 2014; Edelstein and Smythies, 2014). The Xeroderma Pigmentosum group B and D (XPB and XPD respectively) helicases of the 10-subunit transcription factor TFIIH then unwind the helix surrounding the lesion, creating short stretches of single stranded DNA (ssDNA) around the lesion (Qian et al., 2015).

The creation of ssDNA facilitates the recruitment of Xeroderma Pigmentosum group A (XPA) and Replication Protein A (RPA), ssDNA binding proteins, which verify DNA damage and prevent complimentary repair by aberrant NER complexes formed on undamaged DNA (Simpson, 2014). Once DNA damage is verified, Xeroderma Pigmentosum complementation group G (XPG) and the Excision Repair Cross-Complementation group 1 – Xeroderma Pigmentosum group F (ERCC1-XPF) protein complex of structure-specific endonucleases, cut the DNA strand containing CPDs at the 3' and 5' side of the lesion respectively (Corware et al., 2014; Janssen et al., 2014; Yang et al., 2014). When the oligonucleotide, approximately 30nt in length, containing the lesion has been removed, Proliferating Cell Nuclear Antigen (PCNA) is loaded onto the DNA by Replicating Factor C (RFC) protein, as is the case in normal DNA replication. DNA polymerases δ and ϵ then mediate DNA repair synthesis across the gap using the undamaged strand as a template (Bailey et al., 2015; Murphy et al., 2015). Finally, the remaining nick is sealed by DNA ligase I.

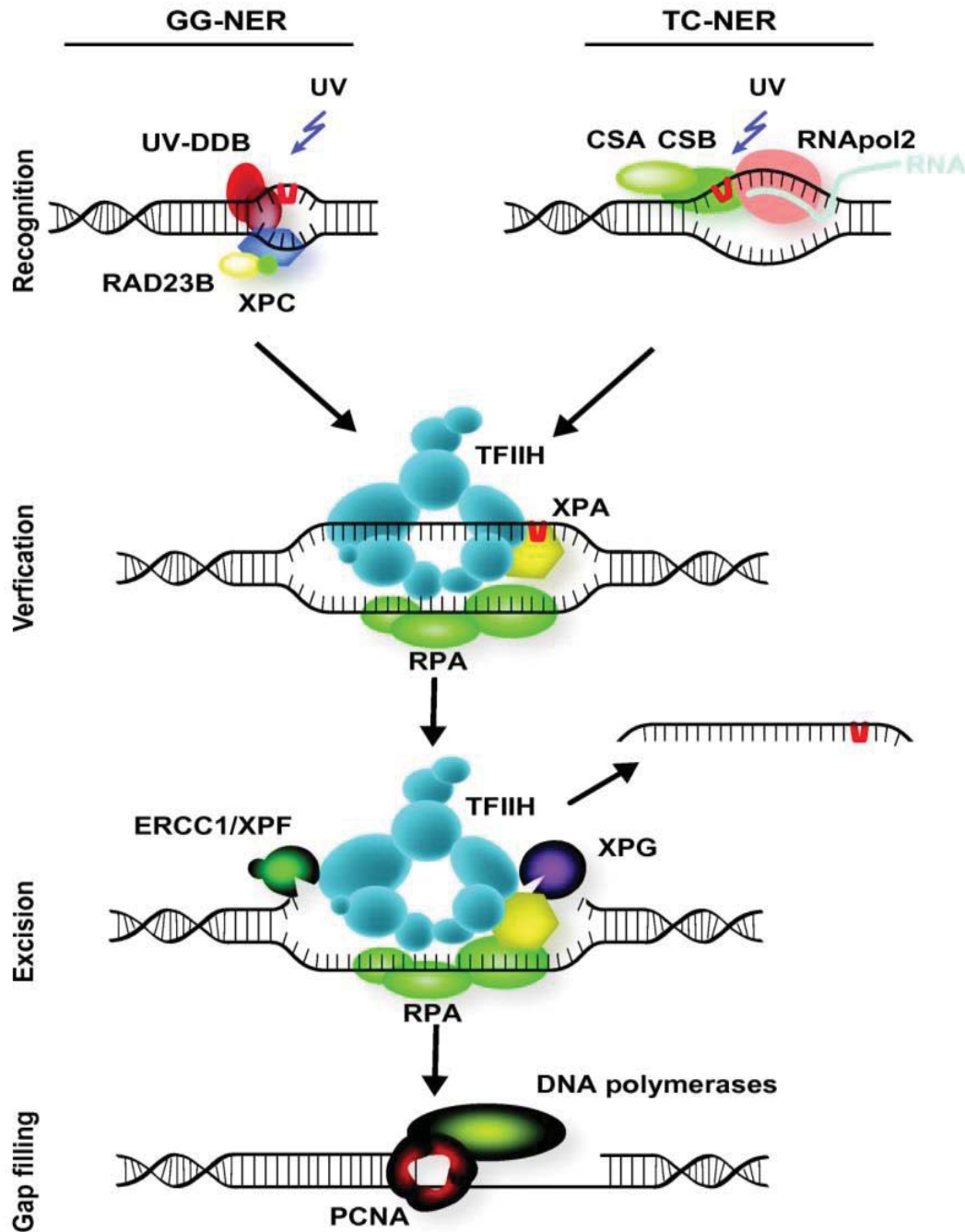


Figure 1.4: The Nucleotide Excision Repair (NER) System. The molecular events involved in nucleotide excision repair (NER) mechanism for DNA damage recognition and repair in mammalian cells. Global genome-NER (GG-NER) and Transcription coupled-NER (TC-NER) mediate excision of the DNA strand containing UVB-induced lesions (Erdmann et al., 2014).

By contrast, GG-NER is mediated by the protein complex XPC-hHR23B (RAD23B), which probes the complete genome for deformation of the DNA double helix (Kobow and Blumcke, 2014; Nakajima and Kunimoto, 2014; Wang et al., 2014). As a result, GG-NER is highly important for the recognition of the highly helix-distorting 6-4PPs, and their immediate repair (Wang et al., 2014). However, this particular nucleotide excision repair system poorly recognizes and removes CPDs which only mildly distort the DNA structure (Bilal et al., 2014). In humans, this oversight in DNA damage surveillance is compensated by the activation of the p53 protein, an important regulator of UV-induced cellular stress response (Bodega and Orlando, 2014; Saleh et al., 2000). The p53 protein induces upregulation of the p48 subunit of the DNA damage binding (DDB) protein to help in the recognition of CPDs and to mediate their repair (Asgari, 2014; van den Elsen et al., 2014).

From the information outlined above, it is clear that pyrimidine dimers are a main form of solar UV-induced DNA damage and that NER is the main line of defence against the genetic alterations that these dimers may cause in keratinocytes (**Figure 4**). Furthermore, it is important to note that these repair mechanisms facilitate an error-free repair of DNA using the template strand remaining at the CPD site. If the cellular damage persists, UVB elicits a fail-safe mechanism evidenced by the formation of sunburn cells (SBC), representing keratinocytes undergoing apoptosis.

Apoptosis Following UV Irradiation

As previously mentioned, when the DNA lesion is too extensive and beyond repair, cell apoptosis occurs and sunburn cells are formed to ensure the disposal of the damaged cells. The main function of sunburn cells, formed by a complex multifactorial process, is to reduce the risk of malignant transformation (Brash et al., 1996; Tian and Xu, 2015). Sunburn cells exhibit morphology characteristically observed in apoptotic cells, including pyknotic nuclei and cytoplasmic shrinkage (Laethem et al., 2005). Sunburn cells typically accumulate in the suprabasal and mid epidermal layers, particularly at later time points after UVB exposure (Babu et al., 2015). The morphological features of sunburn cells result from activation of the pro-apoptosis cysteine-protease family of caspases, which induce cleavage of cellular substrates and rapid disassembly of keratinocytes. Caspase activation in mammalian cells occurs via two principal pathways – extrinsic and intrinsic apoptosis pathway (Assefa et al., 2003; Kroemer et al., 1997; Lee et al., 2013).

The extrinsic caspase-activation pathway is initiated when ligands bind to membrane death receptors (DR), and an apoptosis-inducing signalling complex (DISC) is formed (Kar et al., 2014). DISC induces the recruitment of pro-caspase initiators, procaspase-8 and/or 10, which leads to the activation of caspase-8 and caspase-10, which in turn promote the processing and activation of downstream effectors, procaspases-3, 6 and/or 7 (Aragane et al., 1998; Reidl et al., 2007).

The “intrinsic” apoptosis pathway is induced via the permeabilisation of the outer mitochondrial membrane and the release of intermembrane space proteins, such as cytochrome *c*, which triggers the formation of the “apoptosome” (Clement-Lacroix et al., 1996; Wu and Cederbaum, 2008). Apoptosomes are molecular platforms composed of apoptosis protease-activating factor-1 (Apaf-1), dATP and cytochrome *c*, which recruit and activate procaspase-9. Caspase-9 then cleaves and activates the effector procaspases-3,-6,-7 (Kroemer et al., 1997; Laethem et al., 2005). Intrinsic apoptosis is critically regulated by pro-apoptotic genes such as *BAX*, *BAK* and *BAD*, and anti-apoptotic *Bcl-2* family members (Holley and St Clair, 2009; Wu and Cederbaum, 2008). Due to the ability of UVB to induce DNA damage, induction of sunburn cell formation is achieved via both procaspase activation pathways.

As previously mentioned, the presence of sunburn cells signifies a successful termination of DNA-damaged keratinocytes. Thus, extensive activation of caspase-3 and/or upregulation of pro-apoptotic genes, such as *BAX*, indicates that DNA damaged keratinocytes are appropriately controlled and are in the process of being eliminated from the epidermis. Increased presence of sunburn cells, as well as increased numbers of keratinocytes with active caspase-3 protein, are often observed after extensive UV exposure, and are indicative of the damaging effects of this radiation on the DNA (Assefa et al., 2003; Kroemer et al., 1997; Lee et al., 2013).

UV-Induced Keratinocyte Apoptosis and the p53 Protein

It is important to note that apoptosis following UVB exposure depends on the differentiation state of the keratinocyte, the dose of UVB irradiation and on the balanced presence of survival and death factors in the keratinocyte microenvironment. It is known that p53 is also an important factor in UVB-induced cell death in murine skin and cultured human keratinocytes (Brash et al., 1996; Henseleit et al., 1997). Differentiating populations of keratinocytes require p53 to mediate apoptosis as a result of UVB irradiation. Paradoxically, p53 or p53-regulated proteins also enhance DNA repair in the basal layer so as to maintain the proliferative potential of this cellular compartment (Narine et al., 2010; Tron et al., 1998).

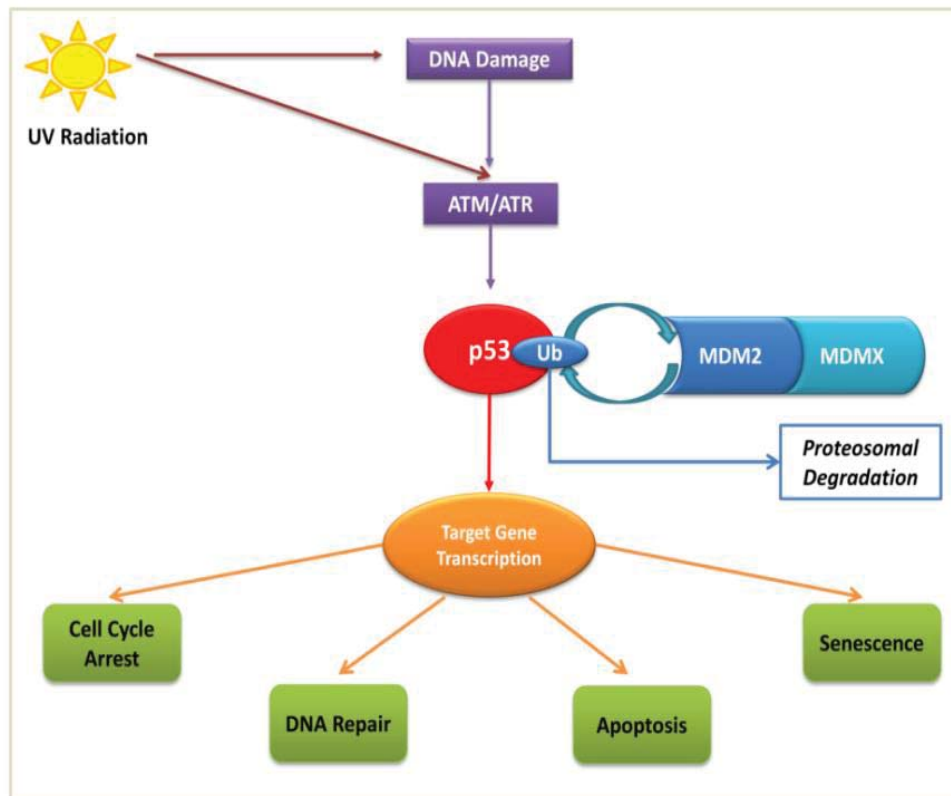


Figure 1.5: The p53 Signaling Pathway. Activation of the p53 protein (red) is initiated by checkpoint enzymes ATM/ATR. Activated p53 binds to target genes to regulate cellular processes. MDM2 induces ubiquitination (Ub) of p53, leading to the degradation of this protein. (Adapted from: (Chakraborty et al., 2011))

Upon UV-induced DNA damage, the tumour suppressor p53 proteins are stabilised, mainly through phosphorylation-mediated disruption of the MDM2-p53 complex, and translocate to the nucleus (Jimenez et al., 1999). In a cell- and stress-specific manner, the transcription factor p53 can trigger either cell-cycle arrest facilitating DNA repair, senescence or apoptosis, thus preventing damaged cells from becoming cancerous. It is important to note that p53-null mice (Attardi and Jaks, 1999) or p53-S389A mutants (Bruins et al., 2004), are more prone to develop UV-induced skin cancers and show a dramatic reduction in the number of apoptotic cells in the epidermis. Thus, the p53 protein appears to be a critical regulator of DNA sunburn cell induction. Due to its role in cell cycle arrest and DNA damage repair, *TP53* is one of the most commonly mutated genes in keratinocytes, and is considered an essential factor for the malignant transformation of keratinocytes.

The p53 Signalling Pathway in Keratinocytes

The *TP53* gene encodes the p53 transcription factor, a cellular protein that is a key tumour-suppressor (2008). As previously mentioned, this protein is involved in maintaining genomic stability, and can enforce a G1 cell cycle arrest or induce apoptosis in response to cellular stresses (Harris and Levine, 2005). As outlined above, cell cycle arrest at the G1 phase is very important as it allows for cellular repair pathways to remove damage before DNA synthesis and cell mitosis can begin (Terzian et al., 2010). Conversely, p53-induced apoptosis helps eliminate potential tumour cell progenitors. Thus, p53 protein activity is important for regulating keratinocyte biology and, in turn, maintaining skin integrity. P53 protein activity and its multiple roles are regulated by post-translational modifications, particularly phosphorylation and acetylation.

Post-translational Modifications and p53 Activation

While the p53 protein is normally expressed at low levels in normal cells, activation and expression of this protein increases in response to DNA damage induced by environmental stressors, particularly UV radiation (Smith et al., 2000). In particular, UVB-induced thymine dimer formation alters the integrity of the DNA template, resulting in the activation of UV damage signal transducers and checkpoint kinases such as Ataxia Telangiectasia (ATM), ATM and Rad-3 (ATR), Checkpoint Kinase 1 (Chk1) and (Chk2) enzymes (**Figure 1.5**). These enzymes phosphorylate p53 at one or more serine residues at the N- or C- terminus, resulting in the activation of the protein (Vogelstein et al., 2000). Once activated, the p53 protein translocates to the nucleus, where it binds to specific DNA sequences comprised of 1-21 nucleotides, termed the p53-responsive element, at the promoter of its target genes, to regulate target gene expression (**Figure 1.6**).

In addition to phosphorylation, acetylation of p53 at various lysine sites within its C-terminus is fundamental for p53 activation. Histone acetyltransferases CBP (KAT3A) and p300 (KAT3B) mediate the acetylation of the p53 protein at six lysine sites, including K381 and K382, which can enhance the DNA-binding ability of p53 and prevent its export from the nucleus (Li et al., 2011). Furthermore, acetylation of p53 by these enzymes also opposes the recruitment of MDM2 and MDM4, which are known repressors of p53, preventing p53 protein degradation.

The action taken by p53 is dependent on the level of DNA damage within cells (Cui et al., 2007). If minor DNA damage is detected, p53 protein expression increases and accumulates to inhibit cell division and promote repair before cell replication begins (Harris and Levine, 2005). If damage is excessive, the p53 protein actively induces transcription of genes that may direct cells to undergo apoptosis (Mullerat et al., 2003). As long as the TP53 gene is free from mutations or otherwise active, this system is maintained. However, once the gene itself accumulates mutations or the protein is otherwise incapacitated, by for example aberrant post-translation modifications, the system is disrupted and damaged cells may escape surveillance (Hussein et al., 2003).

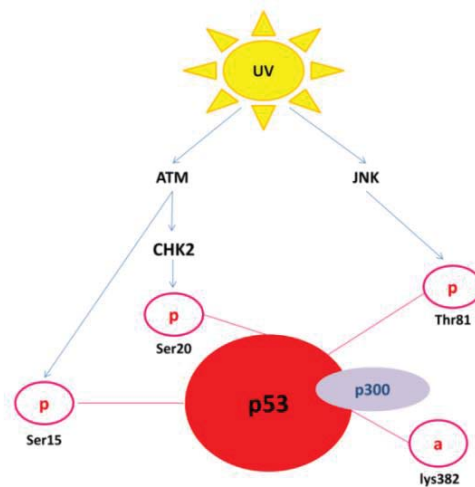


Figure 1.6: Activation of p53 by Post-Translational Modification. Checkpoint enzymes ATM/ATR and Jnk phosphorylate p53 and initiate activation of the p53 protein (red). p300 also induce activation of p53 via acetylation of lysine 382 residue at the p53 c-terminus. a=acetylation, p=phosphorylation, lys 382=lysine at position 382, Ser15 or Ser20=serine at position 15 or 20, Thr81= threonine at position 382

UV radiation, p53- inactivating mutations and Keratinocyte-derived skin cancers

The TP53 gene is commonly found to be mutated in the majority of human cancers. As mentioned above, expression of p53 in the nucleus is elevated after UV irradiation following a genotoxic insult, and is involved in cell cycle arrest (late G1 and G2/M), apoptosis and NER (Athar et al., 2000; Seo and Jung, 2004). In SCC and BCC pathogenesis, the TP53 gene accumulates UVB-induced point mutations, particularly at di-pyrimidic sites associated with C to T transitions and 5–10% of CC to TT tandem mutations. Extensive mouse experiments have confirmed that the TP53 gene is a target in UV-induced carcinogenesis, and it has been found that the wavelength dependency of the induction of SCC closely parallels that of the induction of pyrimidine dimers in the skin, especially those wavelengths within the UVB bands.

Experiments with hairless mice show that clusters of epidermal cells with mutant p53 occur long before SCC becomes visible. Similarly, clusters of mutant p53 have also been found in human skin (Dumaz et al., 1997; Grujil et al., 2001; Kanjilal et al., 1993). The presence of a dysfunctional p53 impairs protective responses against DNA damage and oncogenic signalling. Thus, the presence of *TP53* mutations may facilitate malignant progression of keratinocytes. Interestingly, a study has shown that BCC and SCC frequently show chromosomal aberrations such as loss of heterozygosity (LOH), originally thought to be the result of mutations in *TP53* (Rass and Reichrath, 2008; Ren et al., 1996b). In BCC, LOH is restricted to chromosome arm 9q. However, LOH is found to be more diverse in SCC, where aberrations are found at 3p, 9p, 13q, 17p, 17q. Actinic keratoses (AKs), which are considered precursors of SCCs, also carry extensive LOH and p53 mutations (Grujil et al., 2001; Ren et al., 1996a).

Although it was previously suspected that LOH is attributable to the presence of mutant p53, studies have shown that LOH can occur without any *TP53* gene mutations. These studies then beg the question whether LOH is caused by UVB-induced DNA damage directly, given the significant relationship between AKs, SCC and sun exposure. However, it is important to note that mutations in the *TP53* gene are not enough to cause BCC or SCC. At the very least some oncogenic pathway has to be activated (Brash, 2006)(de Grujil, 2008). In BCCs and SCCs, the deregulation of the Sonic hedgehog (SHh) pathway is commonly observed. SHh normally contributes to self-renewal of the skin and its appendages in adults, so its oncogenic constitutive activation leads to uncontrolled cell proliferation (Reifenberger et al., 2005).

1.3 Heat Stress and Skin Cancers

Apart from UV, heat stress is also hypothesised to be involved in skin carcinogenesis. The possible role of heat stress in skin carcinogenesis was postulated given the observed increased incidence of skin cancer among people constantly exposed to intense temperatures, such as mine workers (Fortes, 2008). Furthermore, epidemiological studies have shown significant correlation between elevated environmental temperatures and increased skin cancer incidences in the US (Freedman et al., 2015; van der Leun et al., 2008). However, the exact contribution of heat stress to skin carcinogenesis has yet to be determined.

Heat control is an important factor in homeostasis and is necessary for maintaining cellular functions (Kregel, 2002). The core body temperature of 37°C, sustains activity and transcription of various genes that are vital in cell differentiation and proliferation. While this temperature is maintained by innate homeostatic mechanisms that regulate internal temperature (Sonna et al., 2002), it is important to note that surface skin temperature, which is normally at 32°C, tends to be affected by environmental temperature. As a matter of fact, surface skin temperature has been found to increase to 40°C with direct exposure to sunlight (Lee et al., 2006). Thus, as the body is constantly exposed to thermal fluctuations in the ambient environment, some cells, particularly those located in the skin, may be subjected to increased temperatures (Jonak et al., 2006).

Cell exposure to elevated temperatures (heat shock), can trigger extensive denaturation, degradation and aggregation of critical intracellular proteins which can lead to cell death (Kregel, 2002). The deleterious consequences of intense temperatures is ameliorated by an adaptive response to ensure cell survival (Soti et al., 2005). The cell survival pathway activated by heat shock is the heat shock response, a cascade of events that lead to induction of heat shock proteins (HSPs) (Ciocca and Calderwood, 2005).

The Heat Shock Response

The heat shock response is primarily controlled by heat shock factor-1 (HSF-1) at the transcription level (Jolly et al., 2004). HSF-1 is present in the cytoplasm of normal cells as a monomer, which has no binding capabilities (King et al., 2001). However, when cells are under duress, monomeric HSFs combine to form trimers which translocate to the nucleus where they bind to heat shock elements (HSEs) (Morimoto, 2002), consensus sequences located in promoters upstream of heat shock genes (Kim et al., 2005). In the nucleus, HSF-1 is phosphorylated which allows it to bind to DNA, leading to transcription of heat shock genes and expression of HSPs, particularly HSP72 (**Figure 1.7**) (Guo et al., 2007).

Expression of HSPs protect cells from apoptosis and from further damage, via interaction with signalling pathways that control cell proliferation and apoptosis (Soti et al., 2005). HSPs are highly conserved proteins that are expressed in all cells. There are many types of heat shock proteins, but HSP90 and HSP72/HSp70 are the main proteins involved in the heat shock response (Volloch and Rits, 1999; Yaglom et al., 2007). These peptides have important roles in various cellular processes but their main function is to act as molecular chaperones, i.e. they bind to other proteins and mediate their folding, transport and protein-protein interactions (Jonak et al., 2006). In addition, HSPs have the ability to direct re-folding of denatured proteins, damaged as a result of heat shock, thus suppressing further damage. As a result, cells are stabilised and protected against damage (De

Maio, 2011). When the stresses are removed, HSP72 binds to HSF-1 to inactivate it through a negative feedback loop. HSF-1 then returns back to the cytoplasm as a monomer (Ciocca and Calderwood, 2005). The role of heat shock response in cell survival and evasion of apoptosis is further discussed in Chapter 2 of this thesis.

