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The importance of β-catenin during melanoma formation

N. Demirkan[1] and L. Larue[2]

[1] *Department of Pathology, Pamukkale University School of Medicine, Kinikli, Denizli, Turkey*

[2] *Developmental Genetics of Melanocytes, UMR 146 CNRS-Institut Curie, Bat. 110, 91405, Orsay Cedex, France*

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β-Catenin has an important role in the differentiation of melanocytes and progression to melanomas. This protein is involved in cell-cell adhesion, cell signaling and gene transcription that are disrupted during malignant transformation. β-Catenin has a cell-cell adhesion function mediated by cadherins. The intercellular signaling is regulated with glycogen synthase 3β (GSK3β)/Wnt pathway and the gene transcription activity is mediated by DNA-binding LEF/TCF proteins. The oncogenic activity of β-catenin is directly associated with its participation in multiple protein-protein interactions and its role during gene expression. The microphthalmia-associated transcription factor (MITF), a target for β-catenin, has a role in melanoblast proliferation and in its differentiation. In this review β-catenin interactions and targets that are involved in the differentiation and transformation of melanocytes and its particular role in the expression of MITF are described.

Keywords: β-catenin, cadherin, microphthalmia-associated transcription factor, cell-cell adhesion, melanocyte

Development of melanocyte and interaction with keratinocyte

Melanoblasts are derived from neural crest cells. These cells migrate along the dorso-lateral pathway, between the somites and the ectoderm, then they cross the basement membrane separating the dermis from the epidermis, and invade the epidermis, afterwards they are called melanocytes once they produce melanin.[1] The melanocyte in human skin is normally embedded in the basal layer of keratinocytes. Interactions between melanocytes and keratinocytes are thought to be important in the control of melanocyte growth.[2] Human melanocytes and keratinocytes express functional E-cadherin, which mediates their heterotypic interactions.[3–5] The cell-cell adhesion function of E-cadherin depends on the presence of cytoplasmic proteins that include α, β and γ catenin. Disruption of the expression of E-cadherin and catenins has been implicated in tumor progression and metastasis.[6] Melanocytes may transform to melanoma, in some cases after forming a nevus and progresses to radial growth phase and to vertical growth phase. Melanocytes, but not nevus cells or melanoma cells express E-cadherin. During the malignant transformation, the classical melanocyte-keratinocyte interaction is lost for a homotypic melanocyte-melanocyte interaction in which cell-cell adhesion molecules are involved and affected.[6,7]

The main proteins involved in cell-cell adhesion are those of the cadherin/catenin complex that have a key role during normal and pathological differentiation of melanocytes. We shall discuss some interactions of these molecules with β-catenin in the progression of human melanoma.

Functions of β-catenin in different cellular compartments

Depending on its localization and its interactions, β-catenin is involved in cell-cell adhesion, cell signaling and gene transcription. The first known function of β-catenin is in cell-cell adhesion. Cadherins are anchored within cells by dynamic associations with catenins, which link them to the actin filaments. Cadherins constitute a superfamily of Ca^{2+}-dependent cell-cell adhesion molecules. They are transmembrane proteins composed of an extracellular domain, a membrane-spanning region, and a highly conserved cytoplasmic part. The cytoplasmic domain of cadherins directly interact with either β-catenin / a-catenin and γ-catenin (=plakoglobin)/a-catenin bound to actin filaments via alfa-catenin.[8] In normal epidermis, β-catenin expression and its cellular localization together with E-cadherins can be shown by their membranous immunohistochemical staining.

