

Molecular biology of normal melanocytes and melanoma cells

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ABSTRACT

Malignant melanoma is one of the most aggressive malignancies in humans and is responsible for 60–80% of deaths from skin cancers. The 5-year survival of patients with metastatic malignant melanoma is about 14%. Its incidence has been increasing in the white population over the past two decades. The mechanisms leading to malignant transformation of melanocytes and melanocytic lesions are poorly understood. In developing malignant melanoma, there is a complex interaction of environmental and endogenous (genetic) factors, including: dysregulation of cell proliferation, programmed cell death (apoptosis) and cell-to-cell interactions. The understanding of genetic alterations in signalling pathways of primary and metastatic malignant melanoma and their interactions may lead to therapeutics modalities, including targeted therapies, particularly in advanced melanomas that have high mortality rates and are often resistant to chemotherapy and radiotherapy. Our knowledge regarding the molecular biology of malignant melanoma has been expanding. Even though several genes involved in melanocyte development may also be associated with melanoma cell development, it is still unclear how a normal melanocyte becomes a melanoma cell. This article reviews the molecular events and recent findings associated with malignant melanoma.

MOLECULAR BIOLOGY OF NORMAL MELANOCYTES

Melanocytes are pigment producing cells of the skin in humans and other vertebrates.¹ Melanoma can occur in the oesophagus, meninges, oral mucosa, nasal cavity mucosa, anus/rectum, penis, vulva, vagina and conjunctiva, where the normal counterpart resides.^{2–4} Melanocytes originate from the neural crest with pluripotential cells that gradually become lineage specific during development.^{5–6} Following neural crest induction partly dependent on intact BMP signalling,⁷ neural crest stem cells undergo epithelial–mesenchymal transition (EMT) with loss of adhesion to neighbouring cells and eventually cell migration. In this process, Snail/Slug transcriptional factors repress E-cadherin expression and subsequent cell detachment and movement.⁸ Commitment of a pluripotential neural crest progenitor/stem cell to become a melanoblast, and eventually a mature melanocyte, involves Wnt (*Wingless* and a related gene termed *int-1* in mice) signalling, which mediates a fate switch from glial-melanocyte lineage towards melanogenesis through β -catenin expression.^{6–9–11} Several genes, including mitf (microphthalmia transcription factor), c-kit, snail/slug, sox10, and endothelins, which are important in melanocyte

development, have been identified.¹ By activating a few pigment-producing genes, including dct (dopachrome tautomerase) and tyrosinase, mitf gene regulates the melanocyte lineage.¹² Melanocyte development, function, migration, and survival, depending on the species, are all dependent on expression of the tyrosine kinase receptor c-kit gene.¹³ Pigment machinery of the skin contains a complex set of reactions.¹⁴ Melanin pigment is produced by melanocytes within cytoplasmic melanosomes, in which tyrosinase acts on tyrosine and results in dopa and dopaquinone.^{15–16} Melanin pigment is transferred to an average of 36 adjacent keratinocytes and the key receptor in this transfer is protease-activated receptor 2 (PAR-2) on the keratinocytes' surface.^{17–18}

