We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

An Overview of Important Genetic Aspects in Melanoma

Rohinton S. Tarapore

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55257>

1. Introduction

Cancer of the skin is the most common form of malignancy in humans and is divided into two categories – non-malignant skin cancer and cutaneous melanoma. Non-melanoma skin cancer (basal cell and small cell carcinoma) make up a vast majority of skin cancers. According to data from National Cancer Institute (NCI) in 2012, more than 2 million new cases of non-melanomas will be identified with less than a 1000 deaths. Despite accounting for only 4% of all cases, melanoma is the deadliest of skin cancers resulting in over 79% of skin cancer deaths [1]. In the United States, melanoma is the fifth most common cancer in men and the sixth most common in women. In 2011, 70,230 new melanoma cases were identified with 8,790 deaths. The median age of diagnosis is between 45-55; although 25% of melanomas occur in individuals over 40 years.

2. Types of skin cancer

- a. **Basal Cell Carcinoma (BCC):** This is the most common form of skin cancer and accounts for more than 90% of all skin cancers in the United States. BCC causes damage by growing and invading the surrounding tissue and usually does not metastasize to other parts of the body. Intermittent sun exposure (especially early in life), age and light colored skin are important factors in the development of BCC. Approximately a fifth of BCCs, develop in regions that are not sun-exposed such as chest, arms, neck, back and scalp [2]. Weakening of the immune system on account of the disease or immune-suppressive drugs is known to promote the risk of developing BCCs. Usually BCC begins as a small, dome-shaped bump and is covered by small superficial blood vessels called telangiectases and its texture is often shiny and translucent. Hereditary predisposition to BCC [3,4] occurs

among individuals with albinism and Xeroderma Pigmentosum. These disorders can be linked to either instability of the skin or diminished pigmentation.

- b. **Squamous Cell Carcinoma (SCC):** This cancer begins in the squamous cells that form the surface of the skin, lining of hollow organs of the respiratory and digestive tracts. The earliest form of SCC is called as actinic keratosis (AK) [2] that appear as rough, red bumps on the scalp, face, ears and back of the hands. The rate at which the bumps (keratosis) invade deeper in the skin to become fully developed squamous cell carcinoma is estimated to be 10-20% over a 10 year period. Actinic keratinosis that becomes thicker and more tender could increase the possibility of getting transformed to an invasive squamous cell carcinoma phenotype. The most important risk factor is sun exposure. Lesions appear after years of sun damage in the forehead, cheeks as well as the backs of hands. Other minor factors like exposure to hydrocarbons, arsenic, heat or X-rays could predispose to SCCs. Unlike BCC, SCCs can metastasize to other parts but are easy to treat.
- c. **Melanoma:** This is the cancer of the melanocytes, the “skin-color producing cells” of the body. An estimated 132,000 new cases of melanoma occur worldwide every year [5,6,7] with approximately 65,161 deaths according to estimates from the World Health Organization (WHO). The high mortality rate of melanoma is remarkably high considering the fact that melanoma is nearly always curable in its early stages; the high mortality rate can be attributed to late diagnosis in which the cancer spreads to other parts of the body [5]. Melanoma incidence has increased more rapidly than that of any other cancer, yet our ability to treat disseminated disease has been lagging [8,9,10]. The predicted 1 year survival for Stage IV melanoma ranges between 41% to 59% [11]. At a very early point in the progression of melanoma, the cancer gains metastatic potential.

3. Risk factors

There are multiple risk factors that contribute to escalating incidence of melanomas in humans (Table 1). Ultraviolet (UV) radiation especially UVA (315-400 nm) and UVB (280-315 nm) from sunlight is an important contributing factor for melanoma progression. A study by Glanz et al [5,12] revealed that 90% of all melanomas are attributed to exposure to ultraviolet radiation.

The damaging effects of UV radiation (UVR) is on account of direct cellular damage and alterations in immunologic functions. UVR causes DNA damage (by formation of pyrimidine dimers), gene mutations, oxidative stress, immunosuppressive and inflammatory responses. All these effects play an important role in photoaging of the skin and predispose to skin cancer [13]. UVR creates mutations in p53, a key tumor suppressor gene that plays an important role in DNA repair and apoptosis. Thus if p53 is mutated, the cells lose the DNA repair process leading to the deregulation of apoptosis, expression of mutated keratinocytes and initiation of skin cancer [13,14]. Darker skinned individuals have lower incidence of cutaneous melanoma primarily as a result of increased epidermal melanin. Studies indicate that epidermal melanin in African-American individuals filters twice as much UVB radiation than in Caucasians. This

is on account of the larger, more melanized melanosomes located in the epidermis of dark skin individuals that absorb and scatter more light energy than the smaller, less melanized melanosomes of white skin. The incidence rate of skin cancer (both melanoma and non-melanoma) has increased significantly in the last decade [15]; particularly among young women. For most individuals, exposure to UVR from the sun is the main source of skin cancer. Nonetheless, some individuals are exposed to high UV doses through artificial sources – sunbeds and sunlamps used for tanning purposes. Indoor tanning is widespread in most developed countries in Northern Europe, Australia and the United States [16]. Intense early sunburns and blistering sunburns are closely associated with melanoma development [17,18, 19]. Statistics indicate that one severe childhood sunburn is associated with a two-fold increase in melanoma risk [20]. Chronic UV exposure results in increased skin aging, wrinkles, uneven skin pigmentation, loss of elasticity and a distribution in the skin barrier function [21]. Chronic UVR exposure is an important risk factor in the development of actinic keratosis (precursor of SCCs).

Ultraviolet (UV radiation)
UVA
UVB
Genetic syndromes
Xeroderma pigmentosum
Oculocutaneous albinism
Basal cell nevus syndrome
Ionizing radiation
X-rays
Other risk factors
Artificial UV radiation (tanning)
Smoking
Color of skin (having fair skin, especially with blue or hazel eyes)
History of precursor lesions
Chronically injured or non-healing wounds
Working outdoors
Increasing age

Table 1. Risk factors for skin cancer [1-8]

Candidate Gene	Protein	Location on chromosome	Stage of melanoma	References
Gene losses <i>PTEN</i>	Pten	10q	Primary & metastatic	[135,136,137]
<i>CDKN2A</i>	p16Ink4a/p14Arf	9p	Primary & metastatic	[135,136]
<i>ITGB3BP</i>	Beta-3-endonexin	1p31	Uveal	[138]
Gene amplification <i>BRAF</i>	Braf	7q21.3	Cutaneous	[135]
<i>CCND1</i>	cyclinD1	11q13	Acral	[136]
<i>CDK4</i>	cdk4	12q14	Acral & mucosal	[136]
<i>CDH2</i>	N-cadherin	18q	Uveal & metastatic	[137,139]
<i>c-MYC</i>	c-myc	8q23	Ocular, primary & metastatic	[135,137,140,141]
<i>MITF</i>	Mitf	3p14	Cutaneous, metastatic	[142,143]

Table 2. Chromosomal aberrations involving important genes found in melanoma

4. Roadway to melanoma

Malignant melanomas arise from epidermal melanocytes or the melanocyte precursor cell which are derived from the neural crest and migrate to the skin and hair follicles [22]. Melanoma initiation and progression is accompanied by a series of histological changes. The five distinct changes are: 1) nevus – benign lesion characterized by an increased number of nested melanocytes; 2) dysplastic nevus – which is characterized by random, discontinuous and atypical melanocytes; 3) radial-growth phase (RGP) melanoma where the cells acquire the ability to proliferate intraepidermally; 4) vertical growth phase (VGP) melanoma – characterized by melanoma cells acquiring the ability to penetrate through the basement membrane (BM) into underlying dermis and subcutaneous tissue; and 5) metastatic melanoma – characterized by the spread of melanoma cells to other areas of the skin and other organs. The most critical event in melanoma progression is the RGP-VGP transition which involves the escape from keratinocyte mediated growth control. This is consistent with tumor thickness being a strong predictor of metastatic disease and adverse clinical outcome [23].

