Insights into the Role of PAX-3 in the Development of Melanocytes and Melanoma

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Abstract: Melanoma is the deadliest form of skin cancer in the United States with an increasing prevalence. However, the development of melanoma from a melanocyte precursor is still poorly defined. Understanding the molecules responsible for melanoma progression may lead to improved targeted therapy. One potential molecule is the paired box-3 (PAX-3) protein, which has been implicated in the development of melanocytes and malignant melanoma. In melanoma, the expression of PAX-3 is believed to be differentially regulated, and has been linked with malignancies and staging of the disease. The loss of PAX-3 regulation has also been associated with the loss of transforming growth factor-beta (TGF-β) activity, but its effect on PAX-3 in differentiated melanocytes as well as metastatic melanoma remains unclear. Understanding PAX-3 regulation could potentially shift melanoma to a less aggressive and less metastatic disease. This review summarizes our current knowledge on PAX-3 during melanocyte development, its regulation, and its implications in the development of novel chemo-immunotherapeutics against metastatic melanoma.

Keywords: PAX-3, melanocyte, melanoma, SOX10, MITF, UVR, TGF-β.

INTRODUCTION

Melanoma is an aggressive skin cancer that has an occurrence rate of approximately 1 in every 50 Americans [1]. It is the 6th most common cancer with a lifetime risk of 2.04% for men and 1.38% for women [2, 3]. According to the American Cancer Society, in 2009 the number of new cases of melanoma rose to 68,720 with 8,650 new deaths attributed to the disease [4]. There are many known factors that contribute to the formation of melanoma. One major factor influencing the increase in melanoma cases is increased exposure to ultra-violet radiation (UVR); exposure resulting in severe sunburns leads to an increased incidence of melanoma [3]. Other risk factors include skin type, hair/eye color, the presence of dysplastic nevi and/or increased nevi, and a family history of melanoma [3]. Also, genetic mutations in BRAF, CDKN2A, and CDK4 have all been attributed to melanoma development. BRAF mutations have been found in 70% of all melanomas and greater than 90% of these mutations carry a single missense mutation [5, 6]. CDKN2A is a gene involved in melanoma pathogenesis and is a germline mutation found in younger patients [7]. CDK4 is involved in cell-cycle arrest and has been identified in 10% of observed melanomas [8]. These mutations are mostly detected in non-Chronic Sun-induced Damage melanomas, while Chronic Sun-induced Damage melanomas are more commonly observed.

When melanoma arises, early diagnosis is crucial to survival. With early diagnosis, more than 80% of cases can be treated successfully with surgery [9]. Surgery includes excision of the tumor and surrounding tissue; lymph nodes near the tumor may also be removed if the cancer has metastasized. Other treatments for melanoma include radiation, used particularly in cases of metastasis, and chemotherapy, given when the cancer is highly aggressive and metastatic [4]. Side effects from these treatments include fatigue, malaise, and an increased susceptibility to non-melanoma cancers [10]. Also, these therapies are severely toxic to the patients, suggesting a need for improved and less toxic treatment options that specifically target melanoma tumors. Immunotherapy, such as IL-2 administration and adoptive T cell transfer, looks promising in filling this gap in treating metastatic melanoma [11]. Whole cell vaccines using melanoma antigens have also been studied in many forms with varied success [12-14]. But problems may occur with these Ags due to spontaneous cysteinylation of Ags, and disruption of HLA class II presentation [15-17]. Adoptive T cell transfer consists of an ex vivo expansion of autologous tumor reactive lymphocytes followed by re-infusion of these cells into the patient [18]. Adoptive T cell transfer has been successful in treating stage II melanoma, but not late stage melanoma [19]. Immunostimulating drug therapies have provided recent advances in targeting malignant melanoma. Ipilimumab is a human monoclonal antibody (IgG1) that targets CTLA-4 to promote antitumor immunity, and has shown improved outcome in metastatic melanoma patients [20]. Even with all of the current immunotherapy treatments, other potential targets that can be used to fight and treat late stage melanoma remain unknown.

One potential target may be the paired box-3 (PAX-3) protein, which is a member of the paired box family and an important regulator in melanocyte development. PAX-3 is a key transcription factor that plays a vital role in embryogenesis, but is also implicated in tumorigenesis [21]. It is expressed in early development, but inhibited in adult
malignant melanocytes. PAX-3 has been recognized as a key component in melanocyte development, yet its role in adult melanocytes and in melanoma remains unclear. In this review, we will discuss the nature of PAX-3 during the development and differentiatation of melanocytes. We will also focus on the role of major regulators of PAX-3 in different stages of melanoma and its potential as a target for future therapies.