MOLECULAR BIOLOGY AND GENETIC ALTERATIONS IN MALIGNANT MELANOMA

There are several endogenous and exogenous (environmental) risk factors associated with developing malignant melanoma, of which previous history of melanoma, family history of melanoma, and multiple dysplastic or benign (common/banal) nevi reveal the strongest association.¹⁹ Other risk factors include: long term sun exposure (probably intermittent exposure), tendency to burn easily and tan poorly, ultraviolet exposure (such as PUVA therapy), blond or red hair, pale skin, large congenital nevi, xeroderma pigmentosum, immunosuppression, scars, chemical exposures and Marjolin ulcers.^{20–28} To some degree, we know the association of these factors to the underlying molecular process. In all, 25–40% of malignant melanomas in a familial setting are associated with mutations in CDKN2A (cyclin-dependent kinase inhibitor 2A)^{29–30} and less commonly mutations in CDK4 (cyclin-dependent kinase 4).³¹ CDKN2A encodes two tumour suppressor proteins, p16 (INK4A/inhibitor of kinase 4A) and p14 (ARF/alternate reading frame), both involved in cell cycle regulation.^{32–34} Other genes, including MC1R (melanocortin-1 receptor) and DNA repair genes, are likely to be more important in determining the susceptibility for melanomas in the general population.³⁵ Even though the results of the study conducted by Kanetsky *et al*³⁶ showed that MC1R is a low-penetrance susceptibility locus for melanoma as previously had been shown, their study did not show strong association between MC1R and melanoma, contrary to previous studies. PTEN/MMAC1 (phosphatase and tensin homologue/mutated in multiple advanced cancers 1) plays a central role in restricting cellular proliferation or promoting apoptosis.³⁷ Tsao *et al* found high levels of PTEN expression in cutaneous muscle, nerve and muscular arteries, and moderate-to-high levels of PTEN in the



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epidermis, follicular epithelium, sebaceous and eccrine glands. They found uniformly strong PTEN in the cytoplasm in almost all benign and dysplastic nevi. They concluded that the presence of PTEN in benign melanocytic tumours and absence of PTEN in a significant proportion of primary cutaneous melanomas support a role of PTEN loss in the pathogenesis of malignant melanoma.³⁷ Inactivating mutation of tumour suppressor gene, PTEN, is seen in 25–50% of non-familial melanomas.^{38–41} One of the most important genetic alterations in malignant melanoma involves receptor tyrosine kinases and the downstream NRAS/BRAF/ERK (neuroblastoma rat sarcoma/v-raf murine sarcoma homologue B/extracellular-related kinase) and phosphatidylinositol 3'-kinase (PI3K)/PTEN pathways.⁴² Upregulation of MAPK (mitogen-activated protein kinase; Ras-Raf-MEK-ERK) and the phosphoinositide 3-kinase-AKT (also known as protein kinase B/PKB) pathways, both of which playing major roles in melanoma progression, are seen in a majority of malignant melanomas.^{43–44} Lentigo maligna (solar melanoma) is associated with BRAF-activating (70%) or NRAS-activating (20%) mutations. In contrast, mucosal and acral melanoma lack mutations in both NRAS and BRAF. They are associated with mutations or genetic amplification of KIT (CD117) in up to 40% of cases.^{45–46} Although benign nevi and malignant melanomas share initiation genetic alterations such as mutations in BRAF and NRAS, melanomas often show recurrent patterns of chromosomal losses (chromosomes 6q, 8p, 9p and 10q), along with chromosomal gains (1q, 6p, 7, 8q, 17q and 20q) by comparative genomic hybridisation (CGH) or karyotyping. Nevi, however, show no detectable chromosomal aberrations by CGH.^{19–47–49} Gerami *et al* have recently developed a fluorescent in situ hybridisation (FISH) based test. By using four probes, this test targets three loci on chromosome 6 (RREB1 and MYB genes) and one locus on chromosome 11 (cyclin D1 gene). This method of testing that is applicable to formalin-fixed paraffin-embedded tissue is claimed to have the most powerful discriminatory ability to differentiate between nevi and malignant melanomas.^{50–51} Piepkorn⁵² showed that expression of p16 (INK4A) is upregulated in human melanocytes by ultraviolet B irradiation. Alterations in cell cycle proteins cyclin D1, pRb and p16 have a role in transformation and progression in melanocytic tumours. Even though there is an increase in expression of pRb and cyclin D1 in the progression to melanoma cells, these two cell cycle proteins interestingly reveal a relative decrease in thick and metastatic malignant melanoma. Progressive loss of p16 is seen in transformation of benign nevi to melanoma and metastatic melanoma.⁵³ Upregulation of PAR-1 (protease-activated receptor-1) mediates high levels of Cx-43 (connexin molecule), involved in tumour cell diapedesis and attachment to endothelial cells.⁵⁴ Type I collagenase and PAR-1 activating functions of MMP-1 (matrix metalloproteinase-1) are required for melanoma progression. Blackburn *et al*⁵⁵ have suggested that highly expressed MMP-1 is involved in progression of non-invasive melanoma to an invasive vertical growth phase by degrading type I collagen of skin.