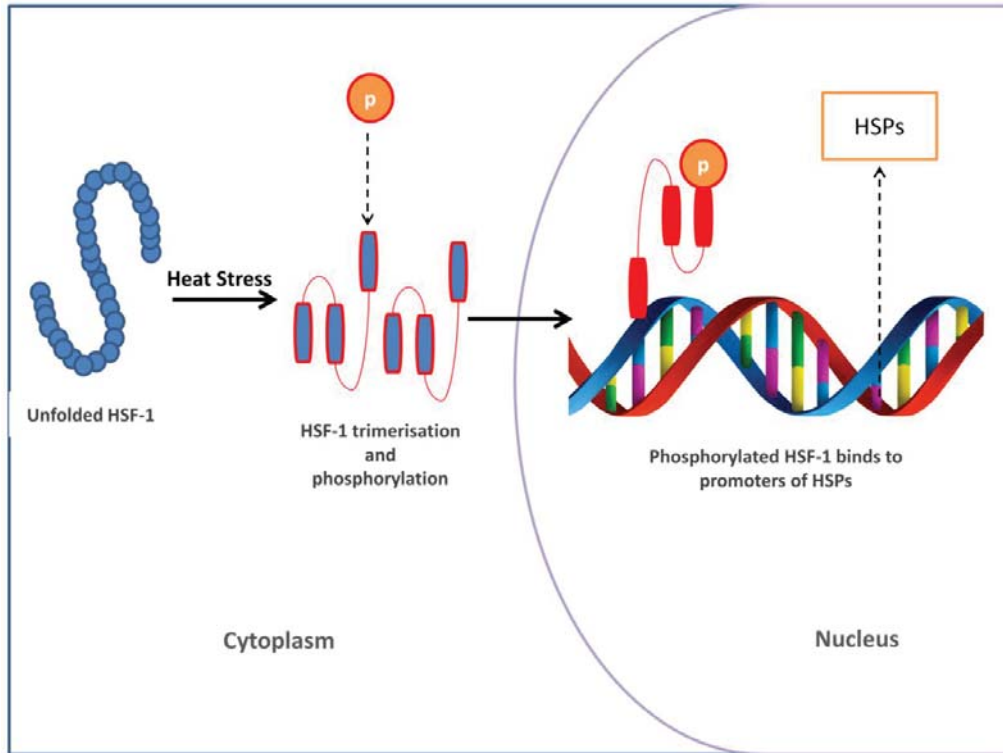


Figure 1.7: The Heat Shock Response

(Adapted from: (Pockley, 2003))

While HSP90 and HSP70 are vital for sustaining cellular integrity and protecting cells against UV-induced apoptosis, they are commonly overexpressed in a variety of cancers, including those of the skin (Nagata et al., 1999). In normal cells, activation of heat shock proteins, particularly HSP90, maintains the native conformation of its various client proteins, including p53, during thermal stress (Chen et al., 2002). Previous studies have shown that full-length HSP90 is able to stabilize p53 against thermal unfolding by binding to the DNA-binding domain of p53, thereby preserving the native conformation of this protein during thermal stress and maintaining its activation-competent state (Park et al., 2011b; Walerych et al., 2004). However, HSP90 can also bind to the DNA-binding domain of mutant p53, which could have implications for skin carcinogenesis (Diaz et al., 2010; Peng et al., 2001).

The HSP90 and mutant p53 interaction represents an altered use of chaperone proteins to protect mutant tumour-suppressor proteins. *TP53* mutations, which are the most common genetic defect observed in human cancers, can result in the expression of a p53 protein with an altered conformation and impaired cell-cycle-checkpoint activity (Whitesell and Lindquist, 2005; Whitesell et al., 1998). The aberrant conformations of mutant p53 do not prevent HSP90 from binding to its DNA-binding domain (Whitesell et al., 1998). The extended interactions, therefore, of p53 with the HSP90-chaperone machinery prevent the normal ubiquitination and subsequent degradation of p53 (Dai et al., 2007). This results in accumulation of dysfunctional protein within the pre-cancerous and in tumour cells, being a hallmark of cancer (Whitesell and Lindquist, 2005; Whitesell et al., 1998).

Previous studies have also shown that mutant p53 protein bound to HSP90 does not function as a tumour suppressor, and that the HSP90-mutant p53 complex can interfere with the functions of normal p53 by inducing formation of heterodimers, between normal and mutant p53, and inappropriate transactivation of p53-regulated target genes (Lin et al., 2008). Impairment of wild-type p53 functions, as a result of interference induced by the HSP90-mutant p53 complex, may help facilitate the upregulation of pro-proliferation and survival genes, normally controlled by this protein (De Maio, 2011; Whitesell et al., 2003). Thus, heat stress, via HSP90, may help contribute to skin cancer formation by promoting the dominant-negative and positive tumour-promoting effect of mutant p53. Thus, heat stress may not act as a primary driver of tumourigenesis but rather it may exacerbate the carcinogenic effects of UV, a known mutagen of *TP53*.

SIRT1 and the Heat Shock Response

The heat shock response can also be regulated by factors that facilitate chromatin stability, particularly SIRT1, a NAD⁺/NADH dependent histone deacetylase (Aarenstrup et al., 2008; Cao et al., 2009; Glozak and Seto, 2007; Westerheide et al., 2009). SIRT1 is capable of inducing compacted chromatin reformation through the removal of acetyl groups, and is highly important for maintaining chromatin stability (Fritah et al., 2009; Lou et al., 2009; Raynes et al., 2013a). However, SIRT1 is also capable of deacetylating various transcription factors, including HSF1, which is essential for cellular response to heat stress (Westerheide et al., 2009).

Recent studies have shown that SIRT1 deacetylates HSF1, resulting in prolonged binding of HSF1 to the *hsp70* promoter and subsequent prolonged induction of the heat shock response (Donmez et al., 2012; Fritah et al., 2009; Lou et al., 2015; Raynes et al., 2013a; Westerheide et al., 2009). Conversely, downregulation of SIRT1 promotes the attenuation of the heat shock response via increased HSF1 acetylation, which inhibits its DNA binding ability. Thus, SIRT1 activation may be an intrinsic component of the heat shock response.

Overexpression of SIRT1 was also found to mediate increased tolerance to high-temperature heat shock in 293T and A53T cells (Westerheide et al., 2009). SIRT1 has also been shown to work together with HSF1 to protect against α -synuclein pathology in Parkinson's Disease (Donmez et al., 2012). A recent study using a transgenic mouse model bearing the human α -synuclein gene with an A53T mutation, which causes familial early-onset Parkinson disease, showed that when A53T-mice were crossed with SIRT1 transgenic mice, SIRT1 prolonged the life span of the mice and decreased the level of α -synuclein aggregates in the mouse brain (Donmez et al., 2012). Interestingly, the molecular mechanism involved SIRT1 induced deacetylation of HSF1, leading to increased HSP70 levels in the brains of the A53T-mice. It is clear then that SIRT1 affects HSF1 activity, via deacetylation, resulting in increased HSP expression under cell stress conditions.

In addition, the interaction of SIRT1 with HSF1 has also been shown to be regulated by heat stress, as endogenous SIRT1 co-immunoprecipitates with HSF1 upon heat shock in mouse embryonic fibroblasts (MEFs) (Donmez et al., 2012). Furthermore, previous studies have shown significant correlation between SIRT1 protein overexpression and increased survival of cells, even at high temperatures (Raynes et al., 2013b; Westerheide et al., 2009). Thus, SIRT1 may play a role in mediating cell survival in the presence of heat. However, the exact mechanism as to how heat stress induces SIRT1 activation is yet to be determined. Nevertheless, recent reports showed that SIRT1 phosphorylation is important for survival of cells under stress conditions. Phosphorylation of SIRT1 has been found to increase its enzymatic activity, and has been shown to induce activation of HSF1 (Monteiro and Cano, 2011; Sasaki et al., 2008).

It is important to note that SIRT1 is also capable of deacetylating p53, particularly at K373 and K382, and affecting the DNA-binding ability of this protein (Aarenstrup et al., 2008; Cao et al., 2009; Glozak and Seto, 2007; Westerheide et al., 2009). Due to its active role in regulating cell proliferation and differentiation of keratinocytes (Boukamp, 2011), studies have shown that inhibition or inactivation of TP53 plays a large role in skin carcinogenesis (Madan et al., 2010). Thus, heat-mediated activation of SIRT1 may be involved in heat-mediated effects that lead to skin cancer pathogenesis.

As previously mentioned, inactivation of *TP53* by activating mutations prevents interaction of p53 to its downstream target genes, blocking p53-mediated transcriptional activation of these effectors and preventing induction of cell cycle arrest or apoptosis of DNA damaged cells. Mutations are not the only types of molecular alteration to the p53 protein that can inactivate its tumour suppressor functions. Post-translational modifications of p53, particularly deacetylation, can also affect the efficiency of this protein to bind to its downstream target genes and thus diminish its ability to regulate the cellular stress response. Interestingly, while heat stress has been found to increase SIRT1 activation, UV has been shown to inhibit it (Cao et al., 2009; Li et al., 2011; Westerheide et al., 2009). Thus, via SIRT1, exposure to heat stress, particularly in addition to UV, may result in the disruption of p53 function in epidermal keratinocytes, making them vulnerable to UVB-induced mutations.

Skin Cancer Pathogenesis and Heat Stress

While the role of UV radiation in skin carcinogenesis is undisputed, the effects of heat shock on cutaneous cancer pathogenesis remains controversial and undefined. Recent epidemiological studies in the US have shown significant increases in skin cancer incidence, particularly BCCs and SCCs, in geographical areas of high temperature (van der Leun et al., 2008). As a matter of fact, in their epidemiological report, van der Leun and colleagues (2008) suggested that the carcinogenic effect of UV appears to be potentiated by 2%, per 1°C increase in environmental temperature. Interestingly, Freedman et al (2015) also found a significant trend towards increased risk of BCCs in people living in areas with high UV radiation and elevated temperatures (approximately 34°C). Thus, these epidemiological results suggest that heat stress may have a role in skin carcinogenesis, possibly by exacerbating UV-induced risk of cutaneous tumour formation.

Despite the existence of epidemiological studies correlating heat stress exposure to increased risk of UV-induced cutaneous cancers, there is a paucity of biological studies aiming to uncover the short term and long-term effects of heat on epidermal cells. Nonetheless, previous studies have shown that pre-treatment with heat stress (38-40°C) increases cell viability and decreases thymine dimer formation in murine and human keratinocytes subsequently exposed to UV, suggesting heat-mediated protection of UV-exposed keratinocytes against damage (Kane and Maytin, 1995; Maytin, 1995; Maytin et al., 1993; Trautinger et al., 1996). Cell protection is hypothesised to be maintained by heat shock proteins (HSPs), particularly HSP72, which can directly interact with various survival kinases or proteins such as c-jun N-terminal kinase (Jnk) and Akt, thereby reducing UV lethality (Jantschitsch and Trautinger, 2003).

It is important to note, that previous studies only reported on the effects of a singular exposure to heat stress and UV on keratinocyte biology. Furthermore, the cells were subjected to heat stress prior to UV irradiation. Thus, this exposure model that may have induced the pre-activation of protective mechanisms against UV-induced damage in keratinocytes. Environmental conditions, however, involve continuous exposure to heat stress independently or concurrently and/or sequentially with UV radiation in several geographical locations. Thus, it is more prudent to determine the effects of repeated simultaneous exposure to heat and UV in human keratinocytes.

Of note, previous studies in mouse models have shown that simultaneous exposure to UV and high temperature increases the incidence and rate of cutaneous tumour formation in mice (Bain et al., 1943b; Freeman and Knox, 1964; van der Leun et al., 2008). Bain and colleagues have shown that white ABC mice concurrently exposed to UV plus heat developed high numbers of cutaneous tumours, which manifested after a short period of time, compared to mice exposed to UV radiation at room temperature (25°) or colder (3-5°C). Freeman et al. (1964) similarly showed significant increases in tumour development in white mice irradiated with UV at high temperatures. These biological studies suggest that exposure to heat and UV may increase the risk of skin cancer formation. However, these studies did not elaborate on the exact contribution or mechanism of heat stress in UV-induced skin carcinogenesis. Interestingly, despite the innate heat shock response in cells, exposure to high temperatures causes DNA damage. Heat stress has been observed to deaminate cytosine and hydrolyse glycosyl bonds, leading to genome instability (Bruskov et al., 2002; Lindahl and Nyberg, 1974; Poltev et al., 1990). In addition, exposure to heat stress can induce formation of reactive oxygen species, which can cause G to T transversion mutations (Bruskov et al., 2002; Ehrlich et al., 1986; Smirnova et al., 2005; Takahashi et al., 2004). Thus, exposure to heat stress may potentially increase the risk of skin cancer formation by contributing to the accumulation of mutations.

In order to generate a more accurate measure of the response of epidermal cells, particularly keratinocytes, to combined exposure to UV and heat, studies that aim to determine these effects need to use human skin models. Previous heat-related studies used primary keratinocytes *in vitro* and/or mouse models to determine the effects of thermal stress in these cells. There are biological differences to mice and humans and, thus, any heat-induced changes in mouse keratinocytes may not accurately reflect that of human cells. Furthermore, while *in vitro* studies have contributed greatly to our understanding of the consequences of environmental stress exposure on keratinocytes, use of primary cells pose significant limitations. In particular, primary keratinocytes are not in their natural environment and the lack of epidermal melanin complex, may affect their response to heat stress exposures. Melanocytes and keratinocytes appear to have a symbiotic relationship, which

allows them to maintain a level of protection against environmental stressors, particularly UV. Within a skin model, local immune responses are present and keratinocyte-melanocyte interaction is preserved, and thus, effects of heat stress on keratinocyte biology may be more accurately measured.

It is imperative that accurate heat-mediated molecular changes in epidermal cell biology are identified, in order to gain more understanding of its possible role in UV-induced skin tumourigenesis. Furthermore, by unravelling the mechanisms responsible for heat-mediated alterations in keratinocyte biology, preventative strategies may be developed to abrogate heat-induced changes in these cells, which will perhaps diminish the risk of skin cancer development.

1.4 Conclusion

In conclusion, the increasing incidence of people diagnosed with various forms of skin cancer annually, makes this disease one of the major health concerns across the globe. The lack of cure, especially for the deadly and metastatic forms such as melanoma and SCC, makes it imperative to determine all possible risk factors for skin cancer in order to decrease the probability of developing the disease. So far, studies have confirmed the role of UV in skin cancer formation. The knowledge gained from studying UV has allowed for the development of preventative strategies, which decrease the chances of developing skin cancer. However, the lack of information on heat, suggests that a potential threat currently goes unabated.

Heat stress has a dramatic effect on epidermal cells and can influence the activity of signal transduction pathways, particularly those that are important in regulating keratinocyte and melanocyte proliferation, survival, differentiation and apoptosis, all crucial in skin cancer initiation. Furthermore, heat has been shown to sustain and stabilise the activity of UV-transformed mutant p53, or activation of SIRT1 leading to inhibition of p53 activity indicating that heat can exacerbate the effects of UV. The order of heat and UV exposure maybe crucial to the downstream effects of heat – the dichotomy of this response may be the critical point that we need to understand and identify. For this reason, it is imperative that the role of heat in skin cancer formation be studied, alone or in synergism with UV radiation.

1.5 Theoretical Framework

In recent years, epidemiological studies have shown that heat stress may increase the incidence of UV-induced skin cancers (Freedman et al., 2015; Sun et al., 2008b; van der Leun et al., 2008). Thus, heat stress could be a potential risk factor for skin carcinogenesis. Studies of the effects of heat stress upon keratinocyte biology remain limited (Calapre et al., 2013). The few studies that are in the literature have shown that heat shock prior to UV irradiation can decrease the levels of UV-induced CPD formation and apoptosis in keratinocytes (Kane and Maytin, 1995; Trautinger et al., 1997). The heat-mediated protection against UV-induced DNA damage was suggested to be the result of the heat shock response activation and induction of heat shock proteins. However, the effects of combined exposure to UV and heat remain to be identified.

As chaperone proteins, HSPs can interact with essential DNA damage response proteins, particularly p53, and can increase their stability and maintain their conformation, ensuring the survival of cells (Zylicz et al., 2001). However, it is important to note that in the environment, particularly in geographical locations with arid climates, heat stress occurs concurrently with UV radiation. Thus, it is important to determine the effects of exposure to UV *plus* heat in human epidermal cells.

Previous studies on mice have shown that concomitant exposure to UV and heat stress increases the formation of skin lesions (Bain et al., 1943b; Freeman and Knox, 1964; van der Leun et al., 2008). Furthermore, previous studies have shown that heat stress increases SIRT1 protein activity (Raynes et al., 2013a; Westerheide et al., 2009). SIRT1 is known to deacetylate p53, affecting the ability of this protein to bind to its downstream target genes (Kim et al., 2007). Furthermore, HSP90 appears to affect the ability of wild type and mutant p53 functions to regulate the transcription of its target genes. Thus, with subsequent inhibition of DNA damage repair and apoptosis, exposure to heat stress after or in combination with UV, could lead to impairment of p53-mediated cellular stress responses in keratinocytes, leading to survival of UV-damaged cells.

This study was purposely designed to examine and understand the effects of heat stress, alone or in conjunction with UV irradiation, on human keratinocyte biology, particularly the regulation of DNA damage repair, apoptosis and proliferation in these cells. Furthermore, we also aimed to determine whether heat stress can specifically stimulate activation or inactivation of pathways associated with skin cancer initiation, such as the p53 signalling pathway, or if heat-induced changes are solely mediated by the heat shock response, or heat-induced post-translational modification to the p53 protein. This knowledge is of particular importance given that the majority of the Australian population are constantly exposed to extreme temperatures and UV. Understanding the potential biological consequences of UV and heat on keratinocytes can be used to develop preventative strategies that will decrease the risks associated with heat exposure, which may significantly impact the incidence of skin cancer in Australia.

1.6 Hypothesis

- Repeated exposure to UV *plus* heat will induce a significant increase in DNA damage formation in keratinocytes. Heat enhances the processes activated by UV radiation to induce molecular pathways in keratinocytes which are associated with skin cancer development such as increased survival, proliferation of DNA damaged cells, and a decrease in apoptosis.

1.7 Aims

1. To explore heat-mediated molecular changes and their impact on known regulators of cellular stress response, proliferation and apoptosis in epidermal cells.
2. To determine if repeated exposure to heat stress and/or UV *plus* heat, induces changes in keratinocyte biology, including apoptosis, proliferation and DNA damage formation in keratinocytes.
3. To identify key molecular changes involved in or responsible for heat-mediated changes in keratinocytes, particularly heat-mediated regulation of HSPs and SIRT1 activation, and their effects on the p53 signalling pathway.

At the author's request,
Chapters 2, 3 and 4 are not available in this version of the thesis.

GENERAL DISCUSSION

CHAPTER 5: Heat Stress and Skin Carcinogenesis

The rising incidence of keratinocyte-derived skin cancers (NMSC) is a significant health and economic burden in Australia (Makin, 2011). UV radiation, particularly UVB, is a known risk factor for skin cancers, contributing to approximately 80% of all cutaneous tumour formation (Boros et al., 2015; Courdavault et al., 2005). UVB induces formation of cyclobutane pyrimidine dimers (CPDs), particularly in areas enriched for methylated cytosines in DNA (Berg et al., 1995; Boros et al., 2015; Courdavault et al., 2005; Hochberg et al., 2006). The persistent presence of CPD lesions can lead to morphological and genetic alterations in epidermal keratinocytes, which in turn causes deregulation of signalling pathways associated with regulating cell apoptosis and proliferation, resulting in the formation of cutaneous tumours (Courdavault et al., 2005). Interestingly, epidemiological studies in the US have found that there is a significant correlation between increased rates of skin cancer and geographical locations with high environmental temperatures (van der Leun et al., 2008). Thus, heat stress could also be a possible risk factor for skin carcinogenesis.

There is limited research aimed at determining the biological effects of heat stress on epidermal cells. Nevertheless, previous studies have shown that exposure to heat stress, for 1 hour at 38-40°C, prior to UVB irradiation, protects keratinocytes against UVB-induced DNA damage (Kane and Maytin, 1995; Maytin et al., 1994; Trautinger et al., 1995). However, concomitant exposure to UV and heat in mice lead to increased skin cancer incidence (Bain et al., 1943b; Freeman and Knox, 1964). Since Exposure to UVB and heat stress is often experienced either synchronously and/or consecutively in the environment, it is more crucial to determine the effects of repeated and concomitant exposure to UVB radiation *plus* heat stress on human keratinocytes biology and whether the order of exposures present a significantly different outcome.

Altogether, this project aimed to address the effects of consecutive exposure to UVB *plus* heat in keratinocytes. In order to generate a more accurate measure of the response of keratinocytes to combined exposure to UV and heat, this study aimed to determine these effects using normal human skin models and *in vitro* primary keratinocytes. Keratinocytes were evaluated in particular for DNA damage formation, cell survival and apoptosis. Furthermore, this research also aimed to identify molecular mechanisms that underpin the biological changes induced by sequential exposure to UVB *plus* heat in keratinocytes, in order to gain a better understanding of the possible role of heat in skin carcinogenesis.

5.1 Effects of UVB *plus* Heat Exposure on Keratinocyte Biology

UVB plus Heat and CPD damage

It has been previously shown that cumulative exposure to high doses of UVB radiation induce significant DNA damage, in the form of CPDs, in keratinocytes (Bertrand-Vallery et al., 2010; Rass and Reichrath, 2008). Here we examined keratinocytes two days post multiple UVB then heat exposures, to determine the overall persistent consequences of these exposures to these cells (**Chapter 3**). As expected, significantly high numbers of keratinocytes harboured CPD damage two days post-UVB exposure. Interestingly, a high proportion of UVB *plus* heat-treated keratinocytes, in the skin and *in vitro*, also harboured CPD damage. These results are in direct contradiction to those observed previously, in which pre-treatment with heat shock, and subsequent activation of the heat shock response (HSR) mechanisms, was shown to diminish UVB-induced DNA damage formation in keratinocytes (Jantschitsch et al., 1998; Kane and Maytin, 1995).

Heat-mediated induction of HSR is known to elevate the expression and activity of heat shock proteins (HSP), particularly HSP70 and HSP90 (King et al., 2001; Walerych et al., 2010; Yoshihisa et al., 2012). HSP70 and HP90 are thought to prevent CPD formation by UVB in keratinocytes, and thus diminish the lethality of UVB in these cells (Hunt et al., 2007; Jantschitsch et al., 1998). The persistent presence of CPDs in keratinocytes in our UVB *plus* heat treated samples seem to suggest inactivity of HSP70 and HSP90. However, analysis of HSP90 and HSP70 mRNA levels in UVB *plus* heat-treated keratinocytes soon after exposures (4 hours) showed that HSR was evident, and HSPs were active at this time.

Increased HSP90 mRNA levels (data not shown) was observed in heat and UVB *plus* heat-treated keratinocytes soon after exposures, indicating that the heat shock response in cells is not compromised by combined exposure to UVB and heat stress. However, the upregulation of HSP90 was not sustained 2 days after exposure, nor did heat exposure subsequent to UV prevent CPD damage formation, as UVB and UVB *plus* heat treated sample were observed to have similar proportions of CPD damaged keratinocytes at 4 hours post exposure. Thus, while the heat shock response is functional, its role in cytoprotection appears to be overcome by repeated exposure to UVB then heat stress.