β-catenin if not incorporated in cell-cell adherens junctions is found as a large complex. It includes the protein encoded by the APC tumor suppressor gene, the GSK-3β serine/threonine protein kinase and axin/conductin protein.[9–12] GSK3β can phosphorylate serine/threonine residues and consequent degradation of β-catenin by the ubiquitin-proteosome system. Activation of the Wnt/Wg pathway block this degradation which regulate the amount of free cytoplasmic β-catenin. Increased amount of free β-catenin translocate into the cell nucleus. Nuclear β-catenin may interact with members of the TCF/LEF family of transcription factors and stimulate transcription of a various target genes.[13–15] Loss or gain of transcription factor gene function can notably play a major role in tumor growth and metastasis of melanoma.[16] These genes are involved in cell proliferation such as c-Myc, cyclin D1 or MITF promoters.[16–18] The importance of these different proteins in the biology of the melanocytes is not yet fully appreciated. A critical role played by the Wnt/β-catenin signaling pathway in the progression of melanoma is due to altered equilibrium of different β-catenin complexes in various cellular compartments.[9–11]

MITF expression and its role in melanoma progression

MITF, is a member of bHLH-Zip family transcription factor, it may either decrease or increase gene transcription. In humans, MITF mutation is characterized by hearing deficiencies, white forelock and ocular anomalies (Waardenburg syndrome, Type II).[19] Human MITF is homologous to mouse micropthalmia gene (mi). Among the various MITF isoforms, the M isoform is found in melanocytes. Melanocyte cell differentiation and function are regulated by a wide spectrum of genes among which MITF has a key role. During embryogenesis, MITF is involved in survival and proliferation of melanoblast. In differentiated melanocytes, it regulates the expression of melanocyte differentiation markers, such as tyrosinase.[16] Normal skin melanocyte, nevi, dysplastic nevi and metastatic tumors are detected with nuclear staining using an antibody against human MITF.[20] It appears that MITF expression is a molecular marker for diagnosis and its presence has a good prognosis in melanomas.[20–22] There is also a study suggesting that MITF may be a prognostic marker in patients with intermediate-thickness melanoma.[22] The MITF gene regulation depends at least on Sox10, Pax3 and β-catenin/TCF.[17]

Oncogenic activation of β-catenin

During the transformation of melanocytes to melanomas, β-catenin is no longer mainly located at the cell surface, but rather accumulated in the cytoplasm and may signal directly to the nucleus.[23–26] Aberrant β-catenin expression, with significant loss of membranous expression lead to metastasis of melanoma was also demonstrated previously.[27–28] It is likely that stabilization and overexpression of β-catenin, either as a result of mutation in β-catenin itself or other components of the Wnt signaling pathway play an important role in melanoma progression (Figure 1). Many of those colon cancer with wild-type APC are seen after mutation of the β-catenin gene.[29,30] In a limited number of melanoma cases, point mutations at the level of the serine,[37,45] and truncated form of β-catenin in its N-terminal region were identified.[31,32] Note, however, that the presence of mutations in melanoma cell lines is higher than in

melanoma cases, although there is an accumulation of β-catenin at nucleus.[28,31,32] One possibility, among others yet to be tested, is the increased frequency of mutations during cell culture. Another hypothesis is β-catenin mutation, which is already present in some cutaneous melanoma cells that might facilitate their adaptation to the culture media.[33] A non-functional APC may also fail to export nuclear β-catenin to the cytoplasm.[34] The consequence of either APC inactivation or β-catenin mutation is similar: a failure to degrade β-catenin properly, also aberrant function of β-catenin may result in increased cellular proliferation.[17,35] Upregulation of β-catenin induces the Lef/Tcf transcription factors and lead to continuous activation of target genes. Altogether, it is not surprising that *Myc* and *MITF*, two targets of β-catenin, are frequently not properly regulated in melanomas.[13,17,36]

Conclusions

β-catenin, once activated with or without mutation may constitutively interact with one or more targets related to cell growth, cell proliferation, cell invasion and apoptosis.[37,38] Melanoma cell lines indicate that regulation of melanoma growth by the Wnt β-catenin / Lef pathway is functionally dependent on the MITF transcription factor.[18,39,40] It is possible, that MITF might also participate in β-catenin-mediated growth, invasion and pigmentation control in melanoma cases.[20]

Although Wnt/β-catenin signaling is required early in melanocyte differentiation, possibly through the ability to activate MITF expression, it is not yet clear whether β-catenin is required at later times. β-Catenin-mediated activation of MITF expression is currently emphasized, however other genes may also

