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Review

Developmental pathways activated in melanocytes and melanoma

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ABSTRACT

Cutaneous malignant melanomas originate primarily within epidermal melanocytic cells. Melanoma cells share many characteristics with melanocyte precursors, suggesting that melanoma cells utilize the developmental programs of their normal counterpart for their own progression. The pigmentation system provides an advantageous model to assess survival pathway interactions in the melanocytic lineage, as genetic alterations controlling melanocyte development can be easily detectable by coat color phenotype that do not affect the viability of an animal. By integrating combinatorial gene knockout approaches, cell-based assays and immunohistochemical observations, recent studies have illustrated several genes and pathways that play important roles both in melanocyte specification and maintenance and in melanoma formation and progression. We are reviewing those genes and pathways to understand the connection between normal and cancerous development and to reveal therapeutic potential of targeting developmental pathways for melanoma therapy.

Introduction

Melanocytes are pigment-producing cells, which are derived from neural crest cells and located at the basal layer of the epidermis, hair bulb, eyes, ears, and meninges. During embryonic development, multipotent trunk neural crest cells migrate from the neural plate to the epidermis and dermis, undergoing lineage specification to form melanocyte precursors – melanoblasts, which eventually generate differentiated melanocytes. Taking advantage of transgenic studies using mouse models as well as other species, a number of genes involved in cell lineage specification and melanocyte development have been identified and characterized. Cutaneous melanoma is the deadliest form of skin cancer, which arises from normal melanocytes or their precursors. Although remarkable advances in melanoma therapy were made recently with the approval of several new drugs against MAPK (mitogen-activated protein kinase)[1] pathway, none of them are regarded as inducing cures in terms of targeted therapy. It has been known that tumor cells utilize the properties of their normal counterpart and the progenitors for their own progression. Indeed, the molecular and cellular mechanisms involved in proliferation and migration of melanoblasts during development and of melanoma cells during tumor progression are often closely related. The goal of this brief review is to dissect the signaling pathways operating during melanocyte development and melanoma tumorigenesis, thus providing complementary information.

Normal melanocyte development

Melanocytes are derived from neural crest cells (NCCs), which are highly migratory embryonic cells. After gastrulation, the neural

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[1] *Abbreviations used:* MAPK, mitogen-activated protein kinase; NCCs, neural crest cells; MSCs, melanocyte stem cells; PI3K, phosphatidylinositol 3-kinase; PCP, planar cell polarity; FZD, Frizzled; DVL, Dishevelled; APC, adenomatous polyposis coli; GSK3β, glycogen synthase kinase 3 beta; SNs, supernatants; DAAM1, Dishevelled-associated activator of morphogenesis 1; ROCK, Rho and Rho-associated kinase; PLC, phospholipase C; PDE, phosphodiesterase; PKC, protein kinase C; VEGF, vascular endothelial growth factor; siRNA, small interfering RNA; HMG, high-mobility-group; MITF, microphthalmia-associated transcription factor; GSI, gamma-secretase inhibitor; MC, melanocyte; KO, knockout; NV, nevi; PM, primary melanoma; MM, metastasis melanoma; Mbs, melanoblasts.

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crest is first induced at the border of the neural plate and non-neural ectoderm, and then delaminates from the region between the dorsal neural tube and overlying ectoderm upon neural tube closure. NCCs are initially multipotent but gradually become lineage-restricted in developmental potential, which is determined by where they migrate and settle. NCCs can give rise to a number of differentiated cell and tissue types including sensory neurons and glial cells, melanocytes, craniofacial cartilage and bone, and smooth muscle [19].

The development of a multipotent neural crest stem cell into a mature melanocyte involves the generation of melanoblasts (Mbs) – the precursors of pigmented melanocytes – from a bipotential glial–melanocyte lineage progenitor. After emerging from the neural crest, Mbs begin their journey to the skin by invading the dorsolateral pathway between the somite and the ectoderm. In the hair-covered body skin of mouse, Mbs travel through the epidermis to arrive at newly forming hair follicles. Upon localized within the follicles, Mbs segregate into two distinct subpopulations: differentiated melanocytes that reside within the hair matrix and contribute to hair pigmentation and melanocyte stem cells (MSCs) that localize in the bulge at the lower permanent portion of the hair follicle, and give rise differentiated melanocyte population for subsequent hair growth cycles. In contrast, in human skin, where hair follicles are relatively sparse, melanocytes also reside in the basal layer of interfollicular epidermis which is close to the dermal-epidermal junction, and respond to environmental cues from surrounding keratinocytes or others for differentiation/pigmentation [19,7].

Melanoma progression

Malignant melanoma is an aggressive form of skin cancer and its incidence is increasing worldwide. Early-stage melanomas can be successfully treated mainly through surgical excision of the primary tumor lesion. However, advanced stage melanomas are difficult to treat once the disease has spread beyond the primary site to distant organs as most patients eventually develop resistance to currently available therapies.

