Chapter 3

An Overview of Important Genetic Aspects in Melanoma

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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 according 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

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

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 *PTEN*	Pten	10q	Primary & metastatic	[135,136,137]
CDKN2A	p16Ink4a/p14Arf	9p	Primary & metastatic	[135,136]
ITGB3BP	Beta-3-endonexin	1p31	Uveal	[138]
Gene amplification *BRAF*	Braf	7q21.3	Cutaneous	[135]
CCND1	cyclinD1	11q13	Acral	[136]
CDK4	cdk4	12q14	Acral & mucosal	[136]
CDH2	N-cadherin	18q	Uveal & metastatic	[137,139]
c-MYC	c-myc	8q23	Ocular, primary & metastatic	[135,137,140,141]
MITF	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 *ATM*	ATM kinase	Cell cycle control, DNA damage response	Melanocytic infiltration	[135,136,137]
CDH2	N-cadherin	Cell-cell adhesion	Melanoma invasiveness	[144,145]
MMP-1, 2	Metallo proteinases -1, 2	Degradation of ECM	Tumor progression	[146]
SPP1	Osteopontin	Inductor of MMP	Risk of metastasis	[144,147]
SPARC	Osteonectin	Angiogenesis	Tumor progression	[144]
WNT5A	Wnt5a	Cell signaling	Melanoma invasiveness	[125,148]
CKS2	Cdc28/cdk1 protein kinase subunit 2	Cell cycle control	Poor prognosis	[147]
EIF2gamma	EIF2γ	DNA translation	Tumor progression	[149]
PCNA	PCNA	Cofactor DNA polymerase	Genome destabilization	[150]
Down Regulation *CDH1*	E-cadherin	Cell-cell adhesion	Tumor progression	[144,145,147]
CDH3	P-cadherin	Cell-cell adhesion	Tumor progression	[145,151]
CDH10	Cadherin-10	Cell-cell adhesion	High risk of metastasis	[144]
DSC1	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, CDK2N2A 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 mutations. 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-

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

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-3β (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 accont of mutations in tumor suppressor PTEN (phosphatase and tensin homolog) [62]. In melanoma, los 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-biphosphate 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 transcended 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-3β (GSK-3β). GSK-3β 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-3β 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 Reifenberger 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-1inhibited 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 phorphorylation 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 leas 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 NFκB (NF-kappaB) pathway

The NFκB family in mammals contains 5 members – p105/p50 (NFκB1), p100/p52 (NFκB2), RelA (p65), RelB and c-Rel (J-206, 207). The canonical activation of NFκB pathway involves TNFα stimulation resulting in the subsequent phosphorylation/activation of IKK (IκB kinase). In turn, IKK-mediated phosphorylation of IκB leading to ubiquitination of IκB and its proteosomal degradation, releasing the NFκB complex which activates a host of target genes [116,117]. The type of genes that get trans-activated depends on the composition of activated NFκB 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].

NFκB is activated in various tumors including melanomas and distinct mechanisms have been proposed for the elevated levels of NFκB activity in melanomas. Activation of NFκB in melanomas is also linked to the loss of E-cadherin, a frequent event in melanoma transformation [119]. NIK (NFκB interacting kinase), an activator of IKK is overexpressed in melanoma cells while compared to normal cells. The major contribution of NFκB in melanoma development and progression relates to its function as an important regulator of survival and apoptosis. A study by Meyskens at al demonstrated that in metastatic melanoma cells, an increase in DNA binding activity of NFκB 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 NFκB 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 TGFβ 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

GSK3β - glycogen synthase kinase-3beta

IKK - Inhibitor of IκB kinase

MAPK - mitogen activated protein kinase

MITF - microphthalmia-associated transcription factor

MMP - matrix metalloproteinase

NF-κB - nuclear factor kappa-B

NIK - NFκB-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

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