MCSP (melanoma chondroitin sulfate proteoglycan/human melanoma proteoglycan) and rat homologous NG2 are transmembrane proteoglycans in which the core protein is modified with chondroitin sulfate.^{56–58} Normal melanocytes express little or no MCSP in situ. Increased levels of MCSP are seen in benign and dysplastic nevi.⁵⁶ MCSP expression in acral lentiginous malignant melanoma is associated with poorer prognosis.^{59–60} MCSP expression is also seen in association with increased c-Met and HGF. Inhibition of c-Met limits the growth and motility of melanoma cells in melanoma cell lines.⁶⁰ Mutant active BRAF in melanomas is associated with a high level of constitutive activation of Erk1,2.⁶¹ Results of the

sustained activation of the Erk1,2 pathway include: entry into the cell cycle, increased expression of key melanoma transcription factors, and other important factors for invasion, such as matrix metalloproteinases. By increasing the expression of specific integrin subunits (such as $\beta 3$) and elevated resistance to apoptosis, Erk1,2 activation causes increased adhesion.⁴³

There is evidence revealing that miRNAs are deregulated during melanoma progression.^{62–63} Frequent dysregulation of miRNA expression in lymph node metastasis of melanomas has been reported in association with patient survival and mutational status. There are also findings which show differential expression of miRNA in melanomas in comparison to melanocytes. Caramuta *et al* found that ectopic expression of miRNA-182 promotes lung metastasis in a mouse model. High and low expression of miRNA-193b and miRNA-191, respectively, are significantly associated with poor survival. Low expression of miRNA-193a, miRNA-338 and miRNA-565 is associated with cases of BRAF missense mutation, and low expression of miRNA-663 is associated with NRAS-mutated cases. However, no significant differentially expressed miRNA was found between BRAF versus NRAS mutated melanomas.⁶⁴ It has been shown that high expression of miRNA-193b is associated with a high risk of metastasis in uveal melanoma.⁶⁵

Based on the fact that angiogenesis is one of the most important factors needed for melanoma progression and metastasis, Mehnert *et al* showed that expression of vascular endothelial growth factor (VEGF) and its receptors (VEGF-R1, VEGF-R2, VEGF-R3) is higher in melanomas and advanced melanomas than in benign nevi. VEGF-R2 shows higher expression in metastatic melanomas than in primary melanomas. In contrast, higher expression of VEGF-R3 is seen in primary lesions, supporting the fact that VEGF-R3 is involved in initiation of lymphatic tumour spread.⁶⁶

Downregulation and upregulation of E-cadherin and N-cadherin respectively may be seen in melanoma. This shift in cadherin profiles may play a role in uncontrolled proliferation, invasion and migration of melanoma cells.⁶⁷

Remodelling of actin cytoskeleton is essential for tumour cell invasion and metastasis. Altered expression of actin cytoskeleton components may also have some role in transformation and tumourigenesis.^{68–69} Immunohistochemical study on human tissue samples has shown there is a significantly higher cortactin expression in melanoma than nevi and in metastatic melanoma than invasive primary melanoma.⁷⁰ Cortactin is a multidomain actin-binding protein involved in endocytosis, cell migration, invasion and adhesion.^{71–73}

Protein kinase C (PKC) is activated by diacylglycerol and mediates pathway signals for cell growth and proliferation. PKC is a target of tumour-promoting phorbol esters in malignant transformation.⁷⁴