Gene	Protein	Function	Commentary	References
Up-regulation <i>ATM</i>	ATM kinase	Cell cycle control, DNA damage response	Melanocytic infiltration	[135,136,137]
<i>CDH2</i>	N-cadherin	Cell-cell adhesion	Melanoma invasiveness	[144,145]
<i>MMP-1, 2</i>	Metallo proteinases -1, 2	Degradation of ECM	Tumor progression	[146]
<i>SPP1</i>	Osteopontin	Inductor of MMP	Risk of metastasis	[144,147]
<i>SPARC</i>	Osteonectin	Angiogenesis	Tumor progression	[144]
<i>WNT5A</i>	Wnt5a	Cell signaling	Melanoma invasiveness	[125,148]
<i>CKS2</i>	Cdc28/cdk1 protein kinase subunit 2	Cell cycle control	Poor prognosis	[147]
<i>EIF2gamma</i>	EIF2γ	DNA translation	Tumor progression	[149]
<i>PCNA</i>	PCNA	Cofactor DNA polymerase	Genome destabilization	[150]
Down Regulation <i>CDH1</i>	E-cadherin	Cell-cell adhesion	Tumor progression	[144,145,147]
<i>CDH3</i>	P-cadherin	Cell-cell adhesion	Tumor progression	[145,151]
<i>CDH10</i>	Cadherin-10	Cell-cell adhesion	High risk of metastasis	[144]
<i>DSC1</i>	Desmocollin-1	Desmosomal component	Loss of cell adhesion	[137,139,179]

Table 3. Genetic expression signatures associated with the progression of melanomas [24]

Acquisition of somatic mutations in key regulatory genes is the driving force behind the initiation and progression of melanoma development. For the past few decades numerous research teams around the world have researched on melanoma genetics leading to an overwhelming body of information.

5. Susceptibility genes

Approximately 8-12% of all melanomas are familial – occurring in individuals with a history of familial melanoma [24]. Two genes have been found to be associated with high penetrance susceptibility – CDKN2A and CDK4. Using linkage analysis of families with high melanoma incidences, the first melanoma incidence susceptibility gene, CDKN2A was identified at chromosome 9p21 [25,26]. The gene CDKN2A encodes two unrelated proteins – p16^{Ink4A} and p14^{Arf}. These proteins are tumor suppressors involved in cell cycle regulation. Numerous studies indicate that p16^{Ink4A} inhibits G1 cyclin dependent kinase (cdk4/cdkb) mediated phosphorylation of retinoblastoma protein (pRB) resulting in cell

cycle progression arrest through G1-S; while p14 favors apoptosis and blocks oncogenic transformation by stabilizing p53 levels through the inhibition of Mdm2-mediated p53 ubiquitination [27,28,29,30]. Loss of p16 promotes hyper-phosphorylation of pRb resulting in its inactivation while the loss of p14 inactivates p53 — leading to unrestricted cell cycle progression. Germline mutations in CDKN2A have been found in 40% of families with 3 or more family members affected by melanoma [31]. Not all individuals carrying germline CDKN2A mutations develop melanoma. Individuals with large numbers of pigment lesions or nevi have familial atypical mole-melanoma syndrome (FAMS) are associated with increased risk to developing melanoma [32,33].

The other melanoma susceptibility gene, CDK4 is located at chromosome 12q14 [34,35,36]. Mutations in CDK4 abrogate binding of cdk4 to p16 have been associated with melanoma pathogenesis [32]. This is evidence that links the entire p16^{Ink4A}-cdk4/cdk6-pRb pathway to melanoma indicating that hereditary retinoblastoma patients with germline inactivation of retinoblastoma (Rb1) are predisposed to melanoma [37,38].

6. Acquired genetic alterations in melanoma

Understanding the regulating pathways involved in melanoma development and progression has advanced significantly in recent years. The discovery of genetic alterations that aids in the formation of various cancers has aided in the development of numerous molecularly targeted therapies for individuals with metastatic disease [39,40,41]. These genes are known to be key molecular driver in melanoma; >70% cases harbor activating mutations in these genes. The molecule that is most commonly found to be mutated in melanomas is BRAF (~50% of all cancers) followed by NRAS (20%) and c-kit (1%) [42,43,44]. Melanoma is the result of complex changes in multiple signaling pathways affecting growth, cell mobility, metabolism and the ability to escape cell death progression. The Ras-Raf-Mek-Erk pathway followed by PI3K/Akt pathway is constitutively activated in a significant number of melanoma tumors.

7. The Ras-Raf-Mek-Erk

In 2002, a breakthrough study found that Braf to be mutated in a large percentage of melanomas — triggering new studies that focus on MAPK (mitogen activated protein kinase) signaling in melanomas. Braf is mutated in upto 82% of cutaneous nevi [45,46], 66% of primary melanomas [44] and 40-68% of metastatic melanomas [47,48]. A specific mutation substitution of valine with glutamic acid at residue 600 (BRAF V600E), account for 90% + BRAF mutation. Raf, a downstream effector of RAS is a serine-threonine specific protein kinase that activates Mek, which inturn activates Erk. Humans have 3 Raf genes: A-raf, B-raf and C-raf. The occurrence of mutation in Nras or Braf is 80-90% of all melanomas suggests that constitutive activation of extracellular signal regulated protein kinase (Ras-Raf-Mek-Erk). Most Ras mutations are present in codon 61 of N-Ras with K-Ras and H-

Ras mutations being relatively rare [49,50]. Constitutive activation of Ras-Raf-Mek-Erk cascade has been shown to contribute to tumorigenesis by inhibiting apoptosis and increasing cell proliferation, tumor invasion and metastasis. Activated Erk plays a pivotal role in cell proliferation by controlling the G1- to S-phase transition by negative regulation of p27 inhibition and upregulation of c-myc activity [51,52]. Inhibition of Erk activity is associated with G1 cell cycle arrest by upregulation of p21 and reduced phosphorylation [52]. Activated Erk is also known to stimulate cell proliferation by increasing the transcription and stability of c-Jun which is mediated by CREB (cyclic adenosine monophosphate responsive element-binding) and Gsk-3 β (glycogen synthase kinase-3beta) respectively [53]. Erk is also believed to increase proliferation by inhibiting differentiation. Constitutively active Erk limits differentiation in melanoma by targeting MITF (microphthalmia-associated transcription factor) for degradation [54,55,56]. The activated Erk pathway enhances melanoma specific survival by differentially regulating RSK-mediated phosphorylation and inactivation of the pro-apoptotic protein Bad [57] and inhibiting Jak-Stat pathway [58].

Erk signaling also contributes towards tumor invasion and metastasis by regulating the expression of integrin and matrix metalloproteinases (MMPs). Activated Ras-Mek-Erk pathway drives the production of MMP1 [59,60,61].

8. The PI3K/Akt pathway

The PI3K/Akt pathway is activated in various cancers, mostly on account of mutations in tumor suppressor PTEN (phosphatase and tensin homolog) [62]. In melanoma, loss of PTEN on chromosome 10q 23-24 was first reported by Parmiter et al [63]. The PTEN gene encodes a phosphatase that degrades products of PI3K by dephosphorylating phosphatidylinositol 3,4,5-triphosphate and phosphatidylinositol 3,4-bisphosphate at 3 positions [64]. Loss of PTEN increases AKT phosphorylation and activity leading to increased mitogenic signaling and decreased apoptosis [65]. Various studies suggest that 30-40% of melanoma cell lines and 5-15% of uncultured melanoma specimens carry inactivating mutations or homozygous deletions of Pten [63,66,67].