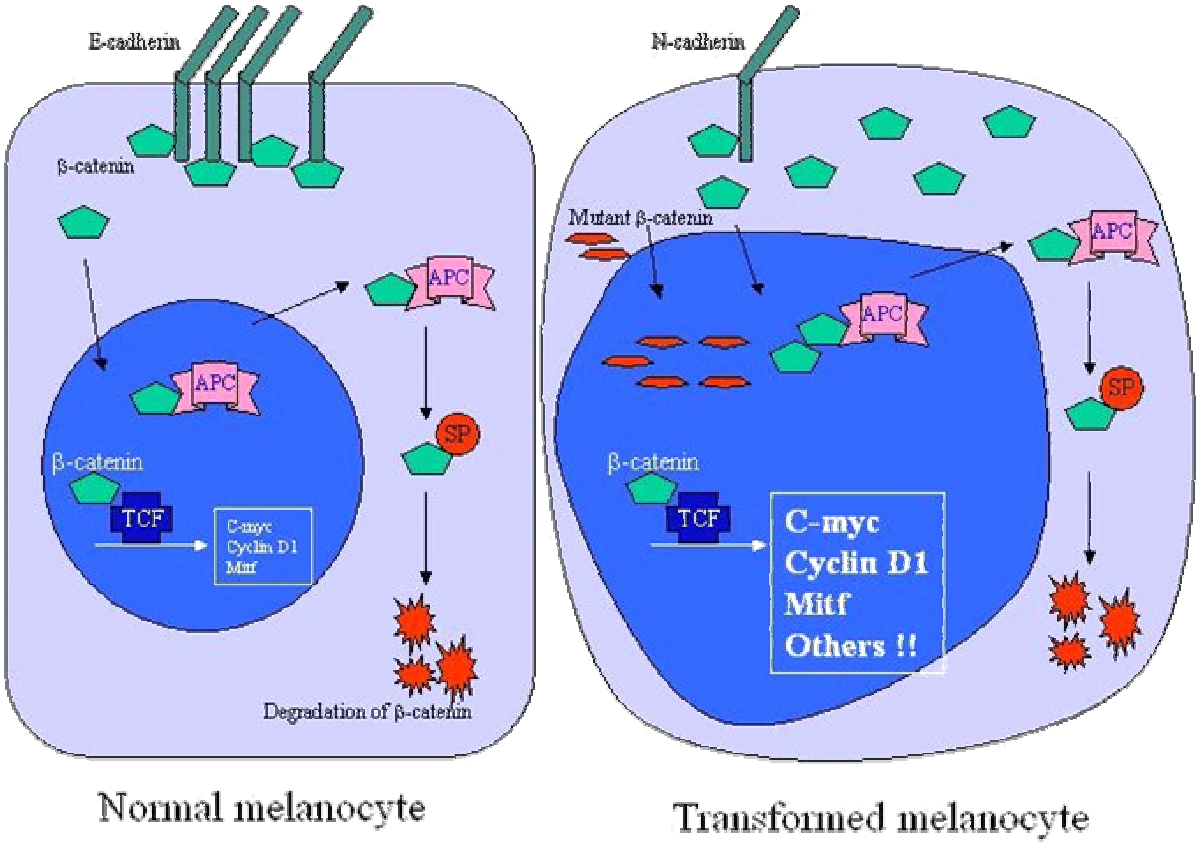

Figure 1: Small pool of β-catenin is bound to the APC–axin-containing protein complex, which targets β-catenin for degradation. In malignant transfomation of melanocyte, active pools of β-catenin in the cytoplasm and nucleus interact with TCF-type transcription factors and stimulate the expression of genes that are targets of the Wnt signaling pathway.

play a primary role in mediating the response of melanocytes to Wnt/β-catenin signaling.[16] β-catenin and its target genes related with Wnt/β-catenin signaling pathway have great importance at melanocyte lineage. The nature of those target genes that may contribute to malignant transformation must be investigated in future studies.

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