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Dual Function of Wnts in Human Cutaneous Melanoma

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1. Introduction

The cellular signaling pathways that respond to Wnts control numerous processes ranging from gastrulation to aging and govern cell fate determination and patterning (Clevers, 2006). Wnt signaling abnormalities often lead to developmental disorders and lethal malignancies. In melanoma aberrant activation of Wnt signaling is often observed. Wnt signaling pathway is a very complex process. Its ligands, called Wnts, can signal via several pathways referred to as the canonical Wnt signaling and two noncanonical Wnt signaling pathways. According to the modern view, the canonical Wnt signaling branch is predominately involved in control of proliferation and differentiation acting at a transcriptional level, whereas the noncanonical ones affect cell motility and cytoskeletal rearrangements. In the case of melanoma, the canonical and the noncanonical Wnt pathways can play opposite roles. The noncanonical Wnt cascade promotes metastasis. Overexpression of Wnt5a correlates with a poor prognosis for patients. The canonical Wnt cascade has long been considered as fully oncogenic. However, a lot of data confirms that it can act as a tumor suppressor by promoting cell differentiation.

2. Wnt proteins and their interactions

Wnt genes were identified both in vertebrates and invertebrates whereas plants, unicellular eukaryotes and prokaryotes appear to lack them (Miller, 2001). In humans, the Wnt family encompasses 19 members. All of them are defined by sequence homology rather than by functional properties (Wnt homepage). *Wnt* genes encode highly modified glycoproteins of 38-43 kDa that have typical features of the secreted growth factors, including a hydrophobic signal sequence, lack of transmembrane domains, an *N*-glycosylation site and a sustained spacing of conserved cysteine residues (McMahon, 1992). It is considered that posttranslational lipid modifications are essential for the Wnt function. Treatment of Wnts with the acylprotein thioesterase that removes palmitates leads to both hydrophobicity and signaling activity reduction (Willert et al., 2003). Moreover, mutations that prevent palmitoylation of cysteine residues in Wnt1, Wnt3a, Wnt5a result in significant decrease of their biological activity, presumably due to inability to bind Frizzled (Fz) receptors. These mutations do not affect secretion. In contrast, a mutation of the conserved serine in Wnt3a abrogates palmitoleic acid addition and blocks secretion. Considering these observations, it

is believed that the palmitoleic acid modification is required for secretion, whereas the palmitate modification is critical for receptor binding (Cadigan & Peifer, 2009).

Complexity of the Wnt signaling is greatly enhanced by abundance of potential receptors. There are several groups of receptor molecules that can bind Wnts. The first group found to transduce the Wnt signal is the Frizzled (Fz) receptor family. The Fz proteins were identified both in vertebrates and invertebrates. In human and mice, the Fz family includes ten members (Wnt homepage). It is believed that Fz proteins are the real G-protein-coupled receptors that can activate the heterotrimeric G-protein to transmit signal from Wnts. Like typical GPCR, they have seven hydrophobic transmembrane domains, glycosylation and phosphorylation sites for cyclic AMP-dependent protein kinase (PKA), protein kinase C (PKC) and casein kinase 2 (CK2), and are able to form homomeric and heteromeric complexes with other members of the Fz family. However, there are several differences between the Fz receptors and other GPCRs. The Fz receptors lack two conserved motifs, Asp-Arg-Tyr and Asn-Pro-X-X-Tyr. The Asp-Arg-Tyr motif is located in the second intracellular loop of several GPCRs and is crucial for G protein coupling. The Asn-Pro-X-X-Tyr motif is present at the end of the seventh transmembrane segment of GPCRs (Angers & Moon, 2009). The classical Fz receptor consists of a long highly glycosylated *N*-terminal extension, that is positioned outside of the cell, seven transmembrane domains and a short cytoplasmic tail. An amino terminal region called a cysteine-rich domain (CRD) is intended for the direct Wnt binding. The carboxyl terminus contains a consensus PDZ domain-binding motif (S/T-X-V) that is important for interaction with cytoplasmic proteins (Miller, 2001). Wnt/Fz combination at the surface of the cell determinates the type of G-protein subunits to be utilized and the kind of cellular response to occur (Liu et al., 1999; Malbon et al., 2001).

In addition to the Fz receptors, Wnt can bind to molecules of the low density lipoprotein (LDL) receptor-related protein (LRP) family. The Wnt-LRP coupling is considered to induce or stabilize the formation of the Fz-LRP-Wnt ternary complex. Wnts have a low affinity to LRP, as compared to Fz, so there is an idea that the coupling occurs between LRP and the Wnt-Fz complex (Tamai et al., 2000). Two members of the vertebrate LRP family, LRP5 and LRP6, are able to bind Wnts. The LRP6 mutant mice phenotype resembles defects caused by several individual *Wnt* genes deficiency (Pinson et al., 2000). LRP overexpression in *Xenopus* leads to activation of the Wnt signaling (Tamai et al., 2000). Moreover, binding of some extracellular inhibitors to LRP leads to abrogation of the Wnt signaling transduction (Itasaki et al., 2003; Glinka et al., 1998). Unlike to the Fz receptors, LRP6 is a single-pass transmembrane protein. It has a highly modular structure consisting of the extracellular domain (ECD) that mediates LRP6-ligand interactions, and the intracellular domain (ICD) that transduces extracellular signals to cytoplasmic effectors. ECD contains the extracellular proteins binding YWTD domains, EGF-like repeats, and LDL repeats. ECD is supposed to act as an autoinhibitory signal, since LRP6 lacking the ECD can constitutively activate the Wnt pathway. In contrast, the ICD domain is sufficient for the activation of the