Pten encodes a negative regulator of extracellular growth signals that are transduced via PI3K-Akt pathway. Akt/protein kinase B (PKB), a serine-threonine kinase, is a core component of the PI3K signaling cascade and is activated through the phosphorylation of Ser 473/474 and Thr 308/309 [68,69]. Activated Akt regulates a network of factors that control cell proliferation and survival and this pathway is hyperactive in most metastatic melanomas [70,71,72]. Akt activates the transcription of a wide variety of genes involved in a wide range of cellular activities – those involved in immune activation, cell proliferation, apoptosis and cell survival [69]. Several studies have documented Akt activation in melanoma. Dai et al undertook a 292 sample study of pAkt levels using tissue microarray & immunohistochemistry strategies and identified strong pAkt expression in 17%, 43%, 49% and 77% of the biopsies in normal nevi, dysplastic nevi, primary melanoma and melanoma metastasis respectively. An important cell

adhesion protein MelCAM that plays critical roles in melanoma development was increased upon active Akt expression [73,74]. PI3K and Akt is known to increase the expression of MMP2 and MMP9 by a mechanism involving Akt activation of NF-kappaB binding to the MMP promoter [75,76]. Akt overexpression led to upregulation of VEGF, increased production of superoxide ROS. Akt can suppress apoptosis by phosphorylating and inactivating many proapoptotic proteins like caspase 9 and Bad [77,78]. PI3K pathway emerges as the central axis that is deregulated in melanoma and along with constitutively active MAPK pathway makes an important role in melanoma development progression. Thus targeting PI3K is expected to be an important therapeutic target modality for melanoma treatment.

9. Wnt/ β -catenin pathway

Beta-catenin (β -catenin) is a key component of the Wnt signaling pathway. Signaling through this pathway controls a wide range of cellular functions and aberrant Wnt/ β -catenin signaling can lead to cancer development and progression [79]. Wnts are glycoproteins that act as ligands to stimulate receptor-mediated signal transduction pathways involved in cell survival, proliferation, behavior and fate. Wnt proteins are known to activate 3 different extracellular pathways – Wnt/ β -catenin, Wnt/planar-polarity and Wnt/ Ca^{2+} pathways [80]. The Wnt/ β -catenin also known as the canonical Wnt pathway plays an important role in melanoma development. In the absence of Wnt ligands, free β -catenin binds to the destructive complex of Axin, adenomatous polyposis coli (APC) and glycogen synthase kinase-3 β (GSK-3 β). GSK-3 β mediates the phosphorylation of β -catenin at specific regulatory sites on the N-terminal side marking β -catenin for ubiquitination and subsequent proteosomal degradation. Upon the binding of Wnt ligand, GSK--catenin for ubiquitination and subsequent proteosomal degradation. Upon the binding of Wnt ligand, GSK-3 β activity is inhibited resulting in accumulation of β -catenin in the cytoplasm and shuttles into the nucleus where it serves as an essential co-activator of the Tcf/Lef (T-cell factor / lymphoid enhancer factor) family [81]. Numerous genes implicated in the tumorigenic process like c-myc and cyclinD1 have been identified as targets of the canonical Wnt signaling.

Increased nuclear localization of β -catenin – an important indication of activated Wnt signaling pathway is observed in over a third of melanoma specimens [82,83,84]. Mutations in β -catenin have been observed in about 23% of melanoma cancer cell lines and these mutations affect phosphorylation sites at Ser33, Ser37, Thr41 and Ser45 [85] at the N-terminal domain. These mutations render β -catenin resistant to phosphorylation and subsequent degradation. Low rates of β -catenin mutation have been observed in primary melanomas and metastasis indicating that activating mutations is a rare event in melanoma tumorigenesis [82,83,84,86,87,88]. Mutations in APC were observed sporadically in primary melanomas [82, 85,88]. While APC promoter 1A hypermethylation was observed in 17% of melanoma biopsies and 13% of melanoma cell lines. Wnt signaling pathways is activated in tumors through aberration in other genes. ICAT (inhibition of β -catenin & T-cell factor), a gene that negatively regulates the association of β -catenin with TCF4 thus repressing the transactivation of β -catenin-Tcf4 target genes [89]. A study by Reifenger J et al suggests that loss of ICAT

expression may contribute to the progression of melanoma [86]. ICAT mRNA expression analysis in two-third melanoma specimens revealed a 20% or less decrease in ICAT transcription [86]. However the mechanism behind the reduced ICAT mRNA level in melanoma is unclear.

Identification of Wnt target genes is also important towards the study of melanoma progression. Brn1, the POU domain transcription factor is directly controlled by Wnt signaling in transgenic mouse models and melanoma cell lines [90]. Studies indicate that overexpression of Brn2 is associated with increased melanoma progression and tumorigenicity [90,91]. MITF (microphthalmia-associated transcription factor), a Wnt target gene, is essential for the development of the melanocyte lineage and has an important role in the control of cell proliferation, survival and differentiation [54,92,93]. The regulation of MITF expression by β -catenin significantly influences the growth and survival behavior of treatment resistant melanoma [94]. A study by Schepsky A et al demonstrated that MITF can directly interact with β -catenin and redirect transcriptional activity away from canonical Wnt signaling-regulated target gene specific for MITF [95]. Induction of Wnt signaling can be blocked by 5 different proteins – Dkk, Wise, sFrp (secreted Frizzled related protein), Wif (wnt inhibitory factor), and Cerebrus that compete for the Wnt ligand or the Lrz-Frp receptor [96]. Interestingly, Dkk1 (Dickkopf 1) expression is negligible in melanomas [97]. Studies by Kuphal et al have demonstrated a downregulated or loss of Dkk-1, -2 and -3 in all melanoma cell lines and most of the melanoma tumor samples that were analyzed [98]. In xenograft mouse model, overexpression of Dkk-1 and Wif-1 inhibited melanoma tissue growth [99,100].

10. The JNK/c-Jun pathway

Activation of Jnks is usually in response to diverse stresses. These kinases play an important role in the regulation of cell proliferation, cell survival, cell death, DNA repair and metabolism. A variety of extracellular stimuli by cytokines, growth factors, hormones, UV radiation and tumor promoters are known to activate Jnks [101]. Sequential protein phosphorylation through a MAP-kinase module (MAP3K-MAP2K-MAPK) is responsible for Jnk activation [102]. Depending upon the cellular context, Jnk has been shown to elicit both positive and negative effects on tumor development [103]. Activation of Jnk is required for Ras-mediated transformation and mediate proliferation and tumor growth [104,105]. These observations are consistent with the findings of constitutively active Jnk in tumor samples and cell lines [103,106]. Jnk mediated the phosphorylation at serine 63 & 73 residues enhancing the ability of transcription factor c-Jun, a component of the AP-1 transcription complex [107]. The activation of Jnk leads to the induction of AP-1 dependent target genes that play important roles in cell proliferation, cell death and inflammation. Other members of the AP-1 transcription complex include c-Jun, Jun B, Jun D, c-Fos, Fra1 and Fra 2. The role of Jnk in oncogenesis is emerging; however c-Jun is a well defined oncogene in cancer. c-Jun is amplified and overexpressed in undifferentiated and aggressive sarcomas [108], breast and lung cancer [109,110]. Since the 1990s, the role of Jnk pathway in melanomas was recognized [111,112]. c-Jun, Jun B, c-fos genes play a role in the transformation of melanocytes into malignant melanomas [111].

The possible role Jnk pathway has led research teams to study the clinical relevance of interfering with this pathway. siRNA or chemical inhibitors of Jnk signaling inhibited proliferation in breast and non-small cell lung cancer (NSCLC) [106,113] and also induced apoptosis in prostate cancer cells [114]. A study by Gurzov E et al demonstrated that knock-down of c-Jun and Jun B in B16F10 melanoma cells by siRNA resulted in increased cell cycle arrest and apoptosis also resulting in extended survival of mice inoculated with these modified melanoma cells[115], suggesting that inactivation of c-Jun and Jun B could provide a valuable strategy for antitumor intervention [115].

11. The NF κ B (NF-kappaB) pathway

The NF κ B family in mammals contains 5 members – p105/p50 (NF κ B1), p100/p52 (NF κ B2), RelA (p65), RelB and c-Rel (J-206, 207). The canonical activation of NF κ B pathway involves TNF α stimulation resulting in the subsequent phosphorylation/activation of IKK (I κ B kinase). In turn, IKK-mediated phosphorylation of I κ B leading to ubiquitination of I κ B and its proteosomal degradation, releasing the NF κ B complex which activates a host of target genes [116,117]. The type of genes that get trans-activated depends on the composition of activated NF κ B complex. For instance, complexes containing c-Rel activates pro-apoptotic genes (Dr4/Dr5, Bcl-x) and inhibits anti-apoptotic genes (cellular inhibitor of apoptosis (cIAP1, cIAP2), survivin). Complexes containing RelA inhibits the expression of DR4/DR5 and upregulates caspase 8, cIAP1 and cIAP2 [118].