**MELANOCTYE DEVELOPMENT**

Melanocytes are derived from the neural crest during embryonic development and act as specialized pigment producing cells that are also the progenitor cells of melanoma [22, 23]. During embryogenesis, melanocytic precursor cells emerge in the neural crest. From there, they migrate to populate various sites including the epidermis, hair follicles, the uveal tract of the eye, and the cochlea [6]. Wnt signaling is responsible for the promotion of neural crest cells into pigment cells and thus decides the fate of the unpigmented precursor melanoblast to mature into a melanocyte [24]. Melanocytes reside in the dermal layer of the skin and produce secretory vesicles called melanosomes, where concentrated and synthesized melamins are contained [25]. Melamins are then exported to the epidermal layer and surround the existing keratinocytes to form a protective layer [26]. The migration of melanin is responsible for the color and pigmentation of the skin. However, with excess UVR exposure, keratinocytes respond by signaling to nearby melanocytes, thus driving a cascade of signals toward differentiation [27]. PAX-3 plays an important role in this signaling cascade ultimately leading to the differentiation of melanocytes [28, 29]. However, specific pathways involved and the direct role of PAX-3 on the signaling of keratinocytes and melanocytes remains unknown.

**PAX-3 AND MELANOCYTOGENESIS**

PAX-3 is a crucial factor in the regulation of melanocytes during embryonic development [21, 30]. PAX-3 contains four domains: the paired domain, the homeodomain, the octapeptide, and the transactivation domain. The paired domain contains two helix-turn-helix (HTH) sub-domains named PAI and RED. The paired domain helps to facilitate protein-protein interactions and DNA binding [31]. The homeodomain, which contains three HTH motifs, may function to regulate the DNA binding ability of the paired domain [29, 32]. The octapeptide is comprised of eight amino acids, where it functions as a protein interaction epitope, linking the paired domain with the homeodomain [29]. The transactivation domain is located at the carboxyl-terminal end and acts as a mediator of PAX-3-DNA interactions. Neural crest cells are the first to express PAX-3 (italicized for gene expression), and expression continues when melanoblasts develop and migrate from the neural crest to hair follicles [33]. Also, when melanoblasts migrate from the neural crest to the epidermal/dermal border, they mature into melanocytes, having Pax-3, Sox10, Mitf, Dct, Tyr, and TRP-1 gene expression, which may also play roles in melanocyte development [34, 35]. PAX-3 is also important in neural crest specification, and later in the expansion of committed melanoblasts formed early in development [36, 37]. Mutations in Pax-3 result in a disease called Waardenburg syndrome that includes abnormalities of the nervous system, eye, nose, as well as pigmentation defects affecting the skin, hair, and otic pigment cells [38, 39]. Many mutations within Pax-3 can result in Waardenburg syndrome, but the most common mutation is in the paired domain [40].

Despite the effect of PAX-3 on neural crest specification, its main and best described role is in the regulation of melanocyte differentiation [29, 37]. PAX-3 acts as both a transcriptional activator and repressor, and functions with other cofactors enhancing activation and repression [40]. The protein Sry-like HMG box 10 (SOX10) helps to enhance PAX-3’s transcriptional activity by direct interaction [41, 42]. SOX10 is a member of the Sox family of transcription factors, and it contains an HMG DNA binding domain that directly interacts with the PAI sub-domain of the paired domain in the PAX-3 protein [32, 42]. SOX10 is expressed in melanoblasts and melanocytes and its main function is in regulation of neural crest and melanocyte differentiation. PAX-3 directly interacts with SOX10, forming a complex which then binds to the promoter of Microphthalmia transcription factor (MITF). MITF is essential for melanoblast survival during and immediately following migration and is considered the major regulator during melanocytogenesis [37]. MITF activation is observed when the melanoblasts migrate to the epidermis [43]. In the melanoblasts, MITF activates the transcription of Dopachrome Tautomerase (Dct), Tyrosinase (Tyr), and Tyrosine related protein-1 (TRP-1), which may aid in early melanoblast differentiatation. Taken together, PAX-3, SOX10, and MITF determine the stages of melanocyte differentiation, and regulate the maintenance of melanoblasts and melanocyte stem cells [34].