The discrepancy between our findings and previously published results is likely a result of the differences in the exposure models. It is important to note that previous studies adhered to a single heat-then-UVB exposure experimental protocol, with a four-hour interval between heat and UVB exposures (Jantschitsch et al., 1998; Kane and Maytin, 1995; Maytin et al., 1994). Pre-treatment to heat stress prior to UVB irradiation activates the heat shock response and HSP70 (Jantschitsch and Trautinger, 2003; Roti Roti, 2007), and thus, these cells were provided with a pre-established protective mechanism against UVB-induced DNA damage. By contrast, our study was based on repeated (4x) instantaneous succession of UVB (1 KJ/m²) and then heat exposure (39°C) for three hours, interestingly produced a significantly different experimental outcome. In a sequential UVB *plus* heat exposure model, the heat shock response is not pre-established in keratinocytes. Furthermore, repeated exposure to high doses of UVB *plus* heat may have overturned the protective effects of heat against CPD formation in keratinocytes. Thus, despite activation of heat shock response, as seen at four hours post-exposure, UVB-induced DNA damage was allowed to accumulate in significant numbers of keratinocytes.

Our results provide evidence to show consecutive exposure to UVB *plus* heat affects the ability of these cells to mount an appropriate response to UVB-mediated DNA damage in keratinocytes. In nature, UVB and heat stress often occur in tandem and can, therefore, synchronously affect epidermal cells. Due to limitations, this study was only able to conduct experiments based on a consecutive UVB *plus* heat exposure. While we tried to ensure that keratinocytes were treated with heat immediately after UVB irradiation, our results may still not be an accurate representation of how the epidermal cells respond to multiple concurrent exposures. It is likely that in a concurrent model, there will be an equal proportion of cells protected from DNA damage and surviving cells with DNA lesions.

It is also important to note that previous heat-related studies commonly used primary keratinocytes *in vitro* and/or mouse models, while this study was the first to use human skin models in a heat and/or UV-related experiments. Due to the biological differences between mice and humans, murine keratinocytes were thought to not necessarily provide an accurate representation of the effects of thermal stress and UVB on these cells. Furthermore, while *in vitro* studies have contributed greatly to our understanding of the consequences of environmental stress exposure on keratinocytes, use of primary cells does pose significant limitations. As mentioned previously, primary keratinocytes *in vitro* lacks interaction with melanocytes, and the lack of an intact epidermal-melanin complex was thought to affect the

response of primary cells to heat and UVB exposures. Interestingly, we found no significant differences in the way skin and *in vitro* keratinocytes responded to UV and heat stress, suggesting that changes induced by combined exposure to these stressors are independent of the epidermal-melanin complex. Our studies thus show that *in vitro* primary keratinocytes are a useful biological model for some UVB *plus* heat-related studies in keratinocytes.

UVB plus Heat, DNA Damage Repair and Cell Survival

This study was the first to report the UVB *plus* heat mediated survival and proliferation of DNA damaged keratinocytes. As mentioned extensively in this thesis, despite the persistent high levels of DNA damage, a high proportion of UVB *plus* heat treated keratinocytes expressed ki67 protein compared to those irradiated with UVB. Thus, despite significant DNA damage, consecutive exposure to heat stress appears to promote proliferation of keratinocytes in UVB *plus* heat treated samples. Interestingly, the high number of CPD keratinocytes that persisted in UVB *plus* heat-treated samples, suggests possible impairment of the DNA damage repair mechanisms. Normally, formation of CPDs induces p53-mediated cell cycle arrest and the activation of nucleotide excision repair (NER) mechanisms (Cheo et al., 1996; Hall et al., 2015).

Nucleotide excision repair facilitates the recognition of DNA damage, via activation of *XPC*, with repair of the DNA lesions and elimination of CPDs in the helix by the *ERCC1* exonuclease (Maytin et al., 1993; Rass and Reichrath, 2008; Rocca et al., 2010; Takahata et al., 2015; van Steeg and Kraemer, 1999). In our study, significant downregulation of *XPC*, a fundamental DNA damage surveillance protein (Cheo et al., 1996; Pines et al., 2009), and *ERCC1*, an exonuclease that facilitates removal of DNA lesions (Nagai et al., 1995; Yang et al., 2007), was observed in UVB *plus* heat treated keratinocytes. These results appear to confirm that exposure to both UVB and heat impairs the ability of keratinocytes to recognise and excise CPD lesions. Impairment of DNA damage repair has not been reported previously as a possible consequence of UVB *plus* heat exposures. This study is therefore the first to show that heat exposure not only ameliorates UVB-induced apoptosis, it also incapacitates mediators of nucleotide excision repair, and thus affects the efficiency of DNA damage surveillance and repair in keratinocytes exposed to UVB *plus* heat.

Heat-mediated survival of UVB-damaged keratinocytes and impairment of the nucleotide excision repair system have potential grave consequences. By allowing keratinocytes with DNA lesions to evade apoptosis, as well as affecting the efficiency of the nucleotide excision repair mechanism to recognise and repair CPDs, heat stress may permit the persistence of mutations and/or contribute to the accumulation of mutations in skin cells. Indeed, Martinconera et al. (2015) recently reported that the burden of UV-signature somatic mutations, averaging at two to six mutations per megabase per cell, are prevalent in 'normal' sun exposed skin. They found that most of the key drivers of cutaneous cell carcinomas are under strong positive selection in normal skin, with mutations found in 18-32% of normal skin cells. Thus, high numbers of cells, carrying cancer-inducing mutations, are present in normal epidermis. Accumulation of driver mutations is the main mechanism that underpins tumour evolution. Thus, with subsequent exposures, heat stress, in addition to UV, may exacerbate the formation of cutaneous malignancies and enhance the frequency of their development.

5.2 Molecular events underpinning UVB plus heat-mediated survival of DNA damaged keratinocytes

Inactivation of p53 signalling

The p53 protein is an important transcription factor involved in maintaining genome integrity upon exposure to UVB, either by enforcing a G1 cell cycle arrest, which induces apoptosis or enhancing nuclear excision repair of damaged cells (Abrahamson et al., 1995; Harris and Levine, 2005; Hermeking et al., 1995; Leontieva et al., 2010; Livingstone et al., 1992; Ozaki and Nakagawara, 2011; Pellegata et al., 1996; Reed and Quelle, 2014; Wan et al., 2015). While expressed at low levels in normal cells, exposure to damaging UVB radiation leads to a rapid increase in p53 protein levels, resulting in accurate chromosome segregation and prevention of replication of cells harbouring DNA damage (Athar et al., 2000; de Gruijl et al., 2001; Geyer et al., 2000; Henseleit et al., 1997; Huang et al., 2013; Nakaya et al., 2000; Prost et al., 1998; Soehnge et al., 1997).

As expected, in our experiments, UVB irradiated keratinocytes of *ex vivo* human skins and *in vitro*, showed increased cellular damage, and activation of cellular stress responses and pro-apoptotic signalling pathways, including significant p53 activation and the high level of caspase-3, a protease involved in apoptosis of damaged cells (Bratton and Salvesen, 2010; Bushell et al., 1999; Cagnol et al., 2011; Porter and Janicke, 1999). Consequently, the

significant increase in cell apoptosis and necrosis in our UVB irradiated samples soon after exposures and at longer time points, suggest that the p53 protein is functional and is inducing the appropriate cellular stress response to UVB-induced DNA damage.

By contrast, keratinocytes treated concurrently with UVB *then* heat exhibited reduced numbers of apoptotic cells. Most importantly, while the whole genome transcriptome analysis, using the Ingenuity Pathway Analysis (IPA) program, revealed a significant persistent activation of p53-mediated stress response in UVB irradiated cells, p53 signalling was found inactivated in UVB *plus* heat treated cells (Chapter 3). Thus, UVB *plus* heat-mediated survival and reduction of apoptosis of keratinocytes with CPDs appears to be a consequence of impairment of p53 signalling in these cells.

The inactivation of p53 signalling was initially thought to be a result of inhibition of the p53 protein transcription. However, soon after exposures when the cellular stress response and transcription of p53 is engaged, the proportion of cells expressing the p53 protein in UVB *plus* heat treated samples was found comparable to those irradiated with UVB. These results indicate that heat stress does not interfere with the induction of p53-mediated response to DNA damage. UVB *plus* heat samples, however, displayed characteristics that are attributable to inactivation of p53 by post-translational modifications at all time points. Diminished efficiency of p53-mediated cellular stress response in UVB *plus* heat appears to be due to possible impairment of the levels of the protein and/or DNA-binding capability of the p53 protein.

As discussed extensively in Chapter 1, acetylation of p53 enriches the DNA-binding ability of this protein to the promoters of its downstream effectors, resulting in significant upregulation of its target genes (Lu et al., 1998; Wang et al., 2004; Nakano et al., 2005). However, deacetylation renders the p53 protein incapable of regulating transcription of downstream target genes, leading to significant inefficient regulation of cell apoptosis and proliferation (Grossman et al., 2001; Lewis et al., 2008; Wang et al., 2004). We observed a lack of acetylated p53 staining in a high proportion of UVB *plus* heat treated keratinocytes, as well as the deregulated expression of downstream target genes, including *BAX* and *Survivin*, in these samples at all time points. Thus, these results provide significant evidence to support that UVB *plus* heat impairs p53 signalling via post-translational modifications to the p53 protein. Increase in *Survivin* activity has previously been found to disrupt cleavage of caspase-3 (Grossman et al., 2001; Lewis et al., 2008; Wang et al., 2004). Thus, heat-mediated upregulation of *Survivin*, because of post-translational modifications to p53, may have induced significant numbers of surviving keratinocytes at early time points and, as a consequence, this may have contributed to the significant cell proliferation observed at later time points.

SIRT1 and SIRT1-mediated p53 deacetylation

Another important factor in p53 signalling is SIRT1, which can affect the acetylation status of the p53 protein (Botta et al., 2012; Cao et al., 2009; Cheng et al., 2003). Significant activation of SIRT1 was observed in UVB *plus* heat treated samples. SIRT1 is a key regulator of cellular processes via its roles in the determination of chromatin structure, chromatin remodelling and regulation of gene expression. SIRT1 functions are achieved by targeted deacetylation of key regulatory proteins, including FOX1, HSF-1 and p53 transcription factors (Glozak and Seto, 2007; Lee and Gu, 2013; Yi and Luo, 2010). SIRT1 facilitates deacetylation of lysine 382 at the c-terminal end of the p53 protein, which renders this protein unable to bind to its downstream gene targets (Botta et al., 2012; Cao et al., 2009; Cheng et al., 2003). Thus, inactivation of p53 signalling in UVB *plus* heat-treated keratinocytes, and consequent lack of apoptosis and cell cycle arrest, was hypothesised to be mediated by SIRT1-induced deacetylation of the p53 protein.

Phosphorylation is a necessary requirement for SIRT1 activation and is important for survival of cells under stress conditions. Phosphorylation of SIRT1 increases its enzymatic activity, and has been shown to induce activation of HSF1 (Monteiro and Cano, 2011; Sasaki et al., 2008). SIRT1 activation was not observed in UVB irradiated samples alone, corresponding to previous studies showing that SIRT1 is inhibited by UVB (Chou et al., 2013a). However, an increase in SIRT1 activation was observed in heat treated samples, which is consistent with previous observations that SIRT1 activity is increased after heat shock (Fritah et al., 2009; Raynes et al., 2013b; Westerheide et al., 2009). It is important to note that in UVB *plus* heat, the balance between these exposures favours the activating effects of heat stress on SIRT1. Thus, SIRT1 activation appears to be an intrinsic component of the UVB *plus* heat-mediated cellular stress response.

Interestingly, in the UVB *plus* heat-treated samples, the significant increase in SIRT1 phosphorylation directly correlated with the increase in p53 deacetylation and deregulation of expression of known p53 downstream gene targets. As a matter of fact, co-expression of both phosphorylated SIRT1 and Survivin protein were observed in UVB *plus* heat treated keratinocytes. Thus, SIRT1 activation appears to mediate survival of UVB *plus* heat-induced survival of keratinocytes by causing deacetylation of p53 and, consequently, inactivation of the p53 signalling pathway.

Inhibition of SIRT1, on the other hand, was found to induce a significant increase in p53 acetylation and activation of p53 signalling. The re-activation of the p53 signalling pathway corresponds to our observed increase in cell apoptosis and necrosis in UVB *plus* heat treated keratinocytes. Thus, these results showed that UVB *plus* heat-mediated survival of DNA damaged keratinocytes was alleviated when SIRT1 was inhibited in these samples. From these results, it is apparent that SIRT1 plays a critical role in the heat-mediated anti-apoptotic and pro-survival effects of UVB *plus* heat exposures on DNA damaged keratinocytes.

UVB plus Heat-induced SIRT1 Pathway in Keratinocytes

In summary, this project provides evidence that exposure to heat stress, in addition to UVB, can induce significant alterations in keratinocyte biology, particularly in the manner in which these cells respond to UVB irradiation. Our results indicate that while UVB *plus* heat stress did not prevent formation of DNA lesions, it promotes an alternative pathway that provides DNA damaged keratinocytes with the capacity to survive and proliferate. In this thesis, we propose a SIRT1 pathway that is mediated by exposure to UVB *plus* heat. As shown in the diagram below (**Figure 5.1**), when keratinocytes are exposed to UVB *plus* heat exposure, the increase in temperature activates SIRT1, which then subsequently deacetylates p53, allowing cells to circumvent apoptosis and survive.

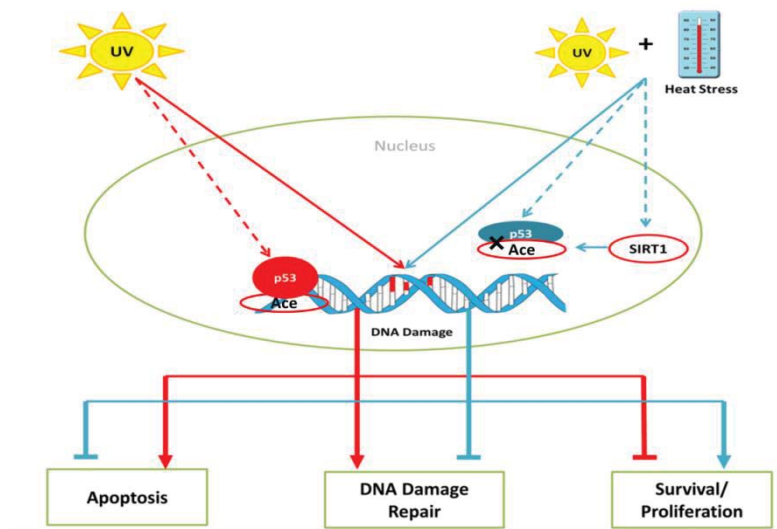


Figure 5.1: *UVB plus Heat-Induced Molecular Pathway for the Survival of DNA Damaged Keratinocytes.* UVB radiation significantly increased p53 protein activity (p53 acetylation, red), leading to increased levels of apoptosis and DNA damage repair together with inhibition of pro-proliferation and survival mechanisms (red line). Heat-mediated SIRT1 activation (red circle) induces inactivity (deacetylation) of p53 (blue), resulting in inhibition of apoptosis and survival of damaged keratinocytes.

While the effects of UVB *plus* heat exposure on p53 signalling were expected, as outlined in our review (Chapter 2), the role of SIRT1 in heat-mediated changes in keratinocytes is an interesting finding. Initially, it was originally hypothesised that any heat-mediated changes in keratinocyte biology would be induced by the heat shock response, an evolutionary conserved mechanism for cytoprotection against high temperatures (de Maio et al., 1996; King et al., 2001; Guo et al., 2007) (Chapter 2). Due to the effects of HSP72 and HSP90 on the JNK, p53 and PI3K/Akt pathways (Hagn et al., 2011; Park et al., 2011a; Walerych et al., 2010), an increase in the activity of these heat shock proteins is thought to mediate the protective effects of heat stress on epidermal cells.

In our study, we observed overexpression or activation of the heat shock response mediators, particularly HSP90, in our UVB *plus* heat-treated samples soon after exposures; however, these changes did not persist at two days post exposure, even though significant differences in keratinocyte apoptosis and necrosis were still prevalent between UVB and UVB *plus* heat treated samples. We did, however, observe a persistent significant upregulation of HSF-1, the mediator of the heat shock response (Chapter 3) and HSP70 (HSPA) at later time points (data not shown), which is known to attenuate UV-induced apoptosis. HSP70 induces activation of Jnk phosphatase, resulting in the subsequent inhibition of Jnk protein activity, and prevents recruitment of procaspase-9, which inhibits subsequent activation of caspase-3, to ensure cell survival (Kregel, 2002; Beeree et al., 2000). Thus, HSPs also appear to contribute to the survival of UVB *plus* heat-damaged keratinocytes in response to heat. However, despite their activity, HSPs do not appear to be the central mechanism associated with UVB *plus* heat-mediated evasion of apoptosis in keratinocytes. Nevertheless, in depth query and better interrogation times need to be defined in future studies to determine whether HSPs have a more substantial role in UVB plus heat-mediated survival of DNA damaged keratinocytes.

Interestingly, inhibition of SIRT1 induced significant downregulation of HSP90, despite the presence of heat stress. This finding was similar to previous reports where diminished expression of HSPs were observed when HeLa cells were exposed to nicotinamide, another Sirt1-inhibitor drug (Westerheide et al., 2009). SIRT1 was found to deacetylate HSF-1, increasing its DNA binding affinity to the promoters of heat shock proteins and thus causing significant increase in HSP expressions (Westerheide et al., 2009). Thus, our study further reinforces the importance of SIRT1 in thermal stress response. However, the exact mechanism as to how heat induces its activation needs to be determined.

5.3 Future Directions: Induction of Skin Cancer by UVB *plus* Heat Stress - SIRT1-Mediated Inactivation of p53 Signalling pathways

This project both re-affirmed some of the reported effects of heat stress, and offered new perspectives on its effects in keratinocyte biology. However, further studies are required in order to validate the findings of this project. In particular, while this study is the first to report a possible role for SIRT1 in UVB *plus* heat mediated changes in keratinocytes, it is still unclear as to how heat stress induces significant SIRT1 activation. Previous studies have hypothesised that an increase in SIRT1 activity after heat stress could be due to significant formation of heat-generated reactive oxygen species, which can cause destabilisation of chromatin and induce activation of the Sirtuin 1 protein (Raynes et al., 2009; Westerheide et al., 2009; Donmez et al., 2012). However, these scientific theories need to be investigated. In addition, cell mediators responsible for SIRT1 activation needs to be identified.

In addition, this study was only able to conduct experiments based on a consecutive UVB *plus* heat exposure model. As previously mentioned, environmental exposure to UVB and heat is not a linear progression of events. Heat stress may be experienced prior to, concurrently or post UVB irradiation. While previous research and this study offer perspectives on the effects of heat stress in keratinocytes when it is provided before or after a UVB respectively, experiments using simultaneous and long-term UVB *plus* heat exposures are necessary to have an accurate measure of the changes induced by exposure to combination of these stressors in human skin physiology. Nonetheless, the dichotomous change in outcome, dependent on the order of exposure is significant and of profound interest to skin cancer biology.

In particular, the significant negative effect of heat stress on the regulation of apoptosis in severely UVB- damaged keratinocytes appears to impose a greater risk of cutaneous malignant transformation, including skin cancers. As previously mentioned, a recent study has shown that 18-32% of normal skin cells are burdened with UV somatic mutations (Martinconera et al., 2015). Thus, heat-mediated circumvention of apoptosis response in keratinocytes, particularly those containing DNA damage, could lead to enhanced accumulation of mutations and transformation of normal keratinocytes to malignant cells (**Figure 5.2**). However, translational experiments, which focus on SIRT1-mediated survival pathways, found prevalent in our UVB *plus* heat treated keratinocytes, are necessary to determine if heat can in fact enhance formation of UVB-induced cutaneous malignancies *in vivo*. Based on our results, we hypothesise that heat will not be the primary inducer of mutations responsible for the deregulation of signalling pathways controlling keratinocyte apoptosis and proliferation. Rather, heat stress, which induces SIRT1 protein activation and then post-translational modification to p53, is thought to aid and exacerbate UVB-induced skin carcinogenesis.

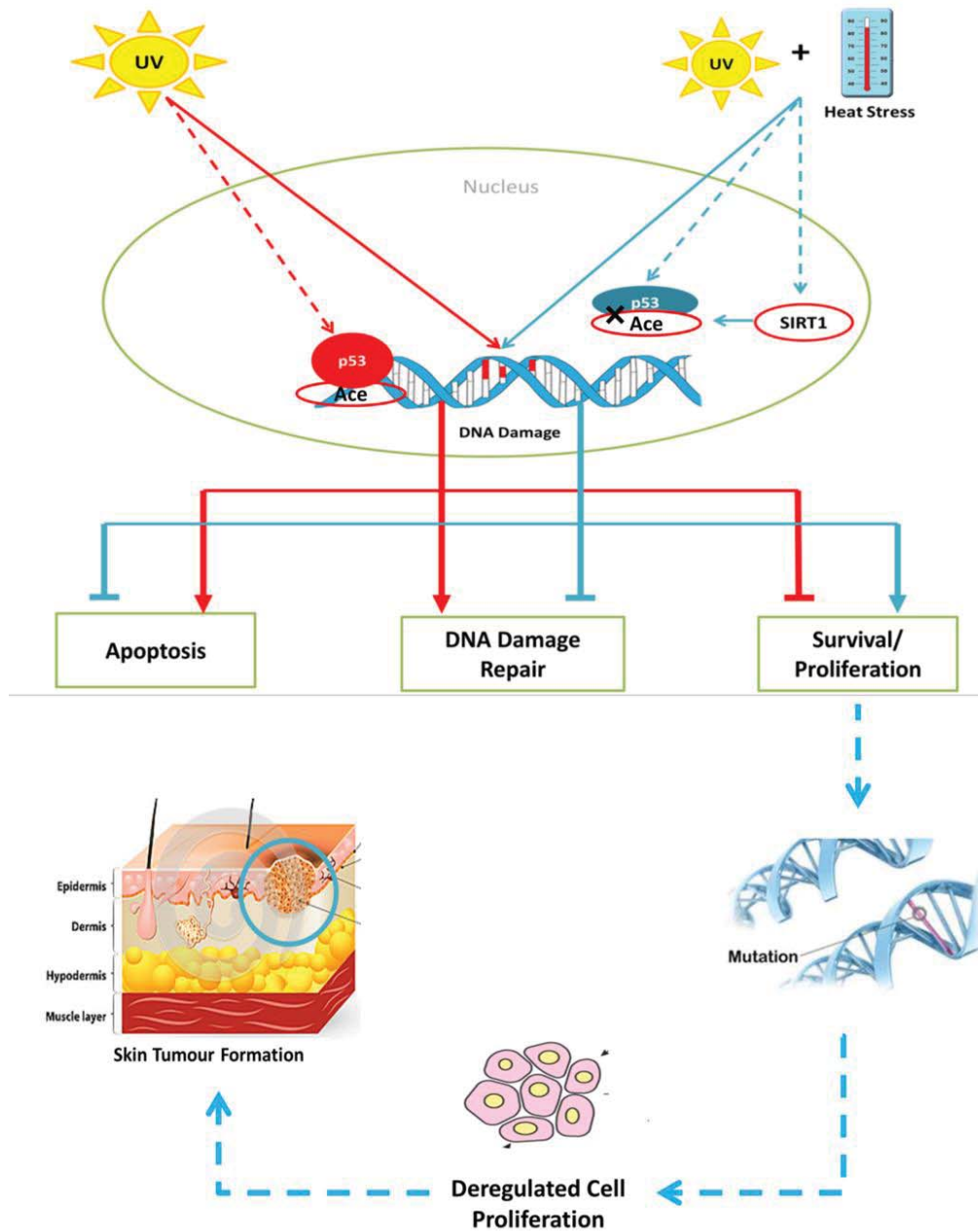


Figure 5.2: *UVB plus Heat-Mediated Skin Carcinogenesis.* Heat-mediated SIRT1 activation is hypothesised to induce inhibition of p53 signalling pathways, leading to survival of damaged keratinocytes, accumulation of mutations in these cells and skin tumour formation.

GENERAL CONCLUSION

In conclusion, the increasing incidence of people diagnosed with various forms of skin cancer annually makes skin cancers one of the major health concerns in Australia. So far, studies have confirmed the role of UVB radiation in skin cancer formation. Now together with previous studies and those described here, knowledge of the role of heat has been advanced and its dramatic effect on cells and influence on the activity of p53 signalling pathways, which are important in coordinating cell cycle arrest and DNA damage repair in keratinocytes while regulating proliferation, survival and apoptosis of these cells, are better understood.