NF κ B is activated in various tumors including melanomas and distinct mechanisms have been proposed for the elevated levels of NF κ B activity in melanomas. Activation of NF κ B in melanomas is also linked to the loss of E-cadherin, a frequent event in melanoma transformation [119]. NIK (NF κ B interacting kinase), an activator of IKK is overexpressed in melanoma cells while compared to normal cells. The major contribution of NF κ B in melanoma development and progression relates to its function as an important regulator of survival and apoptosis. A study by Meyskens et al demonstrated that in metastatic melanoma cells, an increase in DNA binding activity of NF κ B is associated with an increased expression of p50 and RelA resulting in increased expression of anti-apoptotic regulators. Also the expression of c-Rel, the transcriptional activator of pro-apoptotic genes is markedly in melanoma cells compared with normal melanocytes [120]. Strong p50 nuclear staining also correlated with poor prognosis in melanoma patients [121]. Besides eliciting anti-apoptotic activities NF κ B mediates the transcription of MMP2 and MMP9 [121,122]. Overexpression of MMPs is associated with tumor invasion, metastasis and angiogenesis.

12. Melanoma stem cells

Stem cells are cells that can self-renew and the ability to differentiate into various cell lineages. These cells are located in the restrictive niche (environment). The interaction between stem

cells and their microenvironment is important for the self renewal process. These cells are highly clonogenic and slow cycling (quiescent) in response to proliferation and survival stimuli. Stem cells divide asymmetrically giving rise to a daughter cell that remains a stem cell (capable of self renewal) and another daughter cell that can rapidly divide and differentiate. Melanocytes that are found in the skin and in the choroid layer of the eye is derived from the neural crest (NC). Neural crest cells undergo EMT to migrate along the definite pathways in the embryo. NC cells give rise to a large array of differentiated cells – melanocytes, peripheral neurons & glia, endocrine and cartilage cells [123]. Melanoblasts which are melanocytic precursors – unpigmented cells with the potential to produce melanin, invade the skin areas and differentiate into melanocytes.

The cancer stem theory suggests that cancer originates from a small subpopulation of neoplastic stem cells that have the potential to self renew and are primarily responsible for sustaining the tumor and giving rise to progressively differentiating cells that proliferate rapidly and contribute to the cellular heterogeneity of the tumor (F-194). Cancer stem cells arise either from undifferentiated stem cells or from cells that possess stem cell like characteristics. Evidence suggests that aggressive melanoma cells acquire characteristics of embryonic stem cells having a multipotent plastic phenotype [124]. Studies by Bittner MP et al demonstrated that melanoma cells express genes associated with different cell types like endothelial, epithelial, fibroblastic, neuronal, hematopoietic and progenitor cells [125]. Strangely genes specific for melanocytes are downregulated in metastatic melanomas. Tyrosinase & MLANA (melan A), genes associated with pigmentation are greatly downregulated in aggressive melanomas [124]. Aggressive melanoma cells express endothelial-associated genes and form extravascular fluid-conducting networks which allow melanomas to greatly adapt to the hypoxic microenvironment of rapidly proliferating tumors, a phenomenon called as “vascular mimicry” [124,126]. From different melanoma cell lines, cells with stem cell-like features which have the ability to grow as non-adherent cell aggregates known as spheroids/spheres have been isolated (F-196). These cells have the ability to differentiate into various lineages – adipogenic, osteogenic, chondrogenic and melanogenic. A study by Bittner M et al demonstrated a subset of these spheroid cells express the cell surface marker CD20, a unique molecular signature of aggressive melanomas [125]. For the treatment of non-Hodkin’s lymphoma, CD20 is a standard therapeutic target which raises the possibility that CD20 could be used as a potential target for melanoma treatment [127].

Several studies have demonstrated that aggressive melanoma cells share characteristics with embryonic progenitors. Evidence suggests a major role for stromal components in all stages of tumorigenesis (initiation, progression and metastasis) [128]. Noted scientist Stephen Paget had coined the term “seed & soil” hypothesis predicting that metastatic cells only colonize soils (organs) that are permissive to their growth [129,130]. Studies show embryonic microenvironment has the capacity to reverse the metastatic phenotype of cancer cells. The microenvironment of human embryonic stem cells reprograms aggressive melanoma cells towards a less aggressive phenotype [124]. Nodal, an embryonic morphogen of the TGF β family is important for sustaining melanoma aggressiveness and plasticity. Nodal is regained in highly aggressive melanoma cell lines, invasive VGP (vertical growth phase)-stage melanoma and

metastatic melanoma [131], implicating Nodal as a novel diagnostic marker in melanoma progression and could be a therapeutic target for metastatic melanoma treatment [124].

13. Conclusion

Our understanding of melanoma development and progression has evolved tremendously over the past three decades. Unfortunately our understanding of the molecular biology of melanoma is still far from complete despite extensive research and knowledge gained in chromosomal alterations, mutations in important melanoma-associated genes, epigenetic modifications and melanoma microenvironment. Even to this day, the best prognostic significance of primary melanoma is the thickness of the tumor (i.e. RGP → VGP transition) and the presence/absence of ulcerations. Melanoma still remains as a tumor that is refractory to current chemotherapeutic treatments. A further study of the interaction between various signaling pathways will help researchers decipher the complexity of the genetic and epigenetic changes which eventually would lead to better therapeutic modalities for the treatment of primary and metastatic melanomas.

Glossary

AK - actinic keratosis

BCC - basal cell carcinoma

Cdks - cyclin-dependent kinases

CREB - cyclic adenosine monophosphate responsive element-binding

FAMS - familial atypical mole-melanoma syndrome

GSK3 β - glycogen synthase kinase-3 β

IKK - Inhibitor of I κ B kinase

MAPK - mitogen activated protein kinase

MITF - microphthalmia-associated transcription factor

MMP - matrix metalloproteinase

NF- κ B - nuclear factor kappa-B

NIK - NF κ B-interacting kinase

Pten - phosphatase and tensin homolog

Rb - retinoblastoma

RGP - radial growth phase

SCC - Squamous cell carcinoma

Tcf4 - T-cell factor-4

UVR - ultraviolet radiation

VGP - vertical growth phase

WIF1 - Wnt-inhibitory factor 1

Author details

Rohinton S. Tarapore

University of Pennsylvania School of Medicine, Philadelphia, PA, USA

References

- [1] Jemal, A, Bray, F, Center, M. M, Ferlay, J, Ward, E, et al. (2011). Global cancer statistics. *CA Cancer J Clin* , 61, 69-90.
- [2] Marghoob, A. A. (2011). Skin cancers and their etiologies. *Semin Cutan Med Surg* 30: S, 1-5.
- [3] Eichberger, T, Regl, G, Ikram, M. S, Neill, G. W, Philpott, M. P, et al. (2004). FOXE1, a new transcriptional target of GLI2 is expressed in human epidermis and basal cell carcinoma. *J Invest Dermatol* , 122, 1180-1187.
- [4] Iwasaki, J. K, Srivastava, D, Moy, R. L, Lin, H. J, & Kouba, D. J. (2012). The molecular genetics underlying basal cell carcinoma pathogenesis and links to targeted therapeutics. *J Am Acad Dermatol* 66: e, 167-178.
- [5] Narayanan, D. L, Saladi, R. N, & Fox, J. L. (2010). Ultraviolet radiation and skin cancer. *Int J Dermatol* , 49, 978-986.
- [6] Foster, P. J, Dunn, E. A, Karl, K. E, Snir, J. A, Nycz, C. M, et al. (2008). Cellular magnetic resonance imaging: in vivo imaging of melanoma cells in lymph nodes of mice. *Neoplasia* , 10, 207-216.
- [7] Marugame, T, & Zhang, M. J. (2010). Comparison of time trends in melanoma of skin cancer mortality (1990-2006) between countries based on the WHO mortality database. *Jpn J Clin Oncol* 40: 710.
- [8] Ko, J. M, Velez, N. F, & Tsao, H. (2010). Pathways to melanoma. *Semin Cutan Med Surg* , 29, 210-217.