Not only does PAX-3 activate and regulate multiple proteins but it also represses certain proteins during melanocytogenesis (Fig. 1). As previously stated, PAX-3 binds with
SOX10 and together these activate MITF. MITF then activates Dct which encodes a melanogenic enzyme for melanoblasts [44]. However, PAX-3 can also block Dct expression, which then inhibits MITF to bind to the promoter of Dct [44]. This prevents terminal differentiation in the melanoblast, as PAX-3 and MITF share the same binding site on the Dct promoter and compete for binding [34]. Conversely, when beta-catenin is present, PAX-3 is displaced from the promoter of Dct [45]. This allows MITF to continuously activate Dct allowing melanocytes to fully differentiate. Beta-catenin is signaled by Wnt proteins whose function is in neural crest migration, proliferation, and differentiation [46]. Some of the ability of beta-catenin to drive cells toward differentiation is through varying the function of PAX-3 and MITF [34, 47]. This may also explain PAX-3’s role in melanoblast regulation and how PAX-3 is absent or significantly reduced in adult melanocytes.

REGULATION OF PAX-3 IN ADULT MELANOCYTES

PAX-3 expression is observed in early development, but not in adult melanocytes, as previously stated [21]. Regulation of PAX-3 is essential when melanocytes have differentiated into adult cells. One regulator of PAX-3 is TGF-β, a cytokine that controls numerous functions in multiple cells [48, 49]. These functions include cellular behavior, differentiation, survival, migration, proliferation, and/or adhesion [50]. TGF-β also protects homeostasis by acting as a tumor suppressor [51]. In melanoma cells, high levels of TGF-β are secreted within the tumor microenvironment, but tumor cells fail to respond and growth inhibition is prevented [49]. While there are other regulators of PAX-3, TGF-β has an important role in the maintenance of PAX-3 in adult melanocytes (Fig. 2). With limited UVR, keratinocytes residing in the epidermal layer of the skin secrete moderate UVR.
levels of TGF-β, which signals through serine/threonine kinase receptor complexes (RI-RII) on the surface of melanocyte [52]. This binding at the cell membrane activates the Smad proteins to accumulate in the nucleus, which then controls targets of the TGF-β/Smad complex [26, 53]. Once TGF-β is bound to the RI-RII receptor, R-Smad is phosphorylated and forms a complex with Smad4 and Ski [54]. Ski forms this complex in melanocytes and directly inhibits the transcription of PAX-3 [55]. A recent study also showed that treating melanocytes with varying concentrations of TGF-β lead to the down-regulation of PAX-3 mRNA and protein expression [55]. This relationship between TGF-β and PAX-3 will be further investigated in this review.

PAX-3 AND MELANOMA

Studies suggest that PAX-3 plays a critical role in melanocytogenesis [29, 34, 37, 47, 55]. However, it is not clear whether PAX-3 is involved in differentiated melanocytes and in melanoma. The expression of PAX-3 has been found in healthy melanocytes, in benign nevi, and in melanoma tumors [45, 56-58]. Because it is found in both benign and malignant lesions, the re-occurrence of PAX-3 expression may be involved in the transformation of normal melanocytes into malignant melanoma. PAX-3 expression in melanocytes of normal skin has been observed, which may result from TGF-β activity loss in keratinocytes. Interestingly, PAX-3 has been used as a staging marker for melanoma and in the detection of circulating melanoma cells [37, 59]. PAX-3 is also recognized as an immunogenic protein in melanoma [60, 61]. It has also been found in primary tumors as well as in distinct stages of metastatic melanomas such as stages I and IV [62, 63]. This indicates that the regulation of PAX-3 could be a key factor in melanoma initiation, progression, and metastasis.

PAX-3 molecules could be involved in the progression of melanoma because of its role in cellular resistance to apoptosis. It has been shown that PAX-3 directly regulates the tumor suppressor protein PTEN, at least in myogenesis [64]. PTEN is involved in the progression through the G1 cell cycle checkpoint by negatively regulating the PI3K/AKT signaling pathway [65]. This signaling pathway is responsible for regulating cell proliferation and resistance to apoptosis [29]. PAX-3 interacts with an alleged homeodomain binding motif within the PTEN promoter, which provides an opportunity for PAX-3 to regulate PTEN expression and ultimately allow for resistance to apoptosis [64]. This may be significant because PAX-3 expression in melanoma could induce resistance to apoptosis observed in highly malignant melanoma.