In this study, we showed that the efficiency of response to cellular damage mediated by UVB is diminished in the presence of heat and, for the first time, provide a molecular mechanism that explains these effects of heat in the presence of UVB-induced DNA damage. With the novel use of an *ex vivo* human skin model, this study showed that heat stress prevents human keratinocytes, damaged by UVB irradiation, from undergoing apoptosis. Furthermore, UVB *plus* heat exposure mediates SIRT1 activation which has been found to induce deacetylation of p53 and, consequently, the inactivation of p53 signalling pathway. SIRT1 activation is considered the main molecular mechanisms driving UVB *plus* heat-induced survival of DNA damaged keratinocytes.

Overall, the results of this study suggest that by allowing the survival of CPD damaged keratinocytes, via induction of SIRT1 activation, heat stress can exacerbate the carcinogenic effects of UVB. Thus, exposure to heat stress, in addition to UVB, could increase the accumulation of mutations in keratinocytes, possibly leading to the transformation of normal cells into pre-cancerous cells. However, further research is warranted to determine the role of UVB *plus* heat in skin cancer pathogenesis. Knowledge of the effects of UVB *plus* heat stress on skin carcinogenesis can be utilised to decrease risk exposures particularly for people exposed to combinations of these environmental hazards in workplaces such as the mining, construction and petroleum industries.

REFERENCES

- (2013). Climate Data Online, A.B.o. Meteorology, ed. (New South Wales: Australian Bureau of Meteorology).
- Aarenstrup, L., Flindt, E.N., Otkjaer, K., Kirkegaard, M., Andersen, J.S., and Kristiansen, K. (2008). HDAC activity is required for p65/RelA-dependent repression of PPARdelta-mediated transactivation in human keratinocytes. *J Invest Dermatol* *128*, 1095-1106.
- Abbas, T., and Dutta, A. (2009). p21 in cancer: intricate networks and multiple activities. *Nat Rev Cancer* *9*, 400-414.
- Abdel-Malek, Z., Scott, M.C., Suzuki, I., Tada, A., Im, S., Lamoreux, L., Ito, S., Barsh, G., and Hearing, V.J. (2000). The melanocortin-1 receptor is a key regulator of human cutaneous pigmentation. *Pigment Cell Res* *13 Suppl 8*, 156-162.
- Abrahamson, J.L., Lee, J.M., and Bernstein, A. (1995). Regulation of p53-mediated apoptosis and cell cycle arrest by Steel factor. *Mol Cell Biol* *15*, 6953-6960.
- Agthong, S., Kaewsema, A., and Chentanez, V. (2012). Inhibition of p38 MAPK reduces loss of primary sensory neurons after nerve transection. *Neurol Res* *34*, 714-720.
- Aigal, S., Claudinon, J., and Romer, W. (2015). Plasma membrane reorganization: A glycolipid gateway for microbes. *Biochim Biophys Acta* *1853*, 858-871.
- Akerfelt, M., Trouillet, D., Mezger, V., and Sistonen, L. (2007). Heat shock factors at a crossroad between stress and development. *Ann N Y Acad Sci* *1113*, 15-27.
- Alonso, C., Perez, R., Bazaga, P., Medrano, M., and Herrera, C.M. (2015). MSAP markers and global cytosine methylation in plants: a literature survey and comparative analysis for a wild-growing species. *Mol Ecol Resour*.
- Amin, J., Ananthan, J., and Voellmy, R. (1988). Key features of heat shock regulatory elements. *Mol Cell Biol* *8*, 3761-3769.
- Amundson, S.A., Patterson, A., Do, K.T., and Fornace, A.J., Jr. (2002). A nucleotide excision repair master-switch: p53 regulated coordinate induction of global genomic repair genes. *Cancer Biol Ther* *1*, 145-149.
- Ananthaswamy, H.N., and Pierceall, W.E. (1990). Molecular mechanisms of ultraviolet radiation carcinogenesis. *Photochem Photobiol* *52*, 1119-1136.
- Andersen, J.B. (2015). Molecular pathogenesis of intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* *22*, 101-113.
- Andreassi, L. (2011). UV exposure as a risk factor for skin cancer. *Expert Rev Dermatol* *6*, 445-454.
- Anink-Groenen, L.C., Maarleveld, T.R., Verschure, P.J., and Bruggeman, F.J. (2014). Mechanistic stochastic model of histone modification pattern formation. *Epigenetics Chromatin* *7*, 30.
- Aragane, Y., Kulms, D., Metze, D., Wilkes, G., Poppelmann, B., Luger, T.A., and Schwarz, T. (1998). Ultraviolet light induces apoptosis via direct activation of CD95 (Fas/APO-1) independently of its ligand CD95L. *J Cell Biol* *140*, 171-182.
- Arnold, A.W., and Itin, P.H. (2010). Laptop computer-induced erythema ab igne in a child and review of the literature. *Pediatrics* *126*, e1227-1230.

Asada, K., Nakajima, T., Shimazu, T., Yamamichi, N., Maekita, T., Yokoi, C., Oda, I., Ando, T., Yoshida, T., Nanjo, S., *et al.* (2015). Demonstration of the usefulness of epigenetic cancer risk prediction by a multicentre prospective cohort study. *Gut* *64*, 388-396.

Asgari, S. (2014). Epigenetic modifications underlying symbiont-host interactions. *Adv Genet* *86*, 253-276.

Assefa, Z., Garmyn, M., Vantieghem, A., Declercq, W., Vandenaabeele, P., Vandenneede, J.R., and Agostinis, P. (2003). Ultraviolet B radiation-induced apoptosis in human keratinocytes: cytosolic activation of procaspase-8 and the role of Bcl-2. *FEBS Lett* *540*, 125-132.

Athar, M., Kim, A.L., Ahmad, N., Mukhtar, H., Gautier, J., and Bickers, D.R. (2000). Mechanism of ultraviolet B-induced cell cycle arrest in G2/M phase in immortalized skin keratinocytes with defective p53. *Biochem Biophys Res Commun* *277*, 107-111.

Attardi, L.D., and Jacks, T. (1999). The role of p53 in tumour suppression: lessons from mouse models. *Cell Mol Life Sci* *55*, 48-63.

Babu, M., Durga Devi, T., Makinen, P., Kaikkonen, M., Lesch, H.P., Junttila, S., Laiho, A., Ghimire, B., Gyenesei, A., and Yla-Herttuala, S. (2015). Differential Promoter Methylation of Macrophage Genes Is Associated With Impaired Vascular Growth in Ischemic Muscles of Hyperlipidemic and Type 2 Diabetic Mice: Genome-Wide Promoter Methylation Study. *Circ Res* *117*, 289-299.

Bailey, S.T., Westerling, T., and Brown, M. (2015). Loss of estrogen-regulated microRNA expression increases HER2 signaling and is prognostic of poor outcome in luminal breast cancer. *Cancer Res* *75*, 436-445.

Bain, J.A., Rusch, H.P., and Kline, B.E. (1943a). The effect of temperature upon ultraviolet carcinogenesis with wavelengths 2,800-3,400 A. *Cancer Res* *3*, 610-612.

Bain, J.A., Rusch, P., and Kline, B.E. (1943b). The effect of temperature upon ultraviolet carcinogenesis with wavelengths 2800-3400 A. *Cancer Res* *3*, 610-612.

Banerji, U., Affolter, A., Judson, I., Marais, R., and Workman, P. (2008). BRAF and NRAS mutations in melanoma: potential relationships to clinical response to HSP90 inhibitors. *Mol Cancer Ther* *7*, 737-739.

Barascu, A., Le Chalony, C., Pennarun, G., Genet, D., Imam, N., Lopez, B., and Bertrand, P. (2012). Oxidative stress induces an ATM-independent senescence pathway through p38 MAPK-mediated lamin B1 accumulation. *EMBO J* *31*, 1080-1094.

Basset-Seguin, N., Sharpe, H.J., and de Sauvage, F.J. (2015). Efficacy of Hedgehog pathway inhibitors in basal cell carcinoma. *Mol Cancer Ther*.

Bath-Hextall, F., Leonardi-Bee, J., Smith, C., Meal, A., and Hubbard, R. (2007). Trends in incidence of skin basal cell carcinoma. Additional evidence from a UK primary care database study. *Int J Cancer* *121*, 2105-2108.

Baud, M.G., Lin-Shiao, E., Cardote, T., Tallant, C., Pschibul, A., Chan, K.H., Zengerle, M., Garcia, J.R., Kwan, T.T., Ferguson, F.M., *et al.* (2014). Chemical biology. A bump-and-hole approach to engineer controlled selectivity of BET bromodomain chemical probes. *Science* *346*, 638-641.

Beckerman, R., and Prives, C. (2010). Transcriptional regulation by p53. *Cold Spring Harb Perspect Biol* *2*, a000935.

- Beere, H.M., Wolf, B.B., Cain, K., Mosser, D.D., Mahboubi, A., Kuwana, T., Taylor, P., Morimoto, R.I., Cohen, G.M., and Green, D.R. (2000). Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol* 2, 469-475.
- Benjamin, C.L., Ullrich, S.E., Kripke, M.L., and Ananthaswamy, H.N. (2008). p53 tumor suppressor gene: a critical molecular target for UV induction and prevention of skin cancer. *Photochem Photobiol* 84, 55-62.
- Bennett, D. (2005). Familial Melanoma Genes, Melanocyte Immortalisation, and Melanoma Initiation. In *From Melanocyte to Melanoma: The progression to Malignancy*, V. Hearing, and S. Leong, eds. (NJ: Humana Press inc.), pp. 183-196.
- Berg, R.J., de Laat, A., Roza, L., van der Leun, J.C., and de Gruijl, F.R. (1995). Substitution of equally carcinogenic UV-A for UV-B irradiations lowers epidermal thymine dimer levels during skin cancer induction in hairless mice. *Carcinogenesis* 16, 2455-2459.
- Bernabo, P., Rebecchi, L., Jousson, O., Martinez-Guitarte, J.L., and Lencioni, V. (2011). Thermotolerance and hsp70 heat shock response in the cold-stenothermal chironomid *Pseudodiamesa branickii* (NE Italy). *Cell Stress Chaperones* 16, 403-410.
- Bertrand-Vallery, V., Boilan, E., Ninane, N., Demazy, C., Friguet, B., Toussaint, O., Poumay, Y., and Debacq-Chainiaux, F. (2010). Repeated exposures to UVB induce differentiation rather than senescence of human keratinocytes lacking p16(INK-4A). *Biogerontology* 11, 167-181.
- Besarutinia, A., Bates, S.E., Synold, T.W., and Pfeifer, G.P. (2004). Similar mutagenicity of photoactivated porphyrins and ultraviolet A radiation in mouse embryonic fibroblasts: Involvement of oxidative lesions in mutagenesis. *Biochemistry* 43, 15557-15566.
- Bianchi, I., Carbone, M., Lleo, A., and Invernizzi, P. (2014). Genetics and epigenetics of primary biliary cirrhosis. *Semin Liver Dis* 34, 255-264.
- Bilal, I., Chowdhury, A., Davidson, J., and Whitehead, S. (2014). Phytoestrogens and prevention of breast cancer: The contentious debate. *World J Clin Oncol* 5, 705-712.
- Bivik, C., Rosdahl, I., and Ollinger, K. (2007). Hsp70 protects against UVB induced apoptosis by preventing release of cathepsins and cytochrome c in human melanocytes. *Carcinogenesis* 28, 537-544.
- Bodega, B., and Orlando, V. (2014). Repetitive elements dynamics in cell identity programming, maintenance and disease. *Curr Opin Cell Biol* 31, 67-73.
- Boros, G., Miko, E., Muramatsu, H., Weissman, D., Emri, E., van der Horst, G.T., Szegedi, A., Horkay, I., Emri, G., Kariko, K., *et al.* (2015). Identification of Cyclobutane Pyrimidine Dimer-Responsive Genes Using UVB-Irradiated Human Keratinocytes Transfected with In Vitro-Synthesized Photolyase mRNA. *PLoS One* 10, e0131141.
- Botta, G., De Santis, L.P., and Saladino, R. (2012). Current advances in the synthesis and antitumoral activity of SIRT1-2 inhibitors by modulation of p53 and pro-apoptotic proteins. *Curr Med Chem* 19, 5871-5884.
- Boukamp, P. (2011). Pathogenesis of Nonmelanoma Skin Cancer. In *Skin Cancer: A World-Wide Perspective* (Springer-Verlag), pp. 45-54.
- Brant, J.O., Riva, A., Resnick, J.L., and Yang, T.P. (2014). Influence of the Prader-Willi syndrome imprinting center on the DNA methylation landscape in the mouse brain. *Epigenetics* 9, 1540-1556.

- Brantsch, K.D., Meisner, C., Schonfisch, B., Trilling, B., Wehner-Caroli, J., Rocken, M., and Breuninger, H. (2008). Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. *Lancet Oncol* 9, 713-720.
- Brash, D.E. (2006). Roles of the transcription factor p53 in keratinocyte carcinomas. *Br J Dermatol* 154 Suppl 1, 8-10.
- Brash, D.E., Ziegler, A., Jonason, A.S., Simon, J.A., Kunala, S., and Leffell, D.J. (1996). Sunlight and sunburn in human skin cancer: p53, apoptosis, and tumor promotion. *J Investig Dermatol Symp Proc* 1, 136-142.
- Bratton, S.B., and Salvesen, G.S. (2010). Regulation of the Apaf-1-caspase-9 apoptosome. *J Cell Sci* 123, 3209-3214.
- Brenner, M., Coelho, S.G., Beer, J.Z., Miller, S.A., Wolber, R., Smuda, C., and Hearing, V.J. (2009). Long-lasting molecular changes in human skin after repetitive in situ UV irradiation. *J Invest Dermatol* 129, 1002-1011.
- Bria, E., Furlanetto, J., Carbognin, L., Brunelli, M., Caliolo, C., Nortilli, R., Massari, F., Pedron, S., Manfrin, E., Pellini, F., *et al.* (2015). Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: Heat Shock Protein 90 Overexpression, Ki67 Proliferative Index, and Topoisomerase II-alpha Co-amplification as Predictors of Pathologic Complete Response to Neoadjuvant Chemotherapy With Trastuzumab and Docetaxel. *Clin Breast Cancer* 15, 16-23.
- Bruins, W., Zwart, E., Attardi, L.D., Iwakuma, T., Hoogervorst, E.M., Beems, R.B., Miranda, B., van Oostrom, C.T., van den Berg, J., van den Aardweg, G.J., *et al.* (2004). Increased sensitivity to UV radiation in mice with a p53 point mutation at Ser389. *Mol Cell Biol* 24, 8884-8894.
- Bruskov, V.I., Malakhova, L.V., Masalimov, Z.K., and Chernikov, A.V. (2002). Heat-induced formation of reactive oxygen species and 8-oxoguanine, a biomarker of damage to DNA. *Nucleic Acids Res* 30, 1354-1363.
- Bushell, M., McKendrick, L., Janicke, R.U., Clemens, M.J., and Morley, S.J. (1999). Caspase-3 is necessary and sufficient for cleavage of protein synthesis eukaryotic initiation factor 4G during apoptosis. *FEBS Lett* 451, 332-336.
- Cagnol, S., Mansour, A., Van Obberghen-Schilling, E., and Chambard, J.C. (2011). Raf-1 activation prevents caspase 9 processing downstream of apoptosome formation. *J Signal Transduct* 2011, 834948.
- Calapre, L., Gray, E.S., and Ziman, M. (2013). Heat stress: a risk factor for skin carcinogenesis. *Cancer Lett* 337, 35-40.
- Cao, C., Lu, S., Kivlin, R., Wallin, B., Card, E., Bagdasarian, A., Tamakloe, T., Wang, W.J., Song, X., Chu, W.M., *et al.* (2009). SIRT1 confers protection against UVB- and H2O2-induced cell death via modulation of p53 and JNK in cultured skin keratinocytes. *J Cell Mol Med* 13, 3632-3643.
- Cavallari, V., Ciccirello, R., Torre, V., Gagliardi, M.E., Albiero, F., Palazzo, R., Siragusa, M., and Schipis, C. (2001). Chronic heat-induced skin lesions (erythema ab Igne): ultrastructural studies. *Ultrastruct Pathol* 25, 93-97.
- Chakraborty, A., Uechi, T., and Kenmochi, N. (2011). Guarding the 'translation apparatus': defective ribosome biogenesis and the p53 signaling pathway. *Wiley Interdiscip Rev RNA* 2, 507-522.

- Chalhoub, N., and Baker, S.J. (2009). PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol* 4, 127-150.
- Chen, H., Aksoy, I., Gonnot, F., Osteil, P., Aubry, M., Hamela, C., Rognard, C., Hochard, A., Voisin, S., Fontaine, E., *et al.* (2015). Reinforcement of STAT3 activity reprogrammes human embryonic stem cells to naive-like pluripotency. *Nat Commun* 6, 7095.
- Chen, L.L., Wu, N.H., Shen, Y.F., and Guan, X.M. (2002). [The role of p53 binding site on the trans binding of p53 to Hsp90 beta gene]. *Zhongguo yi xue ke xue yuan xue bao Acta Academiae Medicinae Sinicae* 24, 285-288.
- Chen, N., Nomura, M., She, Q.B., Ma, W.Y., Bode, A.M., Wang, L., Flavell, R.A., and Dong, Z. (2001). Suppression of skin tumorigenesis in c-Jun NH(2)-terminal kinase-2-deficient mice. *Cancer Res* 61, 3908-3912.
- Chen, W.Y., Wang, D.H., Yen, R.C., Luo, J., Gu, W., and Baylin, S.B. (2005). Tumor suppressor HIC1 directly regulates SIRT1 to modulate p53-dependent DNA-damage responses. *Cell* 123, 437-448.
- Chen, Y.-R., Chiou, R., Lin, T.-Y., Huang, C.-P., Tang, W.-C., Chen, S.-T., and Lin, S.-B. (2009). Identification of an Alkylhydroquinone from *Rhus succedanea* as an Inhibitor of Tyrosinase and Melanogenesis. *Journal of Agricultural and Food Chemistry* 57, 2200-2205.
- Cheng, H.L., Mostoslavsky, R., Saito, S., Manis, J.P., Gu, Y., Patel, P., Bronson, R., Appella, E., Alt, F.W., and Chua, K.F. (2003). Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. *Proc Natl Acad Sci U S A* 100, 10794-10799.
- Cheo, D.L., Meira, L.B., Hammer, R.E., Burns, D.K., Doughty, A.T., and Friedberg, E.C. (1996). Synergistic interactions between XPC and p53 mutations in double-mutant mice: neural tube abnormalities and accelerated UV radiation-induced skin cancer. *Curr Biol* 6, 1691-1694.
- Chinnathambi, S., Tomanek-Chalkley, A., and Bickenbach, J.R. (2008). HSP70 and EndoG modulate cell death by heat in human skin keratinocytes in vitro. *Cells Tissues Organs* 187, 131-140.
- Chipuk, J.E., and Green, D.R. (2004). Cytoplasmic p53: bax and forward. *Cell Cycle* 3, 429-431.
- Chou, R.H., Hsieh, S.C., Yu, Y.L., Huang, M.H., Huang, Y.C., and Hsieh, Y.H. (2013a). Fisetin inhibits migration and invasion of human cervical cancer cells by down-regulating urokinase plasminogen activator expression through suppressing the p38 MAPK-dependent NF-kappaB signaling pathway. *PLoS One* 8, e71983.
- Chou, W.W., Chen, K.C., Wang, Y.S., Wang, J.Y., Liang, C.L., and Juo, S.H. (2013b). The role of SIRT1/AKT/ERK pathway in ultraviolet B induced damage on human retinal pigment epithelial cells. *Toxicol In Vitro* 27, 1728-1736.
- Chung, K.W., Choi, Y.J., Park, M.H., Jang, E.J., Kim, D.H., Park, B.H., Yu, B.P., and Chung, H.Y. (2014). Molecular Insights into SIRT1 Protection Against UVB-Induced Skin Fibroblast Senescence by Suppression of Oxidative Stress and p53 Acetylation. *J Gerontol A Biol Sci Med Sci*.
- Ciocca, D.R., and Calderwood, S.K. (2005). Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell Stress Chaperones* 10, 86-103.

- Clement-Lacroix, P., Michel, L., Moysan, A., Morliere, P., and Dubertret, L. (1996). UVA-induced immune suppression in human skin: protective effect of vitamin E in human epidermal cells in vitro. *Br J Dermatol* *134*, 77-84.
- Coelho, S.G., Choi, W., Brenner, M., Miyamura, Y., Yamaguchi, Y., Wolber, R., Smuda, C., Batzer, J., Kolbe, L., Ito, S., *et al.* (2009). Short- and long-term effects of UV radiation on the pigmentation of human skin. *J Investig Dermatol Symp Proc* *14*, 32-35.
- Cornford, P.A., Dodson, A.R., Parsons, K.F., Desmond, A.D., Woolfenden, A., Fordham, M., Neoptolemos, J.P., Ke, Y., and Foster, C.S. (2000). Heat shock protein expression independently predicts clinical outcome in prostate cancer. *Cancer Res* *60*, 7099-7105.
- Corware, K., Yardley, V., Mack, C., Schuster, S., Al-Hassi, H., Herath, S., Bergin, P., Modolell, M., Munder, M., Muller, I., *et al.* (2014). Protein energy malnutrition increases arginase activity in monocytes and macrophages. *Nutr Metab (Lond)* *11*, 51.
- Costin, G.E., and Hearing, V.J. (2007). Human skin pigmentation: melanocytes modulate skin color in response to stress. *FASEB J* *21*, 976-994.
- Courdavault, S., Baudouin, C., Charveron, M., Canguilhem, B., Favier, A., Cadet, J., and Douki, T. (2005). Repair of the three main types of bipyrimidine DNA photoproducts in human keratinocytes exposed to UVB and UVA radiations. *DNA Repair (Amst)* *4*, 836-844.
- Cui, R., Widlund, H.R., Feige, E., Lin, J.Y., Wilensky, D.L., Igras, V.E., D'Orazio, J., Fung, C.Y., Schanbacher, C.F., Granter, S.R., *et al.* (2007). Central role of p53 in the suntan response and pathologic hyperpigmentation. *Cell* *128*, 853-864.
- D'Costa, A.M., and Denning, M.F. (2005). A caspase-resistant mutant of PKC-delta protects keratinocytes from UV-induced apoptosis. *Cell Death Differ* *12*, 224-232.
- Dai, C., Dai, S., and Cao, J. (2012a). Proteotoxic stress of cancer: implication of the heat-shock response in oncogenesis. *J Cell Physiol* *227*, 2982-2987.
- Dai, C., Santagata, S., Tang, Z., Shi, J., Cao, J., Kwon, H., Bronson, R.T., Whitesell, L., and Lindquist, S. (2012b). Loss of tumor suppressor NF1 activates HSF1 to promote carcinogenesis. *J Clin Invest* *122*, 3742-3754.
- Dai, C., Whitesell, L., Rogers, A.B., and Lindquist, S. (2007). Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. *Cell* *130*, 1005-1018.
- Daugaard, M., Kirkegaard-Sorensen, T., Ostenfeld, M.S., Aaboe, M., Hoyer-Hansen, M., Orntoft, T.F., Rohde, M., and Jaattela, M. (2007). Lens epithelium-derived growth factor is an Hsp70-2 regulated guardian of lysosomal stability in human cancer. *Cancer Res* *67*, 2559-2567.
- de Gruijl, F.R., van Kranen, H.J., and Mullenders, L.H. (2001). UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. *J Photochem Photobiol B* *63*, 19-27.
- De Maio, A. (1999). Heat shock proteins: facts, thoughts, and dreams. *Shock* *11*, 1-12.
- De Maio, A. (2011). Extracellular heat shock proteins, cellular export vesicles, and the Stress Observation System: a form of communication during injury, infection, and cell damage. It is never known how far a controversial finding will go! Dedicated to Ferruccio Ritossa. *Cell Stress Chaperones* *16*, 235-249.
- DeFedericis, H., Partze, H., Rajecki, M., Budzinski, E., Iijma, H., Dawidzik, J., Evans, M., Greene, K., and Box, H. (2006). Singlet oxygen-induced DNA damage. *Radiat Res* *165*, 445.