- [9] Jemal, A, Thun, M. J, Ries, L. A, Howe, H. L, Weir, H. K, et al. (2008). Annual report to the nation on the status of cancer, 1975-2005, featuring trends in lung cancer, tobacco use, and tobacco control. *J Natl Cancer Inst* , 100, 1672-1694.
- [10] Linos, E, Swetter, S. M, Cockburn, M. G, Colditz, G. A, & Clarke, C. A. (2009). Increasing burden of melanoma in the United States. *J Invest Dermatol* , 129, 1666-1674.
- [11] Wargo, J. A, & Tanabe, K. (2009). Surgical management of melanoma. *Hematol Oncol Clin North Am* x, 23, 565-581.
- [12] Glanz, K, Buller, D. B, & Saraiya, M. (2007). Reducing ultraviolet radiation exposure among outdoor workers: state of the evidence and recommendations. *Environ Health* 6: 22.
- [13] Meeran, S. M, Punathil, T, & Katiyar, S. K. (2008). IL-12 deficiency exacerbates inflammatory responses in UV-irradiated skin and skin tumors. *J Invest Dermatol* , 128, 2716-2727.
- [14] Benjamin, C. L, & Ananthaswamy, H. N. (2007). and the pathogenesis of skin cancer. *Toxicol Appl Pharmacol* 224: 241-248., 53.
- [15] Garbe, C, & Leiter, U. (2009). Melanoma epidemiology and trends. *Clin Dermatol* , 27, 3-9.
- [16] Gandini, S, Autier, P, & Boniol, M. (2011). Reviews on sun exposure and artificial light and melanoma. *Prog Biophys Mol Biol* , 107, 362-366.
- [17] Preston, D. S, & Stern, R. S. (1992). Nonmelanoma cancers of the skin. *N Engl J Med* , 327, 1649-1662.
- [18] Gilchrest, B. A, Eller, M. S, Geller, A. C, & Yaar, M. (1999). The pathogenesis of melanoma induced by ultraviolet radiation. *N Engl J Med* , 340, 1341-1348.
- [19] Gloster, H. M. Jr., Neal K ((2006). Skin cancer in skin of color. *J Am Acad Dermatol quiz* 761-744., 55, 741-760.
- [20] Ma, F, Collado-mesa, F, Hu, S, & Kirsner, R. S. (2007). Skin cancer awareness and sun protection behaviors in white Hispanic and white non-Hispanic high school students in Miami, Florida. *Arch Dermatol* , 143, 983-988.
- [21] Schroeder, P, Haendeler, J, & Krutmann, J. (2008). The role of near infrared radiation in photoaging of the skin. *Exp Gerontol* , 43, 629-632.
- [22] Bennett, D. C. (1993). Genetics, development, and malignancy of melanocytes. *Int Rev Cytol* , 146, 191-260.
- [23] Tsao, H, Atkins, M. B, & Sober, A. J. (2004). Management of cutaneous melanoma. *N Engl J Med* , 351, 998-1012.
- [24] Lomas, J, Martin-duque, P, Pons, M, & Quintanilla, M. (2008). The genetics of malignant melanoma. *Front Biosci* , 13, 5071-5093.

- [25] Hussussian, C. J, Struewing, J. P, Goldstein, A. M, Higgins, P. A, Ally, D. S, et al. (1994). Germline mutations in familial melanoma. *Nat Genet* 8: 15-21., 16.
- [26] Kamb, A, Shattuck-eidens, D, Eeles, R, Liu, Q, Gruis, N. A, et al. (1994). Analysis of the gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. *Nat Genet* 8: 23-26., 16.
- [27] Pomerantz, J, Schreiber-agus, N, Liegeois, N. J, Silverman, A, Alland, L, et al. (1998). The Ink4a tumor suppressor gene product, interacts with MDM2 and neutralizes MDM2's inhibition of p53. *Cell* 92: 713-723., 19Arf.
- [28] Zhang, Y, Xiong, Y, & Yarbrough, W. G. (1998). ARF promotes MDM2 degradation and stabilizes ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell* 92: 725-734., 53.
- [29] Kamijo, T, Weber, J. D, Zambetti, G, Zindy, F, Roussel, M. F, et al. (1998). Functional and physical interactions of the ARF tumor suppressor with and Mdm2. *Proc Natl Acad Sci U S A* 95: 8292-8297., 53.
- [30] Stott, F. J, Bates, S, James, M. C, McConnell, B. B, Starborg, M, et al. (1998). The alternative product from the human CDKN2A locus, ARF), participates in a regulatory feedback loop with p53 and MDM2. *EMBO J* 17: 5001-5014., 14.
- [31] Chin, L, Garraway, L. A, & Fisher, D. E. (2006). Malignant melanoma: genetics and therapeutics in the genomic era. *Genes Dev* , 20, 2149-2182.
- [32] Gandini, S, Sera, F, Cattaruzza, M. S, Pasquini, P, Abeni, D, et al. (2005). Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. *Eur J Cancer* , 41, 28-44.
- [33] Gandini, S, Sera, F, Cattaruzza, M. S, Pasquini, P, Picconi, O, et al. (2005). Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. *Eur J Cancer* , 41, 45-60.
- [34] Zuo, L, Weger, J, Yang, Q, Goldstein, A. M, Tucker, M. A, et al. (1996). Germline mutations in the binding domain of CDK4 in familial melanoma. *Nat Genet* 12: 97-99., 16INK4a.
- [35] Soufir, N, Avril, M. F, Chompret, A, Demenais, F, Bombled, J, et al. (1998). Prevalence of and CDK4 germline mutations in 48 melanoma-prone families in France. The French Familial Melanoma Study Group. *Hum Mol Genet* 7: 209-216., 16.
- [36] Wolfel, T, Hauer, M, Schneider, J, Serrano, M, Wolfel, C, et al. (1995). A CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* 269: 1281-1284., 16INK4a.
- [37] Eng, C, Li, F. P, Abramson, D. H, Ellsworth, R. M, Wong, F. L, et al. (1993). Mortality from second tumors among long-term survivors of retinoblastoma. *J Natl Cancer Inst* , 85, 1121-1128.

- [38] Fletcher, O, Easton, D, Anderson, K, Gilham, C, Jay, M, et al. (2004). Lifetime risks of common cancers among retinoblastoma survivors. *J Natl Cancer Inst* , 96, 357-363.
- [39] Druker, B. J, Talpaz, M, Resta, D. J, Peng, B, Buchdunger, E, et al. (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* , 344, 1031-1037.
- [40] Demetri, G. D, Von Mehren, M, & Blanke, C. D. Van den Abbeele AD, Eisenberg B, et al. ((2002). Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* , 347, 472-480.
- [41] Lynch, T. J, Bell, D. W, Sordella, R, Gurubhagavatula, S, Okimoto, R. A, et al. (2004). Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* , 350, 2129-2139.
- [42] Curtin, J. A, Busam, K, Pinkel, D, & Bastian, B. C. (2006). Somatic activation of KIT in distinct subtypes of melanoma. *J Clin Oncol* , 24, 4340-4346.
- [43] Albino, A. P. Le Strange R, Oliff AI, Furth ME, Old LJ ((1984). Transforming ras genes from human melanoma: a manifestation of tumour heterogeneity? *Nature* , 308, 69-72.
- [44] Davies, H, Bignell, G. R, Cox, C, Stephens, P, Edkins, S, et al. (2002). Mutations of the BRAF gene in human cancer. *Nature* , 417, 949-954.
- [45] Lopez-bergami, P, Fitchman, B, & Ronai, Z. (2008). Understanding signaling cascades in melanoma. *Photochem Photobiol* , 84, 289-306.
- [46] Pollock, P. M, Harper, U. L, Hansen, K. S, Yudt, L. M, Stark, M, et al. (2003). High frequency of BRAF mutations in nevi. *Nat Genet* , 33, 19-20.
- [47] Gorden, A, Osman, I, Gai, W, He, D, Huang, W, et al. (2003). Analysis of BRAF and N-RAS mutations in metastatic melanoma tissues. *Cancer Res* , 63, 3955-3957.
- [48] Kumar, R, Angelini, S, Czene, K, Sauroja, I, Hahka-kemppinen, M, et al. (2003). BRAF mutations in metastatic melanoma: a possible association with clinical outcome. *Clin Cancer Res* , 9, 3362-3368.
- [49] Carr, J, & Mackie, R. M. (1994). Point mutations in the N-ras oncogene in malignant melanoma and congenital naevi. *Br J Dermatol* , 131, 72-77.
- [50] Van Elsas, A, Zerp, S, Van Der Flier, S, Kruse-wolters, M, Vacca, A, et al. (1995). Analysis of N-ras mutations in human cutaneous melanoma: tumor heterogeneity detected by polymerase chain reaction/single-stranded conformation polymorphism analysis. *Recent Results Cancer Res* , 139, 57-67.
- [51] Kortylewski, M, Heinrich, P. C, Kauffmann, M. E, & Bohm, M. MacKiewicz A, et al. ((2001). Mitogen-activated protein kinases control Kip1 expression and growth of human melanoma cells. *Biochem J* 357: 297-303., 27.