The transformation of normal melanocytes into melanoma cells is often considered as a multistep process. The horizontal or radial growth phase is the first step toward the invasive phenotype, in which melanocytes undergo alterations that offer a proliferative and survival advantage [38]. It is followed by a vertical growth phase, in which tumor cells deeply invade into the dermis/hypodermis. Metastatic melanoma cells may eventually break through the endothelium and travel to distant sites [38].

There exist some dominant genetic altering events in melanoma tumorigenesis. Constitutively activating BRAF and NRAS mutations are found in nearly 50% and 20% of melanomas, respectively. These mutations appear to be somatically acquired, as wild-type BRAF and NRAS are detected in normal tissues from melanoma patients. The most common T1799A point mutation in *BRAF* gene causes the V600E amino acid substitution, resulting in a 500-fold increase in inherent BRAF kinase activity that enhances cell division and survival [82]. Notably, many human nevi harbor BRAFV600E or NRAS mutations, which suggests that *BRAF* and *NRAS* mutations are critical but not sufficient for melanoma formation [16,67]. Another common genetic mutation in melanoma is the deletion of the CDKN2A locus, which encodes two tumor suppressor proteins, $p16^{INK4a}$ and $p14^{ARF}$. While $p16^{INK4a}$ is an inhibitor of the cyclin-dependent kinases CDK4 and CDK6 and prevents cell cycle progression, $p14^{ARF}$ functions as a positive regulator of p53. Deletions of the *CDKN2A* locus have been found in up to 50% of melanomas [23].

Correlating with the mutational status in melanoma, RAS-RAF-MAPK signaling pathway is heavily upregulated in melanoma patients. Activation of PI3K/Akt signaling pathway also plays a significant role in melanoma development, frequently in the setting of concurrent activation of the MAPK signaling. On the other hand, deletion of CDKN2A and the associated signaling alterations also contributes to melanoma progression. Owing to space limitations and the focus of this review on pathways important for animal development, we are not going to provide a complete survey of the field, especially exciting progresses related to MAPK, PI3k and CDKN2A pathways.

Developmental pathways in melanocyte and melanoma development

Many genes involved in melanocyte development have also been implicated in melanoma progression. Several genetic pathways are highlighted as follows that regulate various steps in melanocyte development as well as melanoma progression.

Notch

The Notch signaling pathway is evolutionarily conserved in most multicellular organisms. Mammals possess four different notch receptors, Notch1–4, which are single-pass transmembrane receptor proteins. The Notch signaling cascade is triggered upon binding of membrane-bound ligand – Jagged or Delta – to the heterodimeric receptor through cell–cell interactions. Once activated, the intracellular region of the Notch receptor is cleaved through two sequential proteolytic events to release the active intracellular domain of Notch (NIC), which is subsequently translocated into the nucleus to generate a transactivation complex with the RBP-J transcription factor [65]. The resulting transcriptional activation complex promotes transcription of various target genes, including members of the Hairy/Enhancer of Split (Hes) gene and Hairy/E(spl)-related with YRPW (Hey) gene families (Fig. 1). These nuclear proteins antagonize the expression of lineage-specifying genes such as Ascl1, MyoD, Atoh1 and E2A, thus maintaining cells in undifferentiated state [65,9].

Notch proteins during melanocyte development

Using transgenic mouse models, a series of studies have shown that Notch signaling plays a vital role in the maintenance of Mbs as well as MSCs of the skin [59,74,41] (Table 1A). To assess the role of Notch signaling in Mbs, Nishikawa and colleagues conditionally ablated the *RBP-J* gene in the melanocyte lineage using the *Tyr-Cre: RBP-J$^{f/f}$* mouse model [59]. The initial hair pigmentation defects along with premature hair graying in subsequent hair cycles were observed in these mice, which suggests a key role of Notch signaling in the maintenance of both embryonic Mbs and MSCs. This phenotype was reminiscent of the effect caused by pharmacologic inhibition of Notch signaling pathway using a γ-secretase inhibitor (GSI), DAPT, in which apoptosis of Mbs was initiated. Hair derived from DAPT-treated skin organ cultures was unpigmented. The function of Notch in melanocyte development was further supported by the generation of *RBP-Jκ*, *Notch1*, and *Notch2* conditional knockout mice [74]. Conditional deletion of *Notch1* and *Notch2* alleles in the melanocyte lineage results in hair graying in a dose-dependent manner. Dispersed gray hair was detectable when two *Notch* alleles are floxed in *Tyr:Cre/°; Notch1$^{flox/+}$; Notch2$^{flox/+}$*, *Tyr:Cre/°; Notch1$^{+/+}$; Notch2$^{flox/flox}$* and *Tyr:Cre/°; Notch1$^{flox/flox}$; Notch2$^{+/+}$* mice, and pigmentation of hair was almost completely lost in the absence of both Notch1 and Notch2 (*Tyr:Cre/°; Notch1$^{flox/flox}$; Notch2$^{flox/flox}$* mice). Interestingly, while epidermal melanocytes are eliminated in the melanocyte-specific Notch1 and Notch2 double-deficient mice, dermal and