- Delgado-Cruzata, L., Vin-Raviv, N., Tehranifar, P., Flom, J., Reynolds, D., Gonzalez, K., Santella, R.M., and Terry, M.B. (2014). Correlations in global DNA methylation measures in peripheral blood mononuclear cells and granulocytes. *Epigenetics* 9, 1504-1510.
- Dent, P. (2014). Crosstalk between ERK, AKT, and cell survival. *Cancer Biol Ther* 15, 245-246.
- Diaz, S., Cao, A., Villalba, A., and Carballal, M.J. (2010). Expression of mutant protein p53 and Hsp70 and Hsp90 chaperones in cockles *Cerastoderma edule* affected by neoplasia. *Diseases of aquatic organisms* 90, 215-222.
- Donmez, G., Arun, A., Chung, C.Y., McLean, P.J., Lindquist, S., and Guarente, L. (2012). SIRT1 protects against alpha-synuclein aggregation by activating molecular chaperones. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 32, 124-132.
- Donoghue, A.M. (2004). Occupational health hazards in mining: an overview. *Occup Med (Lond)* 54, 283-289.
- Du, Q., Luu, P.L., Stirzaker, C., and Clark, S.J. (2015). Methyl-CpG-binding domain proteins: readers of the epigenome. *Epigenomics*, 1-23.
- Dumaz, N., Duthu, A., Ehrhart, J.C., Drougard, C., Appella, E., Anderson, C.W., May, P., Sarasin, A., and Daya-Grosjean, L. (1997). Prolonged p53 protein accumulation in trichothiodystrophy fibroblasts dependent on unrepaired pyrimidine dimers on the transcribed strands of cellular genes. *Molecular carcinogenesis* 20, 340-347.
- Echchgadda, I., Roth, C.C., Cerna, C.Z., and Wilmink, G.J. (2013). Temporal gene expression kinetics for human keratinocytes exposed to hyperthermic stress. *Cells* 2, 224-243.
- Edelstein, L., and Smythies, J. (2014). The role of epigenetic-related codes in neurocomputation: dynamic hardware in the brain. *Philos Trans R Soc Lond B Biol Sci* 369.
- Ehrlich, M., Norris, K.F., Wang, R.Y., Kuo, K.C., and Gehrke, C.W. (1986). DNA cytosine methylation and heat-induced deamination. *Biosci Rep* 6, 387-393.
- Epstein, E.H. (2008). Basal cell carcinomas: attack of the hedgehog. *Nat Rev Cancer* 8, 743-754.
- Erdmann, R.M., Souza, A.L., Clish, C.B., and Gehring, M. (2014). 5-hydroxymethylcytosine is not present in appreciable quantities in *Arabidopsis* DNA. *G3 (Bethesda)* 5, 1-8.
- Fears, T.R., Scotto, J., and Schneidermann, A. (1977). Mathematical models of age and ultraviolet effects on the incidence of skin cancer among whites in the United States. *American Journal of Epidemiology* 105, 420-427.
- Feng, L., Ma, Y., Sun, J., Shen, Q., Liu, L., Lu, H., Wang, F., Yue, Y., Li, J., Zhang, S., *et al.* (2014). YY1-MIR372-SQSTM1 regulatory axis in autophagy. *Autophagy* 10, 1442-1453.
- Fitch, M.E., Cross, I.V., and Ford, J.M. (2003). p53 responsive nucleotide excision repair gene products p48 and XPC, but not p53, localize to sites of UV-irradiation-induced DNA damage, in vivo. *Carcinogenesis* 24, 843-850.
- Ford, J.M. (2005). Regulation of DNA damage recognition and nucleotide excision repair: another role for p53. *Mutat Res* 577, 195-202.
- Fortes, C. (2008). Nonsolar occupational risk factors for cutaneous melanoma. *The International Society of Dermatology* 47, 319-328.
- Freedman, D.M., Kitahara, C., Linet, M., Alexander, B., Neta, G., Little, M., and Cahoon, E. (2015). Ambient temperature and risk of first primary basal cell carcinoma: A nationwide

- United States cohort study. *Journal of Photochemistry and Photobiology B: Biology* 148, 284-289.
- Freeman, R.G., and Knox, J.M. (1964). Influence of Temperature on Ultraviolet Injury. *Arch Dermatol* 89, 858-864.
- Fritah, S., Col, E., Boyault, C., Govin, J., Sadoul, K., Chiocca, S., Christians, E., Khochbin, S., Jolly, C., and Vourc'h, C. (2009). Heat-shock factor 1 controls genome-wide acetylation in heat-shocked cells. *Mol Biol Cell* 20, 4976-4984.
- Gabai, V.L., Yaglom, J.A., Waldman, T., and Sherman, M.Y. (2009). Heat shock protein Hsp72 controls oncogene-induced senescence pathways in cancer cells. *Mol Cell Biol* 29, 559-569.
- Gaillard, J.B., Chiesa, J., Reboul, D., Arnaud, A., Brun, S., Donadio, D., Exbrayat, C., Wickenhauser, S., Bourquard, P., Jourdan, E., *et al.* (2012). Monosomal karyotype routinely defines a poor prognosis subgroup in acute myeloid leukemia and is frequently associated with TP53 deletion. *Leuk Lymphoma* 53, 336-337.
- Geranton, S.M., and Tochiki, K.K. (2015). Could targeting epigenetic processes relieve chronic pain states? *Curr Opin Support Palliat Care* 9, 138-146.
- Geyer, R.K., Nagasawa, H., Little, J.B., and Maki, C.G. (2000). Role and regulation of p53 during an ultraviolet radiation-induced G1 cell cycle arrest. *Cell Growth Differ* 11, 149-156.
- Gidanian, S., Mentelle, M., Jr., F.M., and Farmer, P. (2008). Melanosomal Damage in Normal Human Melanocytes Induced by UVB and Metal Uptake- A basis for the pro-oxidant state of melanoma. *Photochemistry and Photobiology* 84, 556-564.
- Glozak, M.A., and Seto, E. (2007). Histone deacetylases and cancer. *Oncogene* 26, 5420-5432.
- Gomez, F., Perkins, J.R., Garcia-Martin, E., Canto, G., and Cornejo-Garcia, J.A. (2015). Genetic basis of hypersensitivity reactions to nonsteroidal anti-inflammatory drugs. *Curr Opin Allergy Clin Immunol* 15, 285-293.
- Gonfloni, S., Iannizzotto, V., Maiani, E., Bellusci, G., Ciccone, S., and Diederich, M. (2014). P53 and Sirt1: routes of metabolism and genome stability. *Biochem Pharmacol* 92, 149-156.
- Gorman, S., Tan, J.W., Yerkovich, S.T., Finlay-Jones, J.J., and Hart, P.H. (2007). CD4+ T cells in lymph nodes of UVB-irradiated mice suppress immune responses to new antigens both in vitro and in vivo. *J Invest Dermatol* 127, 915-924.
- Green, D.R. (2006). At the gates of death. *Cancer Cell* 9, 328-330.
- Greinert, R. (2009). Skin cancer: new markers for better prevention. *Pathobiology* 76, 64-81.
- Grossman, D., Kim, P.J., Blanc-Brude, O.P., Brash, D.E., Tognin, S., Marchisio, P.C., and Altieri, D.C. (2001). Transgenic expression of survivin in keratinocytes counteracts UVB-induced apoptosis and cooperates with loss of p53. *J Clin Invest* 108, 991-999.
- Grujil, F.d., Kranen, H.v., and Mullenders, L. (2001). UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. *Journal of Photochemistry and Photobiology B: Biology* 63, 19-27.
- Guo, S., Wharton, W., Moseley, P., and Shi, H. (2007). Heat shock protein 70 regulates cellular redox status by modulating glutathione-related enzyme activities. *Cell Stress Chaperones* 12, 245-254.

- Hacker, E., Boyce, Z., Kimlin, M.G., Wockner, L., Pollak, T., Vaartjes, S.A., Hayward, N.K., and Whiteman, D.C. (2013). The effect of MC1R variants and sunscreen on the response of human melanocytes in vivo to ultraviolet radiation and implications for melanoma. *Pigment Cell Melanoma Res* 26, 835-844.
- Hafner, C., Landthaler, M., and Vogt, T. (2010). Activation of the PI3K/AKT signalling pathway in non-melanoma skin cancer is not mediated by oncogenic PIK3CA and AKT1 hotspot mutations. *Exp Dermatol* 19, e222-227.
- Haggarty, P. (2015). Genetic and metabolic determinants of human epigenetic variation. *Curr Opin Clin Nutr Metab Care* 18, 334-338.
- Hagn, F., Lagleder, S., Retzlaff, M., Rohrberg, J., Demmer, O., Richter, K., Buchner, J., and Kessler, H. (2011). Structural analysis of the interaction between Hsp90 and the tumor suppressor protein p53. *Nature Structural & Molecular Biology* 18, 1086-1093.
- Hall, J.R., Messenger, Z.J., Tam, H.W., Phillips, S.L., Recio, L., and Smart, R.C. (2015). Long noncoding RNA lincRNA-p21 is the major mediator of UVB-induced and p53-dependent apoptosis in keratinocytes. *Cell Death Dis* 6, e1700.
- Harris, S.L., and Levine, A.J. (2005). The p53 pathway: positive and negative feedback loops. *Oncogene* 24, 2899-2908.
- Harrison, M.K., Adon, A.M., and Saavedra, H.I. (2011). The G1 phase Cdks regulate the centrosome cycle and mediate oncogene-dependent centrosome amplification. *Cell Div* 6, 2.
- Hart, P.H., Grimbaldston, M.A., Swift, G.J., Jaksic, A., Noonan, F.P., and Finlay-Jones, J.J. (1998). Dermal mast cells determine susceptibility to ultraviolet B-induced systemic suppression of contact hypersensitivity responses in mice. *J Exp Med* 187, 2045-2053.
- Henseleit, U., Zhang, J., Wanner, R., Haase, I., Kolde, G., and Rosenbach, T. (1997). Role of p53 in UVB-induced apoptosis in human HaCaT keratinocytes. *J Invest Dermatol* 109, 722-727.
- Herbert, K.J., Cook, A.L., and Snow, E.T. (2014). SIRT1 inhibition restores apoptotic sensitivity in p53-mutated human keratinocytes. *Toxicol Appl Pharmacol* 277, 288-297.
- Hermeking, H., Funk, J.O., Reichert, M., Ellwart, J.W., and Eick, D. (1995). Abrogation of p53-induced cell cycle arrest by c-Myc: evidence for an inhibitor of p21WAF1/CIP1/SDI1. *Oncogene* 11, 1409-1415.
- Hildesheim, J., Bulavin, D.V., Anver, M.R., Alvord, W.G., Hollander, M.C., Vardanian, L., and Fornace, A.J., Jr. (2002). Gadd45a protects against UV irradiation-induced skin tumors, and promotes apoptosis and stress signaling via MAPK and p53. *Cancer Res* 62, 7305-7315.
- Hillman, S.L., Finer, S., Smart, M.C., Mathews, C., Lowe, R., Rakyan, V.K., Hitman, G.A., and Williams, D.J. (2015). Novel DNA methylation profiles associated with key gene regulation and transcription pathways in blood and placenta of growth-restricted neonates. *Epigenetics* 10, 50-61.
- Hirobe, T. (2005). Role of keratinocyte-derived factors involved in regulating the proliferation and differentiation of mammalian epidermal melanocytes. *Pigment Cell Res* 18, 2-12.
- Hochberg, M., Kohen, R., and Enk, C.D. (2006). Role of antioxidants in prevention of pyrimidine dimer formation in UVB irradiated human HaCaT keratinocytes. *Biomed Pharmacother* 60, 233-237.

- Holley, A.K., and St Clair, D.K. (2009). Watching the watcher: regulation of p53 by mitochondria. *Future Oncol* 5, 117-130.
- Hsieh, Y.Y., Shen, C.H., Huang, W.S., Chin, C.C., Kuo, Y.H., Hsieh, M.C., Yu, H.R., Chang, T.S., Lin, T.H., Chiu, Y.W., *et al.* (2014). Resistin-induced stromal cell-derived factor-1 expression through Toll-like receptor 4 and activation of p38 MAPK/ NFkappaB signaling pathway in gastric cancer cells. *J Biomed Sci* 21, 59.
- Hu, D.-N. (2008). Methodology for Evaluation of Melanin Content and Production of Pigment Cells in Vitro. *Photochemistry and Photobiology* 84, 645-649.
- Huang, H.C., Chang, T.M., Chang, Y.J., and Wen, H.Y. (2013). UVB irradiation regulates ERK1/2- and p53-dependent thrombomodulin expression in human keratinocytes. *PLoS One* 8, e67632.
- Hube, F., and Francastel, C. (2015). Mammalian introns: when the junk generates molecular diversity. *Int J Mol Sci* 16, 4429-4452.
- Hubner, A., Mulholland, D.J., Standen, C.L., Karasarides, M., Cavanagh-Kyros, J., Barrett, T., Chi, H., Greiner, D.L., Tournier, C., Sawyers, C.L., *et al.* (2012). JNK and PTEN cooperatively control the development of invasive adenocarcinoma of the prostate. *Proc Natl Acad Sci U S A* 109, 12046-12051.
- Hunt, C.R., Pandita, R.K., Laszlo, A., Higashikubo, R., Agarwal, M., Kitamura, T., Gupta, A., Rief, N., Horikoshi, N., Baskaran, R., *et al.* (2007). Hyperthermia activates a subset of ataxia-telangiectasia mutated effectors independent of DNA strand breaks and heat shock protein 70 status. *Cancer Res* 67, 3010-3017.
- Hussein, M.R., Haemel, A.K., and Wood, G.S. (2003). p53-related pathways and the molecular pathogenesis of melanoma. *Eur J Cancer Prev* 12, 93-100.
- Ikehata, H., Kudo, H., Masuda, T., and Ono, T. (2003). UVA induces C-T transitions at Methyl-CpG-associated dipyrimidine sites in mouse skin epidermis more frequently than UVB. *Mutagenesis* 18, 511-519.
- Ikehata, H., and Ono, T. (2011). The mechanisms of UV mutagenesis. *J Radiat Res* 52, 115-125.
- Jang, S.Y., Kim, S.Y., and Bae, Y.S. (2011). p53 deacetylation by SIRT1 decreases during protein kinase CKII downregulation-mediated cellular senescence. *FEBS Lett* 585, 3360-3366.
- Janssen, B.G., Byun, H.M., Cox, B., Gyselaers, W., Izzi, B., Baccarelli, A.A., and Nawrot, T.S. (2014). Variation of DNA methylation in candidate age-related targets on the mitochondrial-telomere axis in cord blood and placenta. *Placenta* 35, 665-672.
- Jantschitsch, C., Kindas-Mugge, I., Metze, D., Amann, G., Micksche, M., and Trautinger, F. (1998). Expression of the small heat shock protein HSP 27 in developing human skin. *Br J Dermatol* 139, 247-253.
- Jantschitsch, C., and Trautinger, F. (2003). Heat shock and UV-B-induced DNA damage and mutagenesis in skin. *Photochem Photobiol Sci* 2, 899-903.
- Jeffrey, K.L., Camps, M., Rommel, C., and Mackay, C.R. (2007). Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. *Nat Rev Drug Discov* 6, 391-403.

- Jimenez, G.S., Khan, S.H., Stommel, J.M., and Wahl, G.M. (1999). p53 regulation by post-translational modification and nuclear retention in response to diverse stresses. *Oncogene* *18*, 7656-7665.
- Jolly, C., Metz, A., Govin, J., Vigneron, M., Turner, B.M., Khochbin, S., and Vourc'h, C. (2004). Stress-induced transcription of satellite III repeats. *J Cell Biol* *164*, 25-33.
- Joly, A.L., Wettstein, G., Mignot, G., Ghiringhelli, F., and Garrido, C. (2010). Dual role of heat shock proteins as regulators of apoptosis and innate immunity. *J Innate Immun* *2*, 238-247.
- Jonak, C., Klosner, G., and Trautinger, F. (2006). Heat shock proteins in the skin. *Int J Cosmet Sci* *28*, 233-241.
- Kane, K.S., and Maytin, E.V. (1995). Ultraviolet B-induced apoptosis of keratinocytes in murine skin is reduced by mild local hyperthermia. *J Invest Dermatol* *104*, 62-67.
- Kanitakis, J., Narvaez, D., Euvrard, S., Faure, M., and Claudy, A. (1997). Proliferation markers Ki67 and PCNA in cutaneous squamous cell carcinomas: lack of prognostic value. *Br J Dermatol* *136*, 643-644.
- Kanjilal, S., Pierceall, W.E., Cummings, K.K., Kripke, M.L., and Ananthaswamy, H.N. (1993). High frequency of p53 mutations in ultraviolet radiation-induced murine skin tumors: evidence for strand bias and tumor heterogeneity. *Cancer Res* *53*, 2961-2964.
- Kar, S., Sengupta, D., Deb, M., Shilpi, A., Parbin, S., Rath, S.K., Pradhan, N., Rakshit, M., and Patra, S.K. (2014). Expression profiling of DNA methylation-mediated epigenetic gene-silencing factors in breast cancer. *Clin Epigenetics* *6*, 20.
- Karin, M., and Gallagher, E. (2005). From JNK to pay dirt: jun kinases, their biochemistry, physiology and clinical importance. *IUBMB Life* *57*, 283-295.
- Kastan, M.B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R.W. (1991). Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* *51*, 6304-6311.
- Katz, T.A., Huang, Y., Davidson, N.E., and Jankowitz, R.C. (2014). Epigenetic reprogramming in breast cancer: from new targets to new therapies. *Ann Med* *46*, 397-408.
- Katzenberger, T., Petzoldt, C., Holler, S., Mader, U., Kalla, J., Adam, P., Ott, M.M., Muller-Hermelink, H.K., Rosenwald, A., and Ott, G. (2006). The Ki67 proliferation index is a quantitative indicator of clinical risk in mantle cell lymphoma. *Blood* *107*, 3407.
- Kayama, M., Nakazawa, T., Thanos, A., Morizane, Y., Murakami, Y., Theodoropoulou, S., Abe, T., Vavvas, D., and Miller, J.W. (2011). Heat shock protein 70 (HSP70) is critical for the photoreceptor stress response after retinal detachment via modulating anti-apoptotic Akt kinase. *Am J Pathol* *178*, 1080-1091.
- Kebir, O., Chaumette, B., Fatjo-Vilas, M., Ambalavanan, A., Ramoz, N., Xiong, L., Mouaffak, F., Millet, B., Jaafari, N., DeLisi, L.E., *et al.* (2014). Family-based association study of common variants, rare mutation study and epistatic interaction detection in HDAC genes in schizophrenia. *Schizophr Res* *160*, 97-103.
- Khalkhali-Ellis, Z., Goossens, W., Margaryan, N.V., and Hendrix, M.J. (2014). Cleavage of Histone 3 by Cathepsin D in the involuting mammary gland. *PLoS One* *9*, e103230.
- Kim, D.H., Jung, Y.J., Lee, J.E., Lee, A.S., Kang, K.P., Lee, S., Park, S.K., Han, M.K., Lee, S.Y., Ramkumar, K.M., *et al.* (2011). SIRT1 activation by resveratrol ameliorates cisplatin-

- induced renal injury through deacetylation of p53. *Am J Physiol Renal Physiol* 301, F427-435.
- Kim, E.J., Kho, J.H., Kang, M.R., and Um, S.J. (2007). Active regulator of SIRT1 cooperates with SIRT1 and facilitates suppression of p53 activity. *Mol Cell* 28, 277-290.
- Kim, S.A., Yoon, J.H., Lee, S.H., and Ahn, S.G. (2005). Polo-like kinase 1 phosphorylates heat shock transcription factor 1 and mediates its nuclear translocation during heat stress. *J Biol Chem* 280, 12653-12657.
- Kim, S.J., Jeong, C.W., Bae, H.B., Kwak, S.H., Son, J.K., Seo, C.S., Lee, H.J., Lee, J., and Yoo, K.Y. (2012). Protective effect of sauchinone against regional myocardial ischemia/reperfusion injury: inhibition of p38 MAPK and JNK death signaling pathways. *J Korean Med Sci* 27, 572-575.
- King, F.W., Wawrzynow, A., Hohfeld, J., and Zylicz, M. (2001). Co-chaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild-type or mutant p53. *EMBO J* 20, 6297-6305.
- Kobow, K., and Blumcke, I. (2014). Epigenetic mechanisms in epilepsy. *Prog Brain Res* 213, 279-316.
- Kolovos, P., van de Werken, H.J., Kepper, N., Zuin, J., Brouwer, R.W., Kockx, C.E., Wendt, K.S., van, I.W.F., Grosveld, F., and Knoch, T.A. (2014). Targeted Chromatin Capture (T2C): a novel high resolution high throughput method to detect genomic interactions and regulatory elements. *Epigenetics Chromatin* 7, 10.
- Kraemer, K.H., Lee, M.M., and Scotto, J. (1987). Xeroderma pigmentosum. Cutaneous, ocular, and neurologic abnormalities in 830 published cases. *Arch Dermatol* 123, 241-250.
- Kratochwil, C.F., and Meyer, A. (2015). Mapping active promoters by ChIP-seq profiling of H3K4me3 in cichlid fish - a first step to uncover cis-regulatory elements in ecological model teleosts. *Mol Ecol Resour* 15, 761-771.
- Kregel, K.C. (2002). Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J Appl Physiol* 92, 2177-2186.
- Kroemer, G., Zamzami, N., and Susin, S.A. (1997). Mitochondrial control of apoptosis. *Immunology today* 18, 44-51.
- Kume, S., Haneda, M., Kanasaki, K., Sugimoto, T., Araki, S., Isono, M., Isshiki, K., Uzu, T., Kashiwagi, A., and Koya, D. (2006). Silent information regulator 2 (SIRT1) attenuates oxidative stress-induced mesangial cell apoptosis via p53 deacetylation. *Free Radic Biol Med* 40, 2175-2182.
- Laethem, A.V., Claerhout, S., Garmyn, M., and Agostinis, P. (2005). The sunburn cell: Regulation of death and survival of the keratinocyte. *The International Journal of Biochemistry & Cell Biology* 37, 1547-1553.
- Lee, C.H., Wu, S.B., Hong, C.H., Yu, H.S., and Wei, Y.H. (2013). Molecular Mechanisms of UV-Induced Apoptosis and Its Effects on Skin Residential Cells: The Implication in UV-Based Phototherapy. *Int J Mol Sci* 14, 6414-6435.
- Lee, E.R., Kim, J.H., Choi, H.Y., Jeon, K., and Cho, S.G. (2011). Cytoprotective effect of eriodictyol in UV-irradiated keratinocytes via phosphatase-dependent modulation of both the p38 MAPK and Akt signaling pathways. *Cell Physiol Biochem* 27, 513-524.