- [52] Lefevre, G, Calipel, A, Mouriaux, F, Hecquet, C, Malecaze, F, et al. (2003). Opposite long-term regulation of c-Myc and through overactivation of Raf-1 and the MEK/ERK module in proliferating human choroidal melanoma cells. *Oncogene* 22: 8813-8822., 27Kip1.
- [53] Lopez-bergami, P, Huang, C, Goydos, J. S, Yip, D, Bar-eli, M, et al. (2007). Rewired ERK-JNK signaling pathways in melanoma. *Cancer Cell* , 11, 447-460.
- [54] Goding, C. R. (2000). Mitf from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage. *Genes Dev* , 14, 1712-1728.
- [55] Wu, M, Hemesath, T. J, Takemoto, C. M, Horstmann, M. A, Wells, A. G, et al. (2000). c-Kit triggers dual phosphorylations, which couple activation and degradation of the essential melanocyte factor Mi. *Genes Dev* , 14, 301-312.
- [56] Kim, D. S, Hwang, E. S, Lee, J. E, Kim, S. Y, Kwon, S. B, et al. (2003). Sphingosine-1-phosphate decreases melanin synthesis via sustained ERK activation and subsequent MITF degradation. *J Cell Sci* , 116, 1699-1706.
- [57] Eisenmann, K. M, Vanbrocklin, M. W, Staffend, N. A, Kitchen, S. M, & Koo, H. M. (2003). Mitogen-activated protein kinase pathway-dependent tumor-specific survival signaling in melanoma cells through inactivation of the proapoptotic protein bad. *Cancer Res* , 63, 8330-8337.
- [58] Krasilnikov, M, Ivanov, V. N, Dong, J, & Ronai, Z. (2003). Erk and p38 negatively regulate STAT-transcriptional activities in human melanoma cells: implications towards sensitization to apoptosis. *Oncogene* , 22, 4092-4101.
- [59] Tower, G. B, Coon, C. C, Benbow, U, Vincenti, M. P, & Brinckerhoff, C. E. (2002). Erk 1/2 differentially regulates the expression from the 1G/2G single nucleotide polymorphism in the MMP-1 promoter in melanoma cells. *Biochim Biophys Acta* , 1586, 265-274.
- [60] Ishii, Y, Ogura, T, Tatemichi, M, Fujisawa, H, Otsuka, F, et al. (2003). Induction of matrix metalloproteinase gene transcription by nitric oxide and mechanisms of MMP-1 gene induction in human melanoma cell lines. *Int J Cancer* , 103, 161-168.
- [61] Ramos, M. C, Steinbrenner, H, Stuhlmann, D, Sies, H, & Brenneisen, P. (2004). Induction of MMP-10 and MMP-1 in a squamous cell carcinoma cell line by ultraviolet radiation. *Biol Chem* , 385, 75-86.
- [62] Steelman, L. S, Bertrand, F. E, & McCubrey, J. A. (2004). The complexity of PTEN: mutation, marker and potential target for therapeutic intervention. *Expert Opin Ther Targets* , 8, 537-550.
- [63] Parmiter, A. H, Balaban, G, & Clark, W. H. Jr., Nowell PC ((1988). Possible involvement of the chromosome region 10q24---q26 in early stages of melanocytic neoplasia. *Cancer Genet Cytogenet* , 30, 313-317.

- [64] Simpson, L, & Parsons, R. (2001). PTEN: life as a tumor suppressor. *Exp Cell Res* , 264, 29-41.
- [65] Wu, H, Goel, V, & Haluska, F. G. (2003). PTEN signaling pathways in melanoma. *Oncogene* , 22, 3113-3122.
- [66] Herbst, R. A, Weiss, J, Ehnis, A, Cavenee, W. K, & Arden, K. C. (1994). Loss of heterozygosity for 10q22-10qter in malignant melanoma progression. *Cancer Res* , 54, 3111-3114.
- [67] Healy, E, Rehman, I, Angus, B, & Rees, J. L. (1995). Loss of heterozygosity in sporadic primary cutaneous melanoma. *Genes Chromosomes Cancer* , 12, 152-156.
- [68] Harlan, J. E, Yoon, H. S, Hajduk, P. J, & Fesik, S. W. (1995). Structural characterization of the interaction between a pleckstrin homology domain and phosphatidylinositol 4,5-bisphosphate. *Biochemistry* , 34, 9859-9864.
- [69] Nicholson, K. M, & Anderson, N. G. (2002). The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal* , 14, 381-395.
- [70] Stahl, J. M, Sharma, A, Cheung, M, Zimmerman, M, Cheng, J. Q, et al. (2004). Deregulated Akt3 activity promotes development of malignant melanoma. *Cancer Res* , 64, 7002-7010.
- [71] Robertson, G. P. (2005). Functional and therapeutic significance of Akt deregulation in malignant melanoma. *Cancer Metastasis Rev* , 24, 273-285.
- [72] Dhawan, P, Singh, A. B, Ellis, D. L, & Richmond, A. (2002). Constitutive activation of Akt/protein kinase B in melanoma leads to up-regulation of nuclear factor-kappaB and tumor progression. *Cancer Res* , 62, 7335-7342.
- [73] Li, G, Kalabis, J, Xu, X, Meier, F, Oka, M, et al. (2003). Reciprocal regulation of Mel-CAM and AKT in human melanoma. *Oncogene* , 22, 6891-6899.
- [74] Johnson, J. P. (1999). Cell adhesion molecules in the development and progression of malignant melanoma. *Cancer Metastasis Rev* , 18, 345-357.
- [75] Kim, D, Kim, S, Koh, H, Yoon, S. O, Chung, A. S, et al. (2001). Akt/PKB promotes cancer cell invasion via increased motility and metalloproteinase production. *FASEB J* , 15, 1953-1962.
- [76] Park, B. K, Zeng, X, & Glazer, R. I. (2001). Akt1 induces extracellular matrix invasion and matrix metalloproteinase-2 activity in mouse mammary epithelial cells. *Cancer Res* , 61, 7647-7653.
- [77] Cardone, M. H, Roy, N, Stennicke, H. R, Salvesen, G. S, Franke, T. F, et al. (1998). Regulation of cell death protease caspase-9 by phosphorylation. *Science* , 282, 1318-1321.