Sviatoha *et al* analysed immunohistochemical expression of the S100A1, S100m, CD44 and Bcl-2 antigens, whose involvement in transformation of melanocytes to melanoma cells has been described. S100 is a dimeric protein composed of α and β subunits that regulates the assembly of cytoskeletal systems of cells such as filaments and microtubules. S100 has been suggested to be involved in cell cycle regulation, division and cell-to-cell communications.^{75–76} One study showed high expression of S100A1 in malignant melanocytic proliferation of conjunctiva.⁷⁷ S100B interacts with p53 tumour suppressor protein and downregulates its function.⁷⁸ Sviatoha *et al* also showed that S100B expression in dysplastic nevi is similar to acquired (banal) nevi, whereas expression of S100A1 tends to be higher. In primary and metastatic melanomas there is a

decrease in expression of S100B and increased expression of S100A1. Even though S100 expression in melanoma is decreased, this expression is higher in areas with increased proliferation.⁷⁹ CD44, an adhesion molecule and a family of transmembrane glycoproteins that is a main ligand for hyaluronic acid, is involved in cell growth signal transmission, cell adhesion to endothelium, cell migration and increased cell motility.^{80–81} CD44 protein is upregulated during melanoma progression.⁸¹ CD44 reveals marked variation in the expression of nevi and melanomas. The same study also showed that in metastatic melanoma, the mean number of CD44 cell fraction was higher in melanoma without metastasis, even though no significant correlation between CD44 protein expression and various histopathological characteristics was seen.⁷⁹ Bcl-2 proto-oncogene, located on chromosome 18, is involved in regulation of apoptosis (programmed cell death).^{82–83} Bcl-2 expression is higher in nevi than melanomas.⁷⁹

Interaction of E2F1 transcription factor with RGFR may act as a driving force in melanoma progression.⁸⁴

There is abnormal expression of major histocompatibility complex (MHC) molecules in melanomas. In the vertical growth phase of melanoma, there is an increased DNA copy number gain of MHC genes, along with increased expression compared to normal melanocytes. However, MHC expression in metastatic melanoma is decreased in comparison to vertical growth phase melanoma, and yet there is still increased DNA copy number gain. In earlier stages of malignant melanoma, there is over-expression of MHC molecules, in contrast to its downregulation in metastatic melanoma.⁸⁵

Loss of pigment epithelium-derived factor (PEDF) is associated with malignant progression and invasion in melanoma.⁸⁶ CXCR1 and CXCR2 chemokines receptors and their ligands are important players in the pathogenesis of malignant melanoma. CXCR1 is constitutively expressed in all melanomas irrespective of their stage and grade. CXCR2 expression, however, is limited to aggressive melanomas. It has been shown that modulation of CXCR1 and CXCR2 expression and/or activity regulate malignant melanoma growth, angiogenesis and metastasis.^{87–89}

ATP-binding cassette (ABC) transporters regulate the transportation of various physiological substances across biological membranes.⁹⁰ ABC transporters' high expression levels have been seen in various malignant tumours, including ovarian carcinoma, lung carcinoma, osteosarcoma and neuroblastoma. ABC transporter mRNA expression profile and differentially regulated ABC transporters are candidate genes involved in malignant melanoma tumourigenesis, progression and treatment resistance.⁹¹

The SOX (sex determining region Y-box) family of transcription factors is involved in the development of normal physiology of numerous tissues including melanocytes. SOX5, SOX9, SOX10 and SOX18 transcription factors affect the key modulatory and regulatory melanocytic genes. This regulation of SOX9 or SOX10 is associated with cancerous transformation.⁹²

Heparin and its derivatives can inhibit angiogenesis and metastasis. Kenessey *et al*⁹³ have shown that fragments of heparin, not involved in haemostasis, may play a role in antimigratory and antimetastatic processes.

Until recently, available strategies on systemic therapy of advanced malignant melanoma have been limited⁹⁴ and have shown only a minimal effect on patient survival. A phase III trial treatment with oral BRAF inhibitor vemurafenib showed significant improvement in both progression-free survival as well as overall survival over chemotherapy.⁹⁵

Even though it is still not completely clear how a normal melanocyte becomes a melanoma cell, the rapid pace of discovering more and more pathways, genetic alterations, and understanding the biology of malignant melanoma in the 'molecular era' are definitely paving the road for finding more specific therapeutic modalities and developing novel targeted therapies.

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