- Lee, H.S., Kwon, S.H., Ham, J.E., Lee, J.Y., Kim, D.H., Shin, K.H., and Choi, S.H. (2012). Zaprinast activates MAPKs, NFkappaB, and Akt and induces the expressions of inflammatory genes in microglia. *Int Immunopharmacol* *13*, 232-241.
- Lee, H.S., Lee, D.H., Cho, S., and Chung, J.H. (2006). Minimal heating dose: a novel biological unit to measure infrared irradiation. *Photodermatology, photoimmunology & photomedicine* *22*, 148-152.
- Lee, J.S., Lee, J.J., and Seo, J.S. (2005). HSP70 deficiency results in activation of c-Jun N-terminal Kinase, extracellular signal-regulated kinase, and caspase-3 in hyperosmolarity-induced apoptosis. *J Biol Chem* *280*, 6634-6641.
- Lee, J.T., and Gu, W. (2013). SIRT1: Regulator of p53 Deacetylation. *Genes Cancer* *4*, 112-117.
- Leontieva, O.V., Gudkov, A.V., and Blagosklonny, M.V. (2010). Weak p53 permits senescence during cell cycle arrest. *Cell Cycle* *9*, 4323-4327.
- Leung, Y.T., Shi, L., Maurer, K., Song, L., Zhang, Z., Petri, M., and Sullivan, K.E. (2015). Interferon regulatory factor 1 and histone H4 acetylation in systemic lupus erythematosus. *Epigenetics* *10*, 191-199.
- Levin, I., Miller, M.D., Schwarzenbacher, R., McMullan, D., Abdubek, P., Ambing, E., Biorac, T., Cambell, J., Canaves, J.M., Chiu, H.J., *et al.* (2005). Crystal structure of an indigoidine synthase A (IndA)-like protein (TM1464) from *Thermotoga maritima* at 1.90 Å resolution reveals a new fold. *Proteins* *59*, 864-868.
- Lewis, D.A., Yi, Q., Travers, J.B., and Spandau, D.F. (2008). UVB-induced senescence in human keratinocytes requires a functional insulin-like growth factor-1 receptor and p53. *Mol Biol Cell* *19*, 1346-1353.
- Li, Y., Matsumori, H., Nakayama, Y., Osaki, M., Kojima, H., Kurimasa, A., Ito, H., Mori, S., Katoh, M., Oshimura, M., *et al.* (2011). SIRT2 down-regulation in HeLa can induce p53 accumulation via p38 MAPK activation-dependent p300 decrease, eventually leading to apoptosis. *Genes Cells* *16*, 34-45.
- Lin, K., Rockliffe, N., Johnson, G.G., Sherrington, P.D., and Pettitt, A.R. (2008). Hsp90 inhibition has opposing effects on wild-type and mutant p53 and induces p21 expression and cytotoxicity irrespective of p53/ATM status in chronic lymphocytic leukaemia cells. *Oncogene* *27*, 2445-2455.
- Lindahl, T., and Nyberg, B. (1974). Heat-induced deamination of cytosine residues in deoxyribonucleic acid. *Biochemistry* *13*, 3405-3410.
- Liu, D.J., Hammer, D., Komlos, D., Chen, K.Y., Firestein, B.L., and Liu, A.Y. (2014a). SIRT1 knockdown promotes neural differentiation and attenuates the heat shock response. *J Cell Physiol* *229*, 1224-1235.
- Liu, L., Rezvani, H.R., Back, J.H., Hosseini, M., Tang, X., Zhu, Y., Mahfouf, W., Raad, H., Raji, G., Athar, M., *et al.* (2014b). Inhibition of p38 MAPK signaling augments skin tumorigenesis via NOX2 driven ROS generation. *PLoS One* *9*, e97245.
- Liu, M., Aneja, R., Liu, C., Sun, L., Gao, J., Wang, H., Dong, J.T., Sarli, V., Giannis, A., Joshi, H.C., *et al.* (2006). Inhibition of the mitotic kinesin Eg5 up-regulates Hsp70 through the phosphatidylinositol 3-kinase/Akt pathway in multiple myeloma cells. *J Biol Chem* *281*, 18090-18097.

- Livingstone, L.R., White, A., Sprouse, J., Livanos, E., Jacks, T., and Tlsty, T.D. (1992). Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell* 70, 923-935.
- Lou, G., Liu, Y., Wu, S., Xue, J., Yang, F., Fu, H., Zheng, M., and Chen, Z. (2015). The p53/miR-34a/SIRT1 Positive Feedback Loop in Quercetin-Induced Apoptosis. *Cell Physiol Biochem* 35, 2192-2202.
- Lou, X., Zhou, Q., Yin, Y., Zhou, C., and Shen, Y. (2009). Inhibition of the met receptor tyrosine kinase signaling enhances the chemosensitivity of glioma cell lines to CDDP through activation of p38 MAPK pathway. *Mol Cancer Ther* 8, 1126-1136.
- Loughlin, J. (2015). Genetic contribution to osteoarthritis development: current state of evidence. *Curr Opin Rheumatol* 27, 284-288.
- Lucas, R., McMichael, T., Smith, W., and Armstrong, B. (2006). Solar ultraviolet radiation: Global burden of disease from solar ultraviolet radiation. In *Environmental Burden of Disease Series* (World Health Organisation).
- Luo, S., Peng, Z., Zheng, Y., Zhang, L., Feng, Y., and Wang, G. (2007). Synergistic effects of acitretin and narrow-band UVB on inducing the expression of heparin-binding epidermal-growth-factor-like growth factor in normal human keratinocytes. *Arch Dermatol Res* 299, 409-413.
- Madan, V., Lear, J.T., and Szeimies, R.M. (2010). Non-melanoma skin cancer. *Lancet* 375, 673-685.
- Maddodi, N., and Selaturi, V. (2008). Role of UV in Cutaneous Melanoma. *Photochemistry and Photobiology* 84, 528-536.
- Makin, J. (2011). Implications of climate change for skin cancer prevention in Australia. *Health Promot J Austr* 22 *Spec No*, S39-41.
- Maloney, A., and Workman, P. (2002). HSP90 as a new therapeutic target for cancer therapy: the story unfolds. *Expert Opin Biol Ther* 2, 3-24.
- Markovic, S., Erickson, L., Rao, R., Weenig, R., Pockaj, B., Bardia, A., Vachon, C., Schild, S., McWilliams, R., Hand, J., *et al.* (2007). Malignant Melanoma in the 21st Century, Part I: Epidemiology, Risk Factors, Screening, Prevention and Diagnosis. *Mayo Clinic Proceedings* 82, 364-380.
- Markovitsi, D., Gustavsson, T., and Banyasz, A. (2010). Absorption of UV radiation by DNA: spatial and temporal features. *Mutat Res* 704, 21-28.
- Marquette, A., Bagot, M., Bensussan, A., and Dumaz, N. (2007). Recent discoveries in the genetics of melanoma and their therapeutic implications. *Arch Immunol Ther Exp* 55, 363-372.
- Marshall, D.J. (2015). Environmentally induced (co)variance in sperm and offspring phenotypes as a source of epigenetic effects. *J Exp Biol* 218, 107-113.
- Martincorena, I., Roshan, A., Gerstung, M., Ellis, P., Van Loo, P., McLaren, S., Wedge, D.C., Fullam, A., Alexandrov, L.B., Tubio, J.M., *et al.* (2015). Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 348, 880-886.
- Matsunaga, T., Hieda, K., and Nikaido, O. (1991). Wavelength dependent formation of thymine dimers and (6-4) photoproducts in DNA by monochromatic ultraviolet light ranging from 150 to 365 nm. *Photochem Photobiol* 54, 403-410.

- Maytin, E.V. (1995). Heat shock proteins and molecular chaperones: implications for adaptive responses in the skin. *The Journal of investigative dermatology* *104*, 448-455.
- Maytin, E.V., Murphy, L.A., and Merrill, M.A. (1993). Hyperthermia induces resistance to ultraviolet light B in primary and immortalized epidermal keratinocytes. *Cancer Res* *53*, 4952-4959.
- Maytin, E.V., Wimberly, J.M., and Kane, K.S. (1994). Heat shock modulates UVB-induced cell death in human epidermal keratinocytes: evidence for a hyperthermia-inducible protective response. *J Invest Dermatol* *103*, 547-553.
- McCarty, G., and Loeb, D.M. (2015). Hypoxia-sensitive epigenetic regulation of an antisense-oriented lncRNA controls WT1 expression in myeloid leukemia cells. *PLoS One* *10*, e0119837.
- Merienne, K., Helmlinger, D., Perkin, G.R., Devys, D., and Trottier, Y. (2003). Polyglutamine expansion induces a protein-damaging stress connecting heat shock protein 70 to the JNK pathway. *J Biol Chem* *278*, 16957-16967.
- Miller, J.H. (1985). Mutagenic specificity of ultraviolet light. *J Mol Biol* *182*, 45-65.
- Ming, L., Wang, P., Bank, A., Yu, J., and Zhang, L. (2006). PUMA Dissociates Bax and Bcl-X(L) to induce apoptosis in colon cancer cells. *J Biol Chem* *281*, 16034-16042.
- Monroe, M., Alam, M., Campbell, W.J., Torre, J.I.d.l., Caputy, G., DeBacker, C., Dryden, R.M., Duffy, M.T., Freema, J., Garza, J.R., *et al.* (2011). Head and Neck Cutaneous Squamous Cell Carcinoma (Medscape).
- Monteiro, J.P., and Cano, M.I. (2011). SIRT1 deacetylase activity and the maintenance of protein homeostasis in response to stress: an overview. *Protein Pept Lett* *18*, 167-173.
- Morimoto, R.I. (2002). Dynamic remodeling of transcription complexes by molecular chaperones. *Cell* *110*, 281-284.
- Mosser, D.D., and Morimoto, R.I. (2004). Molecular chaperones and the stress of oncogenesis. *Oncogene* *23*, 2907-2918.
- Mourad, R., Hsu, P.Y., Juan, L., Shen, C., Koneru, P., Lin, H., Liu, Y., Nephew, K., Huang, T.H., and Li, L. (2014). Estrogen induces global reorganization of chromatin structure in human breast cancer cells. *PLoS One* *9*, e113354.
- Mullard, A. (2015). The Roadmap Epigenomics Project opens new drug development avenues. *Nat Rev Drug Discov* *14*, 223-225.
- Mullerat, J., Deroide, F., Winslet, M.C., and Perrett, C.W. (2003). Proliferation and p53 expression in anal cancer precursor lesions. *Anticancer Res* *23*, 2995-2999.
- Murphy, M., Mabruk, M.J., Lenane, P., Liew, A., McCann, P., Buckley, A., C, O.F., Hevey, D., Billet, P., Robertson, W., *et al.* (2002). Comparison of the expression of p53, p21, Bax and the induction of apoptosis between patients with basal cell carcinoma and normal controls in response to ultraviolet irradiation. *Journal of clinical pathology* *55*, 829-833.
- Murphy, T.M., O'Donovan, A., Mullins, N., O'Farrelly, C., McCann, A., and Malone, K. (2015). Anxiety is associated with higher levels of global DNA methylation and altered expression of epigenetic and interleukin-6 genes. *Psychiatr Genet* *25*, 71-78.
- Nagai, A., Saijo, M., Kuraoka, I., Matsuda, T., Kodo, N., Nakatsu, Y., Mimaki, T., Mino, M., Biggerstaff, M., Wood, R.D., *et al.* (1995). Enhancement of damage-specific DNA binding of

XPA by interaction with the ERCC1 DNA repair protein. *Biochem Biophys Res Commun* 211, 960-966.

Nagata, Y., Anan, T., Yoshida, T., Mizukami, T., Taya, Y., Fujiwara, T., Kato, H., Saya, H., and Nakao, M. (1999). The stabilization mechanism of mutant-type p53 by impaired ubiquitination: the loss of wild-type p53 function and the hsp90 association. *Oncogene* 18, 6037-6049.

Nakajima, H., and Kunimoto, H. (2014). TET2 as an epigenetic master regulator for normal and malignant hematopoiesis. *Cancer Sci* 105, 1093-1099.

Nakaya, N., Lowe, S.W., Taya, Y., Chenchik, A., and Enikolopov, G. (2000). Specific pattern of p53 phosphorylation during nitric oxide-induced cell cycle arrest. *Oncogene* 19, 6369-6375.

Narayanan, D.L., Saladi, R.N., and Fox, J.L. (2010). Ultraviolet radiation and skin cancer. *Int J Dermatol* 49, 978-986.

Narine, K.A., Keuling, A.M., Gombos, R., Tron, V.A., Andrew, S.E., and Young, L.C. (2010). Defining the DNA mismatch repair-dependent apoptotic pathway in primary cells: evidence for p53-independence and involvement of centrosomal caspase 2. *DNA Repair (Amst)* 9, 161-168.

Nickoloff, B.J., Qin, J.Z., Chaturvedi, V., Bacon, P., Panella, J., and Denning, M.F. (2002). Life and death signaling pathways contributing to skin cancer. *J Investig Dermatol Symp Proc* 7, 27-35.

Nowotarski, S.L., Pachaiyappan, B., Holshouser, S.L., Kutz, C.J., Li, Y., Huang, Y., Sharma, S.K., Casero, R.A., Jr., and Woster, P.M. (2015). Structure-activity study for (bis)ureidopropyl- and (bis)thioureidopropylidiamine LSD1 inhibitors with 3-5-3 and 3-6-3 carbon backbone architectures. *Bioorg Med Chem* 23, 1601-1612.

Ozaki, T., and Nakagawara, A. (2011). Role of p53 in Cell Death and Human Cancers. *Cancers* 3, 994-1013.

Pacis, A., Nedelec, Y., and Barreiro, L.B. (2014). When genetics meets epigenetics: deciphering the mechanisms controlling inter-individual variation in immune responses to infection. *Curr Opin Immunol* 29, 119-126.

Pan, X. (2014). Single Cell Analysis: From Technology to Biology and Medicine. *Single Cell Biol* 3.

Paraiso, K.H., Xiang, Y., Rebecca, V.W., Abel, E.V., Chen, Y.A., Munko, A.C., Wood, E., Fedorenko, I.V., Sondak, V.K., Anderson, A.R., *et al.* (2011). PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. *Cancer Res* 71, 2750-2760.

Park, H.G., Han, S.I., Oh, S.Y., and Kang, H.S. (2005). Cellular responses to mild heat stress. *Cell Mol Life Sci* 62, 10-23.

Park, H.S., Lee, J.S., Huh, S.H., Seo, J.S., and Choi, E.J. (2001). Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase. *EMBO J* 20, 446-456.

Park, S.J., Borin, B., Martinez-Yamout, M., and Dyson, H.J. (2011a). The client protein p53 adopts a molten globule-like state in the presence of HSP90. *Nature Structural & Molecular Biology* 18, 537-541.

Park, S.J., Kostic, M., and Dyson, H.J. (2011b). Dynamic Interaction of Hsp90 with Its Client Protein p53. *J Mol Biol* 411, 158-173.

- Pathania, R., Ramachandran, S., Elangovan, S., Padia, R., Yang, P., Cinghu, S., Veeranan-Karmegam, R., Arjunan, P., Gnana-Prakasam, J.P., Sadanand, F., *et al.* (2015). DNMT1 is essential for mammary and cancer stem cell maintenance and tumorigenesis. *Nat Commun* 6, 6910.
- Pellegata, N.S., Antoniono, R.J., Redpath, J.L., and Stanbridge, E.J. (1996). DNA damage and p53-mediated cell cycle arrest: a reevaluation. *Proc Natl Acad Sci U S A* 93, 15209-15214.
- Peng, Y., Chen, L., Li, C., Lu, W., and Chen, J. (2001). Inhibition of MDM2 by hsp90 contributes to mutant p53 stabilization. *J Biol Chem* 276, 40583-40590.
- Perluigi, M., Di Diomenico, F., Blarzino, C., Foppoli, C., Cini, C., Giorgi, A., Grillo, C., De Marco, F., Butterfield, D., Schinina, M., *et al.* (2010). Effects of UVB-induced oxidative stress on protein expression and specific protein oxidation in normal epithelial keratinocytes: a proteomic approach. *Proteome Science* 8, 1-14.
- Phillips, P.A., Dudeja, V., McCarroll, J.A., Borja-Cacho, D., Dawra, R.K., Grizzle, W.E., Vickers, S.M., and Saluja, A.K. (2007). Triptolide induces pancreatic cancer cell death via inhibition of heat shock protein 70. *Cancer Res* 67, 9407-9416.
- Piao, M.J., Hyun, Y.J., Cho, S.J., Kang, H.K., Yoo, E.S., Koh, Y.S., Lee, N.H., Ko, M.H., and Hyun, J.W. (2012). An ethanol extract derived from *Bonnemaisonia hamifera* scavenges ultraviolet B (UVB) radiation-induced reactive oxygen species and attenuates UVB-induced cell damage in human keratinocytes. *Mar Drugs* 10, 2826-2845.
- Pines, A., Backendorf, C., Alekseev, S., Jansen, J.G., de Gruijl, F.R., Vrieling, H., and Mullenders, L.H. (2009). Differential activity of UV-DDB in mouse keratinocytes and fibroblasts: impact on DNA repair and UV-induced skin cancer. *DNA Repair (Amst)* 8, 153-161.
- Piva, R., Belardo, G., and Santoro, M.G. (2006). NF-kappaB: a stress-regulated switch for cell survival. *Antioxid Redox Signal* 8, 478-486.
- Pockley, G. (2003). Heat shock proteins as regulators of the immune response. *The Lancet*.
- Pognonec, P. (2010). ERK and cell death: overview. *FEBS J* 277, 1.
- Poltev, V.I., Shulyupina, N.V., and Bruskov, V.I. (1990). The formation of mispairs by 8-oxyguanine as a pathway of mutations induced by irradiation and oxygen radicals. *J Mol Recognit* 3, 45-47.
- Pons, M., and Quintanilla, M. (2006). Molecular biology of malignant melanoma and other cutaneous tumours. *Clin Trans Oncol* 8, 466-474.
- Porter, A.G., and Janicke, R.U. (1999). Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* 6, 99-104.
- Prost, S., Bellamy, C.O., Clarke, A.R., Wyllie, A.H., and Harrison, D.J. (1998). p53-independent DNA repair and cell cycle arrest in embryonic stem cells. *FEBS Lett* 425, 499-504.
- Putri, R.I., Siregar, N.C., and Siregar, B. (2014). Overexpression and amplification of Murine double minute 2 as a diagnostic tool in large lipomatous tumor and its correlation with Ki67 proliferation index: an institutional experience. *Indian J Pathol Microbiol* 57, 558-563.
- Qian, Y., Tu, J., Tang, N.L., Kong, G.W., Chung, J.P., Chan, W.Y., and Lee, T.L. (2015). Dynamic changes of DNA epigenetic marks in mouse oocytes during natural and accelerated aging. *Int J Biochem Cell Biol*.

- Quenneville, L.A., Trotter, M.J., Maeda, T., and Tron, V.A. (2002). p53-dependent regulation of heat shock protein 72. *Br J Dermatol* *146*, 786-791.
- Rao, V.G., Mandal, S., Ghosh, S., Banerjee, C., and Sarkar, N. (2013). Phase Boundaries, Structural Characteristics, and NMR Spectra of Ionic Liquid-in-Oil Microemulsions Containing Double Chain Surface Active Ionic Liquid: A Comparative Study. *J Phys Chem B* *117*, 1480-1493.
- Rass, K., and Reichrath, J. (2008). UV damage and DNA repair in malignant melanoma and nonmelanoma skin cancer. *Adv Exp Med Biol* *624*, 162-178.
- Ratajczak-Wrona, W., Jablonska, E., Marcinczyk, M., Grabowska, Z., and Piotrowski, L. (2009). Role of p38 MAPK pathway in induction of iNOS expression in neutrophils and peripheral blood mononuclear cells in patients with squamous cell carcinoma of the oral cavity. *J Oral Maxillofac Surg* *67*, 2354-2363.
- Raviram, R., Rocha, P.P., Bonneau, R., and Skok, J.A. (2014). Interpreting 4C-Seq data: how far can we go? *Epigenomics* *6*, 455-457.
- Raynes, R., Brunquell, J., and Westerheide, S.D. (2013a). Stress Inducibility of SIRT1 and Its Role in Cytoprotection and Cancer. *Genes Cancer* *4*, 172-182.
- Raynes, R., Pombier, K.M., Nguyen, K., Brunquell, J., Mendez, J.E., and Westerheide, S.D. (2013b). The SIRT1 modulators AROS and DBC1 regulate HSF1 activity and the heat shock response. *PLoS One* *8*, e54364.
- Reed, S.M., and Quelle, D.E. (2014). p53 Acetylation: Regulation and Consequences. *Cancers (Basel)* *7*, 30-69.
- Reich, A., Lehmann, B., Meurer, M., and Muller, D.J. (2007). Structural alterations provoked by narrow-band ultraviolet B in immortalized keratinocytes: assessment by atomic force microscopy. *Exp Dermatol* *16*, 1007-1015.
- Reidl, J., Starke, J., Omer, D.B., Grinvald, A., and Spors, H. (2007). Independent component analysis of high-resolution imaging data identifies distinct functional domains. *NeuroImage* *34*, 94-108.
- Reifenberger, J., Wolter, M., Knobbe, C.B., Kohler, B., Schonicke, A., Scharwachter, C., Kumar, K., Blaschke, B., Ruzicka, T., and Reifenberger, G. (2005). Somatic mutations in the PTCH, SMOH, SUFUH and TP53 genes in sporadic basal cell carcinomas. *Br J Dermatol* *152*, 43-51.
- Ren, Z.P., Hedrum, A., Ponten, F., Nister, M., Ahmadian, A., Lundeberg, J., Uhlen, M., and Ponten, J. (1996a). Human epidermal cancer and accompanying precursors have identical p53 mutations different from p53 mutations in adjacent areas of clonally expanded non-neoplastic keratinocytes. *Oncogene* *12*, 765-773.
- Ren, Z.P., Ponten, F., Nister, M., and Ponten, J. (1996b). Two distinct p53 immunohistochemical patterns in human squamous-cell skin cancer, precursors and normal epidermis. *Int J Cancer* *69*, 174-179.
- Rerole, A.L., Joly, A.L., Thuringer, D., and Garrido, C. (2010). HSP70 and HSP27: Emerging Targets in Cancer Therapy. In *Apoptosome*, pp. 169-202.
- Rezvani, H.R., Rossignol, R., Ali, N., Benard, G., Tang, X., Yang, H.S., Jouary, T., de Verneuil, H., Taieb, A., Kim, A.L., *et al.* (2011). XPC silencing in normal human keratinocytes triggers metabolic alterations through NOX-1 activation-mediated reactive oxygen species. *Biochim Biophys Acta* *1807*, 609-619.