- [78] Datta, S. R, Dudek, H, Tao, X, Masters, S, Fu, H, et al. (1997). Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* , 91, 231-241.
- [79] Giles, R. H, Van Es, J. H, & Clevers, H. (2003). Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim Biophys Acta* , 1653, 1-24.
- [80] Veeman, M. T, Axelrod, J. D, & Moon, R. T. (2003). A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev Cell* , 5, 367-377.
- [81] Elcheva, I, Tarapore, R. S, Bhatia, N, & Spiegelman, V. S. (2008). Overexpression of mRNA-binding protein CRD-BP in malignant melanomas. *Oncogene* , 27, 5069-5074.
- [82] Rimm, D. L, Caca, K, Hu, G, Harrison, F. B, & Fearon, E. R. (1999). Frequent nuclear/cytoplasmic localization of beta-catenin without exon 3 mutations in malignant melanoma. *Am J Pathol* , 154, 325-329.
- [83] Demunter, A, Libbrecht, L, Degreef, H, & De Wolf-peeters, C. van den Oord JJ ((2002). Loss of membranous expression of beta-catenin is associated with tumor progression in cutaneous melanoma and rarely caused by exon 3 mutations. *Mod Pathol* , 15, 454-461.
- [84] Omholt, K, Platz, A, Ringborg, U, & Hansson, J. (2001). Cytoplasmic and nuclear accumulation of beta-catenin is rarely caused by CTNNB1 exon 3 mutations in cutaneous malignant melanoma. *Int J Cancer* , 92, 839-842.
- [85] Rubinfeld, B, Robbins, P, Gamil, M, Albert, I, Porfiri, E, et al. (1997). Stabilization of beta-catenin by genetic defects in melanoma cell lines. *Science* , 275, 1790-1792.
- [86] Reifemberger, J, Knobbe, C. B, Wolter, M, Blaschke, B, Schulte, K. W, et al. (2002). Molecular genetic analysis of malignant melanomas for aberrations of the WNT signaling pathway genes CTNNB1, APC, ICAT and BTRC. *Int J Cancer* , 100, 549-556.
- [87] Pollock, P. M, & Hayward, N. (2002). Mutations in exon 3 of the beta-catenin gene are rare in melanoma cell lines. *Melanoma Res* , 12, 183-186.
- [88] Worm, J, Christensen, C, Gronbaek, K, Tulchinsky, E, & Guldberg, P. (2004). Genetic and epigenetic alterations of the APC gene in malignant melanoma. *Oncogene* , 23, 5215-5226.
- [89] Tago, K, Nakamura, T, Nishita, M, Hyodo, J, Nagai, S, et al. (2000). Inhibition of Wnt signaling by ICAT, a novel beta-catenin-interacting protein. *Genes Dev* , 14, 1741-1749.
- [90] Goodall, J, Martinozzi, S, Dexter, T. J, Champeval, D, Carreira, S, et al. (2004). Brn-2 expression controls melanoma proliferation and is directly regulated by beta-catenin. *Mol Cell Biol* , 24, 2915-2922.
- [91] Thomson, J. A, Murphy, K, Baker, E, Sutherland, G. R, Parsons, P. G, et al. (1995). The brn-2 gene regulates the melanocytic phenotype and tumorigenic potential of human melanoma cells. *Oncogene* , 11, 691-700.

- [92] Hodgkinson, C. A, Moore, K. J, Nakayama, A, Steingrimsson, E, Copeland, N. G, et al. (1993). Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. *Cell* , 74, 395-404.
- [93] Hughes, M. J, Lingrel, J. B, Krakowsky, J. M, & Anderson, K. P. (1993). A Helix-loop helix transcription factor-like gene is located at the mi locus. *J Biol Chem* , 268, 20687-20690.
- [94] Widlund, H. R, Horstmann, M. A, Price, E. R, Cui, J, Lessnick, S. L, et al. (2002). Beta-catenin-induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. *J Cell Biol* , 158, 1079-1087.
- [95] Schepsky, A, Bruser, K, Gunnarsson, G. J, Goodall, J, Hallsson, J. H, et al. (2006). The microphthalmia-associated transcription factor Mitf interacts with beta-catenin to determine target gene expression. *Mol Cell Biol* , 26, 8914-8927.
- [96] Kawano, Y, & Kypta, R. (2003). Secreted antagonists of the Wnt signalling pathway. *J Cell Sci* , 116, 2627-2634.
- [97] Forget, M. A, Turcotte, S, Beauseigle, D, Godin-ethier, J, Pelletier, S, et al. (2007). The Wnt pathway regulator DKK1 is preferentially expressed in hormone-resistant breast tumours and in some common cancer types. *Br J Cancer* , 96, 646-653.
- [98] Kuphal, S, Lodermeier, S, Bataille, F, Schuierer, M, Hoang, B. H, et al. (2006). Expression of Dickkopf genes is strongly reduced in malignant melanoma. *Oncogene* , 25, 5027-5036.
- [99] Mikheev, A. M, Mikheeva, S. A, Rostomily, R, & Zarbl, H. (2007). Dickkopf-1 activates cell death in MDA-MB435 melanoma cells. *Biochem Biophys Res Commun* , 352, 675-680.
- [100] Lin, Y. C, You, L, Xu, Z, He, B, Yang, C. T, et al. (2007). Wnt inhibitory factor-1 gene transfer inhibits melanoma cell growth. *Hum Gene Ther* , 18, 379-386.
- [101] Weston, C. R, & Davis, R. J. (2007). The JNK signal transduction pathway. *Curr Opin Cell Biol* , 19, 142-149.
- [102] Karin, M. (1995). The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* , 270, 16483-16486.
- [103] Kennedy, N. J, & Davis, R. J. (2003). Role of JNK in tumor development. *Cell Cycle* , 2, 199-201.
- [104] Yang, Y. M, Bost, F, Charbono, W, Dean, N, McKay, R, et al. (2003). C-Jun NH(2)-terminal kinase mediates proliferation and tumor growth of human prostate carcinoma. *Clin Cancer Res* , 9, 391-401.
- [105] Cui, J, Han, S. Y, Wang, C, Su, W, Harshyne, L, et al. (2006). c-Jun NH(2)-terminal kinase 2alpha2 promotes the tumorigenicity of human glioblastoma cells. *Cancer Res* , 66, 10024-10031.

- [106] Khatlani, T. S, Wislez, M, Sun, M, Srinivas, H, Iwanaga, K, et al. (2007). c-Jun N-terminal kinase is activated in non-small-cell lung cancer and promotes neoplastic transformation in human bronchial epithelial cells. *Oncogene* , 26, 2658-2666.
- [107] Adler, V, Schaffer, A, Kim, J, Dolan, L, & Ronai, Z. (1995). UV Irradiation and heat shock mediate JNK activation via alternate pathways. *J Biol Chem* , 270, 26071-26077.
- [108] Mariani, O, Brennetot, C, Coindre, J. M, Gruel, N, Ganem, C, et al. (2007). JUN oncogene amplification and overexpression block adipocytic differentiation in highly aggressive sarcomas. *Cancer Cell* , 11, 361-374.
- [109] Vleugel, M. M, Greijer, A. E, Bos, R, Van Der Wall, E, & Van Diest, P. J. (2006). c-Jun activation is associated with proliferation and angiogenesis in invasive breast cancer. *Hum Pathol* , 37, 668-674.
- [110] Maeno, K, Masuda, A, Yanagisawa, K, Konishi, H, Osada, H, et al. (2006). Altered regulation of c-jun and its involvement in anchorage-independent growth of human lung cancers. *Oncogene* , 25, 271-277.
- [111] Yamanishi, D. T, Buckmeier, J. A, & Meyskens, F. L. Jr. ((1991). Expression of c-jun, jun-B, and c-fos proto-oncogenes in human primary melanocytes and metastatic melanomas. *J Invest Dermatol* , 97, 349-353.
- [112] Rutberg, S. E, Goldstein, I. M, Yang, Y. M, Stackpole, C. W, & Ronai, Z. (1994). Expression and transcriptional activity of AP-1, CRE, and URE binding proteins in B16 mouse melanoma subclones. *Mol Carcinog* , 10, 82-87.
- [113] Mingo-sion, A. M, Marietta, P. M, Koller, E, & Wolf, D. M. Van Den Berg CL ((2004). Inhibition of JNK reduces G2/M transit independent of leading to endoreduplication, decreased proliferation, and apoptosis in breast cancer cells. *Oncogene* 23: 596-604., 53.
- [114] Uzgar, A. R, & Isaacs, J. T. (2004). Enhanced redundancy in Akt and mitogen-activated protein kinase-induced survival of malignant versus normal prostate epithelial cells. *Cancer Res* , 64, 6190-6199.
- [115] Gurzov, E. N, Bakiri, L, Alfaro, J. M, Wagner, E. F, & Izquierdo, M. (2008). Targeting c-Jun and JunB proteins as potential anticancer cell therapy. *Oncogene* , 27, 641-652.
- [116] Dixit, V, & Mak, T. W. (2002). NF-kappaB Signaling Many roads lead to madrid. *Cell* , 111, 615-619.
- [117] Ghosh, S, & Karin, M. (2002). Missing pieces in the NF-kappaB puzzle. *Cell* 109 Suppl: S, 81-96.
- [118] Chen, X, Kandasamy, K, & Srivastava, R. K. (2003). Differential roles of RelA (and c-Rel subunits of nuclear factor kappa B in tumor necrosis factor-related apoptosis-inducing ligand signaling. *Cancer Res* 63: 1059-1066., 65.