The loss of PAX-3 can also be linked with the loss of TGF-β activity (Fig. 2). Levels of TGF-β are reduced when keratinocytes are stimulated with UVR. Studies suggest that TGF-β expression is also suppressed at both the transcriptional and protein level following UVR of the skin [55, 66]. The repression of TGF-β was caused by the UVR-induced expression of both Jun N-terminal kinase (JNK) and the tumor suppressor p53. Collectively, JNK and p53 activate the transcription of activating protein 1 (AP-1) [67, 68]. This allows p53 to stimulate the production of pro-opiomelanocortin/melanocyte stimulating hormone (POMC/MSH) in keratinocytes [27, 69], which releases the αMSH ligand that binds to the melanocortin-1 receptor (MC1R) on the melanocyte cell surface [69]. Once the αMSH ligand binds to MC1R, a signal cascade that works with adenylate cyclase leads to the production of cyclic adenosine monophosphate (cAMP) [6]. As levels of cAMP reach a certain threshold, the phosphorylation of cAMP response element binding transcription factor (CREB) occurs via protein kinase A [69]. CREB stimulates both PAX-3 and SOX10 which together form a complex to activate MITF. MITF, the master regulator of melanocyte differentiation, plays multiple roles in melanoma. Loss of MITF results in cell cycle arrest and apoptosis, moderate levels lead to proliferation and survival, and increased expression results in differentiation [70, 71]. MITF and PAX-3 can also independently initiate the expression of receptor tyrosine kinase MET, which plays a role in cell migration and growth, and in cancer progression and metastasis [72].

TGF-β plays an important role on PAX-3 in melanocytes, but loses its effect on melanoma cells that ultimately leads to melanocyte differentiation, metastasis, and survival [55]. However, the cause of the lost effect of TGF-β on PAX-3 in melanoma is currently unknown. It has been suggested that when Ski localizes to the cytoplasm in melanocytes, effects of TGF-β on the Smad complex are restricted and nuclear localization is prevented [51, 73]. Further study may determine the role of TGF-β in regulating PAX-3 in melanocytes and melanoma. The direct role that PAX-3 plays during intermediate stages of melanoma (i.e., stages II and III), must be identified to better understand the process of melanoma progression. Given PAX-3’s expression in late stage metastatic melanoma but absence of intermediate stages, targeting PAX-3 could potentially shift the nature of melanoma tumors to a less aggressive, less metastatic form.

CONCLUSIONS

Melanoma is a deadly skin cancer that is becoming increasingly common. Treatments such as surgery, radiation, and chemotherapy have failed in curbing late stage metastatic disease. Immunotherapy has emerged to fill this void by providing a novel approach to treating metastatic melanoma [11]. Treatments such as IL-2 administration, whole cell vaccines, adoptive T cell transfer, and immune-stimulating drug treatment have had some success in treating melanoma, but a standard of care remains elusive. The improved targeting of metastatic melanoma is needed for any of these techniques to be fully effective. The melanocyte protein PAX-3 is important in the regulation of melanocyte differentiation and is a potential target in melanoma. It has been implicated as a differentiation factor in melanoma, and has been increasingly used as a staging marker, with expression in stages I and IV. TGF-β regulates PAX-3 through the activation of the Smad pathway. However, upon increased UVR exposure, TGF-β functions are blocked, allowing PAX-3 molecules to activate downstream targets for melanocyte differentiation. In malignant melanoma, TGF-β is still produced, but its effect is lost on PAX-3, allowing the protein to remain active. The targeting of PAX-3 could potentially shift melanoma tumors to less aggressive and less metastatic form of the disease. PAX-3 siRNA could also block the differentiation, progression, and survival of...
the melanoma allowing a secondary treatment, such as immunotherapy or a combination of chemotherapy and immunotherapy to more successfully destroy the cancer. This review suggests that both in vitro and in vivo studies are needed to determine the specific activity of PAX-3 in the differentiation of melanocytes, and staging of malignant melanoma. Another direction of study could focus on the signaling pathways of TGF-β and how its effect on PAX-3 is lost. Understanding the regulation of PAX-3 in the development of melanocytes and melanoma could provide an opportunity for drug targeting and advancement in the treatment of metastatic melanoma.

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ABBREVIATIONS

TGF-β  =  Transforming growth factor-beta
UVR  =  Ultraviolet radiation
CTLA-4  =  Cytotoxic T lymphocyte-associated antigen 4
PAX-3  =  Paired box 3
SOX10  =  Sry-like HMG box 10
HTH  =  Helix-turn-helix
MITF  =  Microphthalmia transcription factor
Det  =  Dopachrome tautomerase

REFERENCES