- Riyahi, S., Sanchez-Delgado, M., Calafell, F., Monk, D., and Senar, J.C. (2015). Combined epigenetic and intraspecific variation of the DRD4 and SERT genes influence novelty seeking behavior in great tit *Parus major*. *Epigenetics* *10*, 516-525.
- Rocca, C.J., Poindessous, V., Soares, D.G., Ouadrani, K.E., Sarasin, A., Guerin, E., de Gramont, A., Henriques, J.A., Escargueil, A.E., and Larsen, A.K. (2010). The NER proteins XPC and CSB, but not ERCC1, regulate the sensitivity to the novel DNA binder S23906: implications for recognition and repair of antitumor alkylators. *Biochem Pharmacol* *80*, 335-343.
- Rosso, S., Sera, F., Segnan, N., and Zanetti, R. (2008). Sun exposure prior to diagnosis is associated with improved survival in melanoma patients: results from a long-term follow-up study of Italian patients. *Eur J Cancer* *44*, 1275-1281.
- Roti Roti, J.L. (2007). Heat-induced alterations of nuclear protein associations and their effects on DNA repair and replication. *Int J Hyperthermia* *23*, 3-15.
- Roti Roti, J.L. (2008). Cellular responses to hyperthermia (40-46 degrees C): cell killing and molecular events. *Int J Hyperthermia* *24*, 3-15.
- Rubin, T.G., Gray, J.D., and McEwen, B.S. (2014). Experience and the ever-changing brain: what the transcriptome can reveal. *Bioessays* *36*, 1072-1081.
- Sabunciyan, S., Maher, B., Bahn, S., Dickerson, F., and Yolken, R.H. (2015). Association of DNA Methylation with Acute Mania and Inflammatory Markers. *PLoS One* *10*, e0132001.
- Saksouk, N., Barth, T.K., Ziegler-Birling, C., Olova, N., Nowak, A., Rey, E., Mateos-Langerak, J., Urbach, S., Reik, W., Torres-Padilla, M.E., *et al.* (2014). Redundant mechanisms to form silent chromatin at pericentromeric regions rely on BEND3 and DNA methylation. *Mol Cell* *56*, 580-594.
- Saleh, H.A., Jackson, H., and Banerjee, M. (2000). Immunohistochemical expression of bcl-2 and p53 oncoproteins: correlation with Ki67 proliferation index and prognostic histopathologic parameters in colorectal neoplasia. *Appl Immunohistochem Mol Morphol* *8*, 175-182.
- Santen, S., Mihaescu, A., Laschke, M.W., Menger, M.D., Wang, Y., Jeppsson, B., and Thorlacius, H. (2009). p38 MAPK regulates ischemia-reperfusion-induced recruitment of leukocytes in the colon. *Surgery* *145*, 303-312.
- Saran, A. (2010). Basal cell carcinoma and the carcinogenic role of aberrant Hedgehog signaling. *Future Oncol* *6*, 1003-1014.
- Sarasin, A. (1999). The molecular pathways of ultraviolet-induced carcinogenesis. *Mutat Res* *428*, 5-10.
- Sasaki, T., Maier, B., Koclega, K.D., Chruszcz, M., Gluba, W., Stukenberg, P.T., Minor, W., and Scrbale, H. (2008). Phosphorylation regulates SIRT1 function. *PLoS One* *3*, e4020.
- Schraml, E., Fuchs, R., Kotzbeck, P., Grillari, J., and Schauenstein, K. (2009). Acute adrenergic stress inhibits proliferation of murine hematopoietic progenitor cells via p38/MAPK signaling. *Stem Cells Dev* *18*, 215-227.
- Schwarz, T. (2005). Biological effects of UV radiation on keratinocytes and Langerhans cells. *Exp Dermatol* *14*, 788-789.
- Seo, Y.R., and Jung, H.J. (2004). The potential roles of p53 tumor suppressor in nucleotide excision repair (NER) and base excision repair (BER). *Experimental & molecular medicine* *36*, 505-509.

- Seripa, D., Panza, F., Daragjati, J., Paroni, G., and Pilotto, A. (2015). Measuring pharmacogenetics in special groups: geriatrics. *Expert Opin Drug Metab Toxicol* *11*, 1073-1088.
- Setlow, R., Grist, E., Thompson, K., and Woodhead, A.D. (1993). Wavelengths effective in induction of malignant melanoma. *Proc Natl Acad Sci USA* *90*.
- Sexton-Oates, A., MacGregor, D., Dodgshun, A., and Saffery, R. (2015). The potential for epigenetic analysis of paediatric CNS tumours to improve diagnosis, treatment and prognosis. *Ann Oncol* *26*, 1314-1324.
- She, Q.B., Chen, N., Bode, A.M., Flavell, R.A., and Dong, Z. (2002). Deficiency of c-Jun-NH(2)-terminal kinase-1 in mice enhances skin tumor development by 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res* *62*, 1343-1348.
- Shellman, Y.G., Howe, W.R., Miller, L.A., Goldstein, N.B., Pacheco, T.R., Mahajan, R.L., LaRue, S.M., and Norris, D.A. (2008). Hyperthermia induces endoplasmic reticulum-mediated apoptosis in melanoma and non-melanoma skin cancer cells. *J Invest Dermatol* *128*, 949-956.
- Simpson, J.T. (2014). Genome informatics 2014. *Genome Biol* *15*, 543.
- Sinha, R.P., and Hader, D.P. (2002). Life under solar UV radiation in aquatic organisms. *Ad Space Res* *30*, 1547-1556.
- Smirnova, V.S., Gudkov, S.V., Chernikov, A.V., and Bruskov, V.I. (2005). [The formation of 8-oxoguanine and its oxidative products in DNA in vitro at 37 degrees C]. *Biofizika* *50*, 243-252.
- Smith, M.L., Ford, J.M., Hollander, M.C., Bortnick, R.A., Amundson, S.A., Seo, Y.R., Deng, C.X., Hanawalt, P.C., and Fornace, A.J., Jr. (2000). p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol Cell Biol* *20*, 3705-3714.
- Smoller, B.R. (2006). Squamous cell carcinoma: from precursor lesions to high-risk variants. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* *19 Suppl 2*, S88-92.
- Soehnge, H., Ouhtit, A., and Ananthaswamy, O.N. (1997). Mechanisms of induction of skin cancer by UV radiation. *Front Biosci* *2*, d538-551.
- Solomon, C., White, E., Kristal, A.R., and Vaughan, T. (2004). Melanoma and Lifetime UV Radiation. *Cancer Causes Control* *15*, 893-902.
- Solomon, J.M., Pasupuleti, R., Xu, L., McDonagh, T., Curtis, R., DiStefano, P.S., and Huber, L.J. (2006). Inhibition of SIRT1 catalytic activity increases p53 acetylation but does not alter cell survival following DNA damage. *Mol Cell Biol* *26*, 28-38.
- Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K., Tignor, M., and Miller, H. (2007). Climate Change 2007: The Physical Science Basis. In Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (United Kingdom and New York: Cambridge University Press).
- Sonna, L.A., Fujita, J., Gaffin, S.L., and Lilly, C.M. (2002). Invited review: Effects of heat and cold stress on mammalian gene expression. *J Appl Physiol* *92*, 1725-1742.
- Soti, C., Nagy, E., Giricz, Z., Vigh, L., Csermely, P., and Ferdinandy, P. (2005). Heat shock proteins as emerging therapeutic targets. *Br J Pharmacol* *146*, 769-780.

- Soumyanath, A., Venkatasamy, R., Joshi, M., Faas, L., Adejuyigbe, B., Drake, A., Hider, R., and Young, A. (2006). UV Irradiation Affects Melanocytes' Stimulatory Activity and Protein Binding of Piperine. *Photochemistry and Photobiology* 82, 1541-1548.
- Sridhar, H., Srinivasan, U., Askins, R.A., Canales-Delgado, J.C., Chen, C.C., Ewert, D.N., Gale, G.A., Goodale, E., Gram, W.K., Hart, P.J., *et al.* (2012). Positive relationships between association strength and phenotypic similarity characterize the assembly of mixed-species bird flocks worldwide. *Am Nat* 180, 777-790.
- Sumimoto, H., Imabayashi, F., Iwata, T., and Kawakami, Y. (2006). The BRAK-MAPK signalling pathway is essential for cancer-immune evasion in human melanoma cells. *The Journal of Experimental Medicine* 203, 1651-1656.
- Sun, B., Zhang, S., Zhang, D., Li, Y., Zhao, X., Luo, Y., and Guo, Y. (2008a). Identification of metastasis-related proteins and their clinical relevance to triple-negative human breast cancer. *Clin Cancer Res* 14, 7050-7059.
- Sun, J., Ramnath, R.D., Zhi, L., Tamizhselvi, R., and Bhatia, M. (2008b). Substance P enhances NF-kappaB transactivation and chemokine response in murine macrophages via ERK1/2 and p38 MAPK signaling pathways. *Am J Physiol Cell Physiol* 294, C1586-1596.
- Takahashi, A., Matsumoto, H., Nagayama, K., Kitano, M., Hirose, S., Tanaka, H., Mori, E., Yamakawa, N., Yasumoto, J., Yuki, K., *et al.* (2004). Evidence for the involvement of double-strand breaks in heat-induced cell killing. *Cancer Res* 64, 8839-8845.
- Takahata, C., Masuda, Y., Takedachi, A., Tanaka, K., Iwai, S., and Kuraoka, I. (2015). Repair synthesis step involving ERCC1-XPF participates in DNA repair of the Top1-DNA damage complex. *Carcinogenesis* 36, 841-851.
- Tallmadge, R.L., Shen, L., Tseng, C.T., Miller, S.C., Barry, J., and Felipe, M.J. (2015). Bone marrow transcriptome and epigenome profiles of equine common variable immunodeficiency patients unveil block of B lymphocyte differentiation. *Clin Immunol* 160, 261-276.
- Tan, S., and Bertucci, V. (2000). Erythema ab igne: an old condition new again. *CMAJ* 162, 77-78.
- Terzian, T., Torchia, E., Dai, D., Robinson, S., Murao, K., Steigman, R., Gonzalez, V., Boyle, G., Powell, M., Pollock, P., *et al.* (2010). p53 prevents progression of nevi to melanoma predominantly through cell cycle regulation. *Pigment Cell Melanoma Res* 23, 781-794.
- Tian, W., and Xu, Y. (2015). Decoding liver injury: A regulatory role for histone modifications. *Int J Biochem Cell Biol*.
- Tody, A., Higgins, E., Kazumasa, W., Ito, S., Brurchill, S., and Marks, J. (1991). Pheomelanin, as well as Eumelanin, are present in human epidermis. *Journal of Investigative Dermatology* 97, 340-344.
- Trautinger, F., Jantschitsch, C., Strohal, R., Pourmojib, M., and Kindas-Mugge, I. (1997). Mispriming and PCR amplification of hsp27. *Cell Stress Chaperones* 2, 276-277.
- Trautinger, F., Kindas-Mugge, I., Barlan, B., Neuner, P., and Knobler, R.M. (1995). 72-kD heat shock protein is a mediator of resistance to ultraviolet B light. *The Journal of investigative dermatology* 105, 160-162.

- Trautinger, F., Knobler, R.M., Honigsmann, H., Mayr, W., and Kindas-Mugge, I. (1996). Increased expression of the 72-kDa heat shock protein and reduced sunburn cell formation in human skin after local hyperthermia. *The Journal of investigative dermatology* *107*, 442-443.
- Tron, V.A., Trotter, M.J., Tang, L., Krajewska, M., Reed, J.C., Ho, V.C., and Li, G. (1998). p53-regulated apoptosis is differentiation dependent in ultraviolet B-irradiated mouse keratinocytes. *Am J Pathol* *153*, 579-585.
- Tronov, V., Artamonov, D., and B Gorbacheva, L. (2010). Genetic Markers of Melanoma. *Russian Journal of Genetics* *46*, 146-156.
- van den Elsen, P.J., van Eggermond, M.C., Puentes, F., van der Valk, P., Baker, D., and Amor, S. (2014). The epigenetics of multiple sclerosis and other related disorders. *Mult Scler Relat Disord* *3*, 163-175.
- van der Leun, J.C., Piacentini, R.D., and de Gruijl, F.R. (2008). Climate change and human skin cancer. *Photochem Photobiol Sci* *7*, 730-733.
- van Leeuwen, I.M., Higgins, M., Campbell, J., McCarthy, A.R., Sachweh, M.C., Navarro, A.M., and Lain, S. (2013). Modulation of p53 C-terminal acetylation by mdm2, p14ARF, and cytoplasmic SirT2. *Mol Cancer Ther* *12*, 471-480.
- van Steeg, H., and Kraemer, K.H. (1999). Xeroderma pigmentosum and the role of UV-induced DNA damage in skin cancer. *Mol Med Today* *5*, 86-94.
- Verdin, E., and Ott, M. (2015). 50 years of protein acetylation: from gene regulation to epigenetics, metabolism and beyond. *Nat Rev Mol Cell Biol* *16*, 258-264.
- Vidal, A.C., Benjamin Neelon, S.E., Liu, Y., Tuli, A.M., Fuemmeler, B.F., Hoyo, C., Murtha, A.P., Huang, Z., Schildkraut, J., Overcash, F., *et al.* (2014). Maternal stress, preterm birth, and DNA methylation at imprint regulatory sequences in humans. *Genet Epigenet* *6*, 37-44.
- Vilahur, N., Bustamante, M., Byun, H.M., Fernandez, M.F., Santa Marina, L., Basterrechea, M., Ballester, F., Murcia, M., Tardon, A., Fernandez-Somoano, A., *et al.* (2014). Prenatal exposure to mixtures of xenoestrogens and repetitive element DNA methylation changes in human placenta. *Environ Int* *71*, 81-87.
- Villanueva, A., Portela, A., Sayols, S., Battiston, C., Hoshida, Y., Mendez-Gonzalez, J., Imbeaud, S., Letouze, E., Hernandez-Gea, V., Cornella, H., *et al.* (2015). DNA methylation-based prognosis and epidrivers in hepatocellular carcinoma. *Hepatology* *61*, 1945-1956.
- Vivanco, I., Palaskas, N., Tran, C., Finn, S.P., Getz, G., Kennedy, N.J., Jiao, J., Rose, J., Xie, W., Loda, M., *et al.* (2007). Identification of the JNK signaling pathway as a functional target of the tumor suppressor PTEN. *Cancer Cell* *11*, 555-569.
- Vogelstein, B., Lane, D., and Levine, A.J. (2000). Surfing the p53 network. *Nature* *408*, 307-310.
- Volloch, V., and Rits, S. (1999). A natural extracellular factor that induces Hsp72, inhibits apoptosis, and restores stress resistance in aged human cells. *Exp Cell Res* *253*, 483-492.
- von Kriegsheim, A., Pitt, A., Grindlay, G.J., Kolch, W., and Dhillon, A.S. (2006). Regulation of the Raf-MEK-ERK pathway by protein phosphatase 5. *Nat Cell Biol* *8*, 1011-1016.
- Wagner, E.F., and Nebreda, A.R. (2009). Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* *9*, 537-549.

- Walerych, D., Gutkowska, M., Klejman, M.P., Wawrzynow, B., Tracz, Z., Wiech, M., Zylicz, M., and Zylicz, A. (2010). ATP binding to Hsp90 is sufficient for effective chaperoning of p53 protein. *J Biol Chem* 285, 32020-32028.
- Walerych, D., Kudla, G., Gutkowska, M., Wawrzynow, B., Muller, L., King, F.W., Helwak, A., Boros, J., Zylicz, A., and Zylicz, M. (2004). Hsp90 chaperones wild-type p53 tumor suppressor protein. *J Biol Chem* 279, 48836-48845.
- Wan, D., Jiang, C., Hua, X., Wang, T., and Chai, Y. (2015). Cell cycle arrest and apoptosis induced by aspidin PB through the p53/p21 and mitochondria-dependent pathways in human osteosarcoma cells. *Anticancer Drugs* 26, 931-941.
- Wang, C., Schroeder, F.A., Wey, H.Y., Borra, R., Wagner, F.F., Reis, S., Kim, S.W., Holson, E.B., Haggarty, S.J., and Hooker, J.M. (2014). In vivo imaging of histone deacetylases (HDACs) in the central nervous system and major peripheral organs. *J Med Chem* 57, 7999-8009.
- Wang, S., Gao, J., Lei, Q., Rozengurt, N., Pritchard, C., Jiao, J., Thomas, G.V., Li, G., Roy-Burman, P., Nelson, P.S., *et al.* (2003). Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. *Cancer Cell* 4, 209-221.
- Wang, Z., Fukuda, S., and Pelus, L.M. (2004). Survivin regulates the p53 tumor suppressor gene family. *Oncogene* 23, 8146-8153.
- Wei, L.K., Sutherland, H., Au, A., Camilleri, E., Haupt, L.M., Gan, S.H., and Griffiths, L.R. (2015). Metylenetetrahydrofolate Reductase CpG Islands: Epigenotyping. *J Clin Lab Anal.*
- Weigmann, H.J., Schanzer, S., Antoniou, C., Sterry, W., and Lademann, J. (2010). Influence of the absorption behavior of sunscreens in the short-wavelength UV range (UVB) and the long-wavelength UV range (UVA) on the relation of the UVB absorption to sun protection factor. *Journal of biomedical optics* 15, 055008.
- Wellbrock, C. (2005). Melanoma Development and Pigment Cell Transformation. In *From Melanocytes to Melanoma: The Progression to Malignancy*, V. Hearing, and S. Leong, eds. (NJ: Humana Press Inc.), pp. 247-261.
- Welsh, M.M., Karagas, M.R., Kuriger, J.K., Houseman, A., Spencer, S.K., Perry, A.E., and Nelson, H.H. (2011). Genetic determinants of UV-susceptibility in non-melanoma skin cancer. *PLoS One* 6, e20019.
- Westerheide, S.D., Anckar, J., Stevens, S.M., Jr., Sistonen, L., and Morimoto, R.I. (2009). Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. *Science* 323, 1063-1066.
- Weston, C.R., and Davis, R.J. (2007). The JNK signal transduction pathway. *Curr Opin Cell Biol* 19, 142-149.
- Whitesell, L., Bagatell, R., and Falsey, R. (2003). The stress response: implications for the clinical development of hsp90 inhibitors. *Current cancer drug targets* 3, 349-358.
- Whitesell, L., and Lindquist, S.L. (2005). HSP90 and the chaperoning of cancer. *Nat Rev Cancer* 5, 761-772.
- Whitesell, L., Sutphin, P.D., Pulcini, E.J., Martinez, J.D., and Cook, P.H. (1998). The physical association of multiple molecular chaperone proteins with mutant p53 is altered by geldanamycin, an hsp90-binding agent. *Mol Cell Biol* 18, 1517-1524.

- Wiech, M., Olszewski, M.B., Tracz-Gaszewska, Z., Wawrzynow, B., Zylicz, M., and Zylicz, A. (2012). Molecular mechanism of mutant p53 stabilization: the role of HSP70 and MDM2. *PLoS One* 7, e51426.
- Wilken, M.S., Brzezinski, J.A., La Torre, A., Siebenthal, K., Thurman, R., Sabo, P., Sandstrom, R.S., Vierstra, J., Canfield, T.K., Hansen, R.S., *et al.* (2015). DNase I hypersensitivity analysis of the mouse brain and retina identifies region-specific regulatory elements. *Epigenetics Chromatin* 8, 8.
- Wilking, M.J., Singh, C., Nihal, M., Zhong, W., and Ahmad, N. (2014). SIRT1 deacetylase is overexpressed in human melanoma and its small molecule inhibition imparts anti-proliferative response via p53 activation. *Arch Biochem Biophys* 563, 94-100.
- Williams, M., and Ouhtit, A. (2005). Towards better understanding of the molecular mechanisms involved in sunlight induced melanoma. *Journal of Biomedical and Biotechnology*, 57-61.
- Winter, M., Moser, M.A., Meunier, D., Fischer, C., Machat, G., Mattes, K., Lichtenberger, B.M., Brunmeir, R., Weissmann, S., Murko, C., *et al.* (2013). Divergent roles of HDAC1 and HDAC2 in the regulation of epidermal development and tumorigenesis. *EMBO J* 32, 3176-3191.
- Wong, R.S., Kapp, L.N., Krishnaswamy, G., and Dewey, W.C. (1993). Critical steps for induction of chromosomal aberrations in CHO cells heated in S phase. *Radiat Res* 133, 52-59.
- Wu, D., and Cederbaum, A. (2008). Cytochrome P4502E1 sensitizes to tumor necrosis factor alpha-induced liver injury through activation of mitogen-activated protein kinases in mice. *Hepatology* 47, 1005-1017.
- Wu, X., Bayle, J.H., Olson, D., and Levine, A.J. (1993). The p53-mdm-2 autoregulatory feedback loop. *Genes Dev* 7, 1126-1132.
- Xia, J., Sellers, L.A., Oxley, D., Smith, T., Emson, P., and Keverne, E.B. (2006). Urinary pheromones promote ERK/Akt phosphorylation, regeneration and survival of vomeronasal (V2R) neurons. *Eur J Neurosci* 24, 3333-3342.
- Xiang, F., Lucas, R., Hales, S., and Neale, R. (2014). Incidence of nonmelanoma skin cancer in relation to ambient UV radiation in white populations, 1978-2012: empirical relationships. *JAMA dermatology* 150, 1063-1071.
- Xu, Y., Li, N., Xiang, R., and Sun, P. (2014). Emerging roles of the p38 MAPK and PI3K/AKT/mTOR pathways in oncogene-induced senescence. *Trends Biochem Sci* 39, 268-276.
- Yaglom, J.A., Gabai, V.L., and Sherman, M.Y. (2007). High levels of heat shock protein Hsp72 in cancer cells suppress default senescence pathways. *Cancer Res* 67, 2373-2381.
- Yamaguchi, Y., Beer, J., and Hearing, V. (2008). Melanin Mediated Apoptosis of Epidermal Cells Damaged by Ultraviolet Radiation: Factors influencing the incidence of skin cancer. Springer-Verlag Publishing 1, 43-50.
- Yang, J., Liu, X., Niu, P., Zou, Y., Gong, Z., Yuan, J., and Wu, T. (2007). Dynamic changes of XPA, XPC, XPF, XPG and ERCC1 protein expression and their correlations with levels of DNA damage in human bronchial epithelia cells exposed to benzo[a]pyrene. *Toxicol Lett* 174, 10-17.

- Yang, X., Yang, J., Li, L., Sun, L., Yi, X., Han, X., Si, W., Yan, R., Chen, Z., Xie, G., *et al.* (2014). PAAT, a novel ATPase and trans-regulator of mitochondrial ABC transporters, is critically involved in the maintenance of mitochondrial homeostasis. *FASEB J* 28, 4821-4834.
- Yi, J., and Luo, J. (2010). SIRT1 and p53, effect on cancer, senescence and beyond. *Biochim Biophys Acta* 1804, 1684-1689.
- Yogianti, F., Kunisada, M., Ono, R., Sakumi, K., Nakabeppu, Y., and Nishigori, C. (2012). Skin tumours induced by narrowband UVB have higher frequency of p53 mutations than tumours induced by broadband UVB independent of Ogg1 genotype. *Mutagenesis* 27, 637-643.
- Yoshihisa, Y., Hassan, M.A., Furusawa, Y., Tabuchi, Y., Kondo, T., and Shimizu, T. (2012). Alkannin, HSP70 inducer, protects against UVB-induced apoptosis in human keratinocytes. *PLoS One* 7, e47903.
- Yu, Z.K., Geyer, R.K., and Maki, C.G. (2000). MDM2-dependent ubiquitination of nuclear and cytoplasmic P53. *Oncogene* 19, 5892-5897.
- Yuan, J., Luo, K., Liu, T., and Lou, Z. (2012). Regulation of SIRT1 activity by genotoxic stress. *Genes Dev* 26, 791-796.
- Zhang, H., and Burrows, F. (2004). Targeting multiple signal transduction pathways through inhibition of Hsp90. *J Mol Med (Berl)* 82, 488-499.
- Zhang, H.S., Chen, X.Y., Wu, T.C., Sang, W.W., and Ruan, Z. (2012). MiR-34a is involved in Tat-induced HIV-1 long terminal repeat (LTR) transactivation through the SIRT1/NFkappaB pathway. *FEBS Lett* 586, 4203-4207.
- Zitzmann, M., Bongers, R., Werler, S., Bogdanova, N., Wistuba, J., Kliesch, S., Gromoll, J., and Tuttelmann, F. (2015). Gene expression patterns in relation to the clinical phenotype in Klinefelter syndrome. *J Clin Endocrinol Metab* 100, E518-523.
- Zylicz, M., King, F.W., and Wawrzynow, A. (2001). Hsp70 interactions with the p53 tumour suppressor protein. *EMBO J* 20, 4634-4638.