- [119] Poser, I, Dominguez, D, De Herreros, A. G, Varnai, A, Buettner, R, et al. (2001). Loss of E-cadherin expression in melanoma cells involves up-regulation of the transcriptional repressor Snail. *J Biol Chem* , 276, 24661-24666.
- [120] Meyskens, F. L. Jr., Buckmeier JA, McNulty SE, Tohidian NB ((1999). Activation of nuclear factor-kappa B in human metastatic melanomacells and the effect of oxidative stress. *Clin Cancer Res* , 5, 1197-1202.
- [121] Boukerche, H, Su, Z. Z, Emdad, L, Sarkar, D, & Fisher, P. B. (2007). mda-9/Syntenin regulates the metastatic phenotype in human melanoma cells by activating nuclear factor-kappaB. *Cancer Res* , 67, 1812-1822.
- [122] Philip, S, & Kundu, G. C. (2003). Osteopontin induces nuclear factor kappa B-mediated promatrix metalloproteinase-2 activation through I kappa B alpha /IKK signaling pathways, and curcumin (diferulolylmethane) down-regulates these pathways. *J Biol Chem* , 278, 14487-14497.
- [123] Dupin, E. Le Douarin NM ((2003). Development of melanocyte precursors from the vertebrate neural crest. *Oncogene* , 22, 3016-3023.
- [124] Hendrix, M. J, Seftor, E. A, Seftor, R. E, Kasemeier-kulesa, J, Kulesa, P. M, et al. (2007). Reprogramming metastatic tumour cells with embryonic microenvironments. *Nat Rev Cancer* , 7, 246-255.
- [125] Bittner, M, Meltzer, P, Chen, Y, Jiang, Y, Seftor, E, et al. (2000). Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature* , 406, 536-540.
- [126] Hendrix, M. J, Seftor, E. A, Hess, A. R, & Seftor, R. E. (2003). Molecular plasticity of human melanoma cells. *Oncogene* , 22, 3070-3075.
- [127] Coiffier, B, Lepage, E, Briere, J, Herbrecht, R, Tilly, H, et al. (2002). CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* , 346, 235-242.
- [128] Lee, J. T, & Herlyn, M. (2007). Microenvironmental influences in melanoma progression. *J Cell Biochem* , 101, 862-872.
- [129] Paget, S. (1989). The distribution of secondary growths in cancer of the breast. 1889. *Cancer Metastasis Rev* , 8, 98-101.
- [130] Fidler, I. J. (2003). The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat Rev Cancer* , 3, 453-458.
- [131] Topczewska, J. M, Postovit, L. M, Margaryan, N. V, Sam, A, Hess, A. R, et al. (2006). Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nat Med* , 12, 925-932.

- [132] Miller, S. A, Hamilton, S. L, Wester, U. G, & Cyr, W. H. (1998). An analysis of UVA emissions from sunlamps and the potential importance for melanoma. *Photochem Photobiol* , 68, 63-70.
- [133] Lautenschlager, S, Wulf, H. C, & Pittelkow, M. R. (2007). Photoprotection. *Lancet* , 370, 528-537.
- [134] Brenner, M, & Hearing, V. J. (2008). The protective role of melanin against UV damage in human skin. *Photochem Photobiol* , 84, 539-549.
- [135] Bastian, B. C. LeBoit PE, Hamm H, Brocker EB, Pinkel D ((1998). Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. *Cancer Res* , 58, 2170-2175.
- [136] Curtin, J. A, Fridlyand, J, Kageshita, T, Patel, H. N, Busam, K. J, et al. (2005). Distinct sets of genetic alterations in melanoma. *N Engl J Med* , 353, 2135-2147.
- [137] Balazs, M, Adam, Z, Treszl, A, Begany, A, Hunyadi, J, et al. (2001). Chromosomal imbalances in primary and metastatic melanomas revealed by comparative genomic hybridization. *Cytometry* , 46, 222-232.
- [138] Hausler, T, Stang, A, Anastassiou, G, Jockel, K. H, Mrzyk, S, et al. (2005). Loss of heterozygosity of 1p in uveal melanomas with monosomy 3. *Int J Cancer* , 116, 909-913.
- [139] White, J. S, Mclean, I. W, Becker, R. L, Director-myska, A. E, & Nath, J. (2006). Correlation of comparative genomic hybridization results of 100 archival uveal melanomas with patient survival. *Cancer Genet Cytogenet* , 170, 29-39.
- [140] Speicher, M. R, & Prescher, G. du Manoir S, Jauch A, Horsthemke B, et al. ((1994). Chromosomal gains and losses in uveal melanomas detected by comparative genomic hybridization. *Cancer Res* , 54, 3817-3823.
- [141] Vajdic, C. M, Hutchins, A. M, Kricker, A, Aitken, J. F, Armstrong, B. K, et al. (2003). Chromosomal gains and losses in ocular melanoma detected by comparative genomic hybridization in an Australian population-based study. *Cancer Genet Cytogenet* , 144, 12-17.
- [142] Garraway, L. A, Widlund, H. R, Rubin, M. A, Getz, G, Berger, A. J, et al. (2005). Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature* , 436, 117-122.
- [143] Jonsson, G, Dahl, C, Staaf, J, Sandberg, T, Bendahl, P. O, et al. (2007). Genomic profiling of malignant melanoma using tiling-resolution arrayCGH. *Oncogene* , 26, 4738-4748.
- [144] Alonso, S. R, Tracey, L, Ortiz, P, Perez-gomez, B, Palacios, J, et al. (2007). A high-throughput study in melanoma identifies epithelial-mesenchymal transition as a major determinant of metastasis. *Cancer Res* , 67, 3450-3460.

- [145] Hoek, K, Rimm, D. L, Williams, K. R, Zhao, H, Ariyan, S, et al. (2004). Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. *Cancer Res* , 64, 5270-5282.
- [146] Seftor, R. E, Seftor, E. A, Koshikawa, N, Meltzer, P. S, Gardner, L. M, et al. (2001). Cooperative interactions of laminin 5 gamma2 chain, matrix metalloproteinase-2, and membrane type-1-matrix/metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma. *Cancer Res* , 61, 6322-6327.
- [147] Jaeger, J, Koczan, D, Thiesen, H. J, Ibrahim, S. M, Gross, G, et al. (2007). Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. *Clin Cancer Res* , 13, 806-815.
- [148] Weeraratna, A. T, Jiang, Y, Hostetter, G, Rosenblatt, K, Duray, P, et al. (2002). Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell* , 1, 279-288.
- [149] Becker, B, Roesch, A, Hafner, C, Stolz, W, Dugas, M, et al. (2004). Discrimination of melanocytic tumors by cDNA array hybridization of tissues prepared by laser pressure catapulting. *J Invest Dermatol* , 122, 361-368.
- [150] Winnepenninckx, V, Lazar, V, Michiels, S, Dessen, P, Stas, M, et al. (2006). Gene expression profiling of primary cutaneous melanoma and clinical outcome. *J Natl Cancer Inst* , 98, 472-482.
- [151] Haqq, C, Nosrati, M, Sudilovsky, D, Crothers, J, Khodabakhsh, D, et al. (2005). The gene expression signatures of melanoma progression. *Proc Natl Acad Sci U S A* , 102, 6092-6097.