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APPENDICES

Ethics



APPLICATION FOR RESEARCH INVOLVING HUMAN PARTICIPANTS

SECTION 1 – PROJECT DETAILS

1.1 TITLE	
PROJECT NUMBER	8569
PROJECT NAME	The Role of Heat in Skin Cancer Initiation
PROJECT SHORT NAME	
PROJECT STATUS	Approved

1.2 DURATION			
WHOLE PROJECT	FROM:	27 February 2012	TO: 31 December 2015
DATA COLLECTION PHASE	FROM:	1 August 2012	TO: 31 December 2014

1.3 APPLICATION TYPE		
MULTICENTRE PROJECT	PERMITS/LICENCES REQUIRED	EXECUTIVE APPROVAL
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

1.5 STUDENT PROJECT	
Is this project for the purposes of a degree (student project)?	Yes

STUDENT NUMBER	FACULTY	DEGREE TYPE
10100338	CHS	PhD or Doctorate

1.6 CLASSIFICATION OF THE PROJECT

CATEGORY	DESCRIPTION
<input type="checkbox"/>	Observation only E.g. observation of students in a classroom
<input type="checkbox"/>	Data/information/samples to be collected anonymously Researcher will NEVER know the identity of the participant e.g. anonymous questionnaire
<input type="checkbox"/>	Creative arts research project E.g. participants will be involved in creating a new work of art
<input type="checkbox"/>	Collection/creation of works of art for an exhibition
<input type="checkbox"/>	Oral history or biographical research project
<input type="checkbox"/>	Qualitative methodology E.g. focus group, semi-structured interview, action research
<input type="checkbox"/>	Evaluation research only Researchers will only conduct the evaluation of an existing program
<input type="checkbox"/>	Comparison or evaluation of existing methods or techniques E.g. comparison of different training methods
<input type="checkbox"/>	Educational research project involving tasks that are the same or similar to those usually completed by students in a standard education setting
<input type="checkbox"/>	Research to be conducted in the researcher's workplace
<input type="checkbox"/>	Administration of standardized psychological tests
<input type="checkbox"/>	Epidemiological studies Projects involving population based research which may include data linkage
<input type="checkbox"/>	Use of identifiable or potentially identifiable personal information about individuals WITHOUT their consent
<input type="checkbox"/>	Psychiatric or clinical psychology research
<input type="checkbox"/>	Research projects involving physiological investigations
<input type="checkbox"/>	Testing of biomechanical devices
<input type="checkbox"/>	Human tissue samples, including blood, tissues, sputum, urine
<input type="checkbox"/>	Human genetic research
<input type="checkbox"/>	Ionising radiation
<input type="checkbox"/>	Clinical research NOT under CTN/CTX scheme
<input type="checkbox"/>	Clinical trial of a drug or device Under CTN/CTX scheme
<input type="checkbox"/>	Research involving assisted reproductive technology or the use of embryos and/or gametes
<input type="checkbox"/>	Surgical procedures
<input checked="" type="checkbox"/>	OTHER Use of commercialised cell lines.

SECTION 2 – APPLICANT DETAILS

2.1 Chief Investigator

NAME	Ms Leslie Calapre	
ADDRESS	78 Sturtridge Rd Lockridge WA 6054	
PHONE	CONTACT NUMBER	MOBILE
	6304 5716	0407246254
ECU EMAIL	l.calapre@ecu.edu.au	
FACULTY	Computing, Health and Science	
SCHOOL	Medical Sciences	
ROLE IN PROJECT	Chief Investigator	

2.2 Responsible Investigator

NAME	Ms Leslie Calapre	
ADDRESS	78 Sturtridge Rd Lockridge WA 6054	
PHONE	CONTACT NUMBER	MOBILE
	6304 5716	0407246254
ECU EMAIL	l.calapre@ecu.edu.au	
FACULTY	Computing, Health and Science	
SCHOOL	Medical Sciences	
ROLE IN PROJECT	Chief Investigator	

2.3 Other Investigator

NAME	A/Prof Mel Ziman	
ADDRESS		
PHONE	CONTACT NUMBER	MOBILE
	6304 5171	
ECU EMAIL	m.ziman@ecu.edu.au	
FACULTY	Computing, Health and Science	
SCHOOL	Medical Sciences	
ROLE IN PROJECT	Supervisor	

2.5 EXPERIENCE OF RESEARCHERS

Leslie Calapre has a Bachelor of Science (Biomedical Science) numerous basic techniques were learnt. Furthermore, the researcher have worked with melanocytes and keratinocytes previously as part of an Honours project. Methodology for cell culturing outlined in the proposal is similar to the method used to obtained data in previous Honours studies.

Both A/Prof Melanie Ziman have extensive knowledge in the field of melanoma research and have widespread experience of techniques involved in research into this field of study.

2.6 CONTACT PERSON FOR THIS PROJECT

Ms Leslie Calapre

SECTION 3 – EXECUTIVE SUMMARY, AIMS AND RESEARCH QUESTION(S)

3.1 EXECUTIVE SUMMARY

A brief summary of the project.

This project aims to determine if exposure of human skin cells, particularly keratinocytes and melanocytes, to increased temperature (heat) and combination of heat and UV radiation can lead to the development of changes commonly associated with skin cancer formation. The role of the cancer associated heat shock protein 72 (HSP72) in relation to skin cancer initiation via exposure to the chosen stressors will also be monitored. Human epidermal keratinocytes and melanocytes will be co-cultured and exposed to the heat and UV plus heat, and the changes that will be monitored will include physiological changes (cell morphology), rate of apoptosis (cell death), rate of proliferation (cell growth) and genetic changes.

3.2 AIMS

The aims of the project and how the project is designed to meet its aims.

This project has four aims:

1. To determine if long exposure to heat stress alone is sufficient to stimulate changes that has been associated with skin cancer development.
2. To determine whether exposure to heat exacerbates the effects of UV on skin cells, resulting in early appearance of changes that are linked to skin cancer development.
3. To determine whether heat exposure enhances UV-induced cellular changes through HSP72 interactions with JNK pathways, the pro-survival proteins Akt and ERK, and p53.

To achieve these aims, human skin cells (keratinocytes and melanocytes) will be co-cultured and exposed to heat and UV plus heat. Changes in keratinocytes and melanocytes' cell morphology, apoptosis and proliferation rates as well as their genetic components will be monitored to and then compared to primary skin cancer cell lines to determine if these cells have adapted dysplastic or cancer properties. The interaction of HSP72, which is activated when exposed to extreme heat and is commonly found in cancer, and other genes such as JNK, p53, Akt and ERK will be given particular focus as this was hypothesised to have a major role in the process of skin cancer formation after exposure to UV and heat.

3.3 RESEARCH QUESTION(S)

The research questions that the research project seeks to answer.

This project is based on these two hypotheses:

- Repeated long exposures to heat will enhance the processes activated by UV radiation to induce changes that are associated with skin cancer development.
- Changes that are associated with skin cancer formation will be observed after constant exposure to UV plus heat due to the interaction of heat-sustained HSP72 expression and the UV-damaged signalling pathways involved in cell apoptosis and survival.

3.4 ANTICIPATED OUTCOMES

The anticipated outcomes of the project – how the project will contribute to new knowledge in the research field.

This project hopes to provide a substantial evidence that exposure to heat stress, alone and/or in combination with UV radiation, can induce changes in epidermal cells that are associated with skin cancer formation. This knowledge is of great importance particularly to people working in mining, construction and petroleum industries, who are constantly exposed to intense heat and UV radiation. By defining the role of heat in skin cancer initiation, strategies aiming to educate and prevent or decrease constant exposure can be formulated and implemented, thus possibly decreasing skin cancer incidences among people working in these environments. Furthermore, the recently concluded Climate Change Adaptation Conference have alluded to the possibility that the normal global temperature in the next decades will be between 40°C - 42°C, therefore it is important to determine the effects of these temperatures on skin cells now, so that in the future further increase in skin cancer incidence may be prevented.

SECTION 4 – PARTICIPANTS

4.1 APPLICATION TYPE				
Use of information from databanks (with no contact with participants)	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
Use of previously collected information	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
Use of previously collected human tissue samples only	YES	<input checked="" type="checkbox"/>	NO	<input type="checkbox"/>
Contact with participants	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>

SECTION 6 – RESEARCH PROCEDURES

6.1 RESEARCH PROCEDURES			
<i>The research procedures that will be part of this project.</i>			
Observation		Interviews	
• With the knowledge of the participants	<input type="checkbox"/>	• Face-to-face	<input type="checkbox"/>
• Without the knowledge of the participants	<input type="checkbox"/>	• Telephone	<input type="checkbox"/>
• Recorded (video or audio)	<input type="checkbox"/>	• Recorded (video or audio)	<input type="checkbox"/>
Questionnaire/Survey (Written)		Focus groups	<input type="checkbox"/>
• Anonymous	<input type="checkbox"/>	Computer testing	<input type="checkbox"/>
• Potentially identifiable (coded)	<input type="checkbox"/>	Questionnaire/Survey (Online)	<input type="checkbox"/>
• Fully identifiable (participant's name is on it)	<input type="checkbox"/>	Photographs	<input type="checkbox"/>
Psychological tests	<input type="checkbox"/>	Physical activity or exercise	<input type="checkbox"/>
Physiological tests or medical tests	<input type="checkbox"/>	Involvement in the development or performance of a creative art work	<input type="checkbox"/>
Other – see explanation	<input checked="" type="checkbox"/>	Standard laboratory techniques will be used to monitor differences in cell morphology, apoptosis and proliferation rates, gene expression and mutation profiles of exposed cultured cells.	

PLEASE ATTACH A COPY OF ALL DATA COLLECTION INSTRUMENTS

NOTE: IF DATA COLLECTION INSTRUMENTS ARE STILL BEING DEVELOPED, PLEASE NOTE THIS BELOW. IT WILL BE A CONDITION OF APPROVAL THAT THESE DATA COLLECTION INSTRUMENTS ARE FORWARDED THE HREC FOR REVIEW AND APPROVAL BEFORE THEY ARE USED WITH PARTICIPANTS.

6.2 RESEARCH PROCEDURES – SUMMARY

A summary of what the participants in this research project will be asked to do.

N/A

Commercial cell lines will be used for this project.

6.3 RESEARCH PROCEDURES – RESPONSIBLE RESEARCHER(S)

The researcher(s) responsible for conducting the research procedures with the participants.

Ms Leslie Calapre

6.4 RESEARCH PROCEDURES – LOCATION

Where will the research / data collection take place?

Australia	YES	<input checked="" type="checkbox"/>	NO	<input type="checkbox"/>
Edith Cowan University Building 17 Level 3, Research Facility				
Overseas	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>

6.5 POSSIBLE EFFECTS OF RESEARCH PROCEDURES

<i>Does the project involve any test or procedures that may indicate the presence of a medical/clinical condition OR indicate that the participant may be "at risk"?</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
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6.6 SPECIFIC TYPES OF RESEARCH PROCEDURES

Privacy considerations Collection of identifiable data <i>without</i> the consent of participants from:	<input type="checkbox"/>
<ul style="list-style-type: none"> • A Commonwealth department or agency • A State department or agency • A private sector organisation 	
Collection and use of human tissue samples	<input type="checkbox"/>
Human genetic research	<input checked="" type="checkbox"/>
Human stem cell research	<input type="checkbox"/>
Research involving pregnant women and/or human foetus	<input type="checkbox"/>
Use of substances/procedures that may be harmful	<input type="checkbox"/>
Clinical research, including clinical trials and innovative therapy or innovation	<input type="checkbox"/>

SECTION 7 – RISKS AND BENEFITS

7.1 LEVEL OF RISKS TO PARTICIPANTS

<i>Details of potential risks to participants.</i>	
Level of risk that the research project may involve	Participants may only experience inconvenience

7.3 OTHER RISKS

<i>Details of any other risks associated with the research project.</i>	
There will be no risk to participants in this research, as the cell lines that will be used are available commercially. There may be a slight risk to the researcher due to the use of standard laboratory chemicals and procedures, however steps have been taken to greatly reduce the risks of harm to the researcher and a risk assessment has been completed.	

7.4 RISK ASSESSMENT

<i>The level of potential risk associated with this research project.</i>			
LIKELIHOOD		CONSEQUENCE	
RARE <i>May only occur in exceptional circumstances</i>	<input checked="" type="checkbox"/>	INSIGNIFICANT <i>No injuries. Little or no impact on participants.</i>	<input checked="" type="checkbox"/>
UNLIKELY <i>Could occur at some time</i>	<input type="checkbox"/>	MINOR <i>First aid treatment and/or counselling required.</i>	<input type="checkbox"/>
POSSIBLE <i>Might occur at some time</i>	<input type="checkbox"/>	SERIOUS <i>Medical treatment required.</i>	<input type="checkbox"/>
LIKELY <i>Will probably occur in most circumstances</i>	<input type="checkbox"/>	DISASTEROUS <i>Death or extensive injuries.</i>	<input type="checkbox"/>
ALMOST CERTAIN <i>Expected to occur in most circumstances</i>	<input type="checkbox"/>	CATASTROPHIC <i>Multiple deaths or severe permanent injuries.</i>	<input type="checkbox"/>
LIKELIHOOD Rating	1	CONSEQUENCE Rating	1

NOTE: THERE MAY BE OTHER CONSEQUENCES OF THE RISK OCCURRING – PLEASE REFER TO THE RISK MANAGEMENT MATRIX:
http://www.ecu.edu.au/RMAA/ecu_only/risk/Risk%20Management%20Definitions%20and%20Delegations%20Revised.xls

LIKELIHOOD	Risk Rating L x C	CONSEQUENCES				
		Insignificant	Minor	Serious	Disastrous	Catastrophic
Rare	1	1 (Low)	2 (Low)	3 (Low)	4 (Low)	5 (Moderate)
Unlikely	2	2 (Low)	4 (Low)	6 (Moderate)	8 (Moderate)	10 (Substantial)
Possible	3	3 (Low)	6 (Moderate)	9 (Moderate)	12 (Substantial)	15 (High)
Likely	4	4 (Low)	8 (Moderate)	12 (Substantial)	16 (High)	20 (Extreme)
Almost Certain	5	5 (Moderate)	10 (Substantial)	15 (High)	20 (Extreme)	25 (Extreme)

7.6 POTENTIAL BENEFITS - PARTICIPANTS*Details of the potential benefits of this research project to the participants.*

N/A

Human participants are not involved in this research. This research will use cell lines that are commercially available.

7.7 POTENTIAL BENEFITS – WIDER COMMUNITY*Details of the potential benefits of this research project to the wider community.*

This project hopes to provide a substantial evidence that exposure to heat stress, alone and/or in combination with UV radiation, can induce changes in epidermal cells that are associated with skin cancer formation. This knowledge is of great importance particularly to people working in mining, construction and petroleum industries, who are constantly exposed to intense heat and UV radiation. By defining the role of heat in skin cancer initiation, strategies aiming to educate and prevent or decrease constant exposure can be formulated and implemented, thus possibly decreasing skin cancer incidences among people working in these environments. Furthermore, the recently concluded Climate Change Adaptation Conference have alluded to the possibility that the normal global temperature in the next decades will be between 40°C - 42°C, therefore it is important to determine the effects of these temperatures on skin cells now, so that in the future further increase in skin cancer incidence may be prevented.

SECTION 8 – DATA COLLECTION – CONFIDENTIALITY AND SECURITY**8.1 DATA RECORDING***Recording of research data or information for this project.*

Computer file/floppy disk/CD/DVD	<input checked="" type="checkbox"/>	Transcripts of tapes/recordings	<input type="checkbox"/>
Paper copy e.g. questionnaires/surveys	<input type="checkbox"/>	Handwritten notes	<input type="checkbox"/>
Photographs	<input type="checkbox"/>	Interview notes	<input type="checkbox"/>
Audiotape/videotape/film recording	<input type="checkbox"/>	Works of art or their documentation	<input type="checkbox"/>
Electronic/digital recording	<input type="checkbox"/>		
Other – see explanation	<input type="checkbox"/>		

8.2 CONFIDENTIALITY DURING THE RESEARCH PROJECTWill participants be given an assurance of confidentiality? YES NO

Data are collected in anonymous format only	<input type="checkbox"/>	Participants have consented to being identified (e.g. oral history project)	<input type="checkbox"/>
Participants will be part of a public performance	<input type="checkbox"/>		
Other – see explanation	<input checked="" type="checkbox"/>	The cell lines that will be used are available commercially; no human participants are involved in the project.	

8.3 SECURITY OF DATA DURING THE RESEARCH PROJECT*Details of where the data collected during the research project will be kept and how security will be maintained*

Data will be stored on a computer that is secured by a password. Additionally, the computer is located in a building with restricted access.

8.4 ACCESS TO DATA*Details of access to the data*

N/A

Only the investigators listed on the application will have access to the data.

8.5 FUTURE USE OF DATAWill data (including samples) be preserved for possible future use in another RESEARCH project? YES NO

The cell lines may be used for future research conducted by the Melanoma Research Group.

8.6 STORAGE OF DATA AFTER THE PROJECT HAS BEEN COMPLETED*Details of data storage.*

Who is storing the data?	Leslie Calapre
Where is the data being stored?	Edith Cowan University, Joondalup Campus, Building 17 Level 3.
How long will the data be stored?	5 years - the minimum recommended period for retention

8.7 DISPOSAL OF DATAWill data ultimately be destroyed? YES NO

Data will be deleted permanently from the computer.

SECTION 9 – RESEARCH OUTCOMES

9.1 RESEARCH OUTCOMES – COMMUNICATION TO PARTICIPANTS

Will the results of the research project be made available to the participants? YES NO

There are no human participants in the study, only the use of cell lines that are commercially available.

9.2 RESEARCH OUTCOMES – COMMUNICATION TO WIDER COMMUNITY

Will the results of the research project be made available to the participants? YES NO

The method(s) to be used to communicate the results of the research project to the wider community.

Oral presentation	<input checked="" type="checkbox"/>	Thesis/dissertation	<input type="checkbox"/>
Conference presentation(s)	<input checked="" type="checkbox"/>	Report to organisation	<input type="checkbox"/>
Journal article(s) and/or book chapter(s)	<input checked="" type="checkbox"/>	Research paper	<input checked="" type="checkbox"/>
Artwork or its documentation	<input type="checkbox"/>		
Other – please provide details	<input type="checkbox"/>		

DECLARATION

PROJECT NUMBER	TITLE OF PROJECT
8569	The Role of Heat in Skin Cancer Initiation

As the Chief Investigator, I declare that

- I have read and understood the guidelines contained in the National Statement on Ethical Conduct in Human Research, the Australian Code for Conducting Responsible Research and the ECU Policy for the Conduct of Ethical Human Research and I accept the legal and ethical responsibilities associated with this application for ethics approval.
- All investigators in this research project, including staff and students, have the necessary experience and competence to undertake this research project to ensure protection of the welfare of the participants OR the necessary training will be obtained before the project commences.
- The project will be conducted in the manner approved by the Ethics Committee, which includes any specific conditions of approval.
- Any further changes to participants, procedures, timeline or staff involved will be notified to the Ethics Committee.

Chief Investigator

NAME	SIGNATURE	DATE
Ms Leslie Calapre		

Supervisor(s) (for student projects ONLY)

As the supervisor, I declare that the student researcher has received (or will receive) the necessary training and resources to undertake this research project.

NAME	SIGNATURE	DATE
A/Prof Mel Ziman		

Other Investigators

NAME	ROLE IN PROJECT	SIGNATURE	DATE

ADDITIONAL SECTIONS

	Details	Attachment required
10	MULTICENTRE RESEARCH PROJECTS	No
11	PERMITS/LICENCES	No
12	EXECUTIVE APPROVAL	No
13	PREVIOUSLY COLLECTED INFORMATION	No
14	PREVIOUSLY COLLECTED HUMAN TISSUE SAMPLES	Yes
15	RESEARCH INVOLVING CHILDREN AND YOUNG PEOPLE	No
16	RESEARCH INVOLVING ABORIGINAL AND TORRES STRAIT ISLANDER PEOPLES	No
17	RESEARCH IN OVERSEAS COUNTRIES	No
18	PRIVACY CONSIDERATIONS	No
19	COLLECTION AND USE OF HUMAN TISSUE SAMPLES	No
20	HUMAN GENETIC RESEARCH	Yes
21	SAFETY CONSIDERATIONS USE OF SUBSTANCES/PROCEDURES THAT MAY BE HARMFUL	No
22	CLINICAL RESEARCH	No

SECTION 14 – PREVIOUSLY COLLECTED HUMAN TISSUE SAMPLES

14.1 ACCESS TO PREVIOUSLY COLLECTED TISSUE SAMPLES

<i>Tissue previously collected by the Chief Investigator for another purpose</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Tissue held in archives or banks</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Tissue removed in the course of a clinical procedures and not required for any clinical purposes</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Other – see explanation</i>	YES	<input checked="" type="checkbox"/>	NO	<input type="checkbox"/>

Details of the previously collected human tissue samples.

The research will involve lines obtained from primary skin cancer lesions and neonatal and adult human epidermal melanocytes that are available commercially. The specific method of cell collection is not known to the researchers. cell lines neonatal and adult human epidermal melanocytes and keratinocytes that are available commercially. The specific method of cell collection is not known to the researchers.

14.2 INFORMATION PROVIDED FOR ORIGINAL COLLECTION AND USE

Details of information provided to individuals.

N/A

The research involves the use of cell lines that are available commercially.

14.3 CONSENT PROVIDED FOR ORIGINAL COLLECTION AND USE

<i>Diagnostic and/or therapeutic purposes</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Previously approved research project – please provide details below</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Consent provided for unspecified research but further consent required</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Consent provided for unspecified research but further consent not required</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Consent was not obtained</i>	YES	<input checked="" type="checkbox"/>	NO	<input type="checkbox"/>
<i>Other – see explanation</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>

14.4 CONSENT PROCESS

<i>Will individuals consent to the use of their tissue for this research project?</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
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<i>Tissue samples are deidentified (the identity of the original participants was never recorded OR the samples have been deidentified)</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>The original participants provided consent for the data or information to be used in further research projects</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>No further consent will be obtained</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Other – see explanation</i>	YES	<input checked="" type="checkbox"/>	NO	<input type="checkbox"/>

14.5 CONSENT PROCESS – HREC WAIVER OF CONSENT

<i>Is the research team requesting that the HREC waive the requirement for consent?</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
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14.6 RESULTS OF THE RESEARCH PROJECT

<i>Will the research project produce information relevant to the health and well being of the individuals who provided the tissue samples?</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
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14.7 SECURITY OF INFORMATION

Details of retention period, security arrangements, and fate of the tissue samples.

Any cells remaining after the research has been conducted that have been purchased for this project will be stored indefinitely in a liquid nitrogen tank for possible use in future projects. The storage facility is located in a restricted access level of the building and can only be accessed by research staff for the School of Medical Science at Edith Cowan University. However, due to the cell lines being available commercially there is no need for strict security.

SECTION 20 – HUMAN GENETIC RESEARCH

20.1 SOURCE OF GENETIC MATERIAL

<i>Genetic material collected from participants recruited for this research project</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Currently stored genetic material</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Genetic material obtained from a tissue bank or archive</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Other – see explanation</i>	YES	<input checked="" type="checkbox"/>	NO	<input type="checkbox"/>

DNA and RNA will be extracted from cell lines from primary and metastatic melanoma and adult and neonatal human epidermal melanocytes that are available commercially. The results of sequencing will be compared to known human DNA and RNA sequence libraries.

20.2 CONSENT PROCESS - INDIVIDUALS

<i>Will individuals consent to the use of their genetic material for this research project?</i>	YES	<input checked="" type="checkbox"/>	NO	<input type="checkbox"/>
<i>Use of genetic material in this research project only</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Use in specified research but further consent will be required</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Use in unspecified research but further consent not required</i>	YES	<input checked="" type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Other – see explanation</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>

20.3 CONSENT PROCESS - FAMILIES

<i>Will the project also involve the families of individuals who provide their genetic material for this research project – either as participants or to confirm family information provided by individuals?</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
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20.4 RESULTS OF THE RESEARCH PROJECT

<i>Will the research project potentially product information that may be relevant to the health and well being of the individuals and/or their family members?</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Will the research project potentially product information that may result in harm to individuals or their families or particular groups of people?</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Will the research project potentially product information that may have the potential to identify non-paternity, non-maternity or non-relationship to siblings?</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>
<i>Will the research project potentially product information that may reveal information about an individual's future health or risk of having children with a genetic disorder?</i>	YES	<input type="checkbox"/>	NO	<input checked="" type="checkbox"/>

20.5 SECURITY OF INFORMATION

<i>Details of retention period, security arrangements, and fate of the tissue samples.</i>
Upon completion of the project, no genetic material will be retained. During this project, no unrequired genetic material will be obtained, only genetic material needed to pursue the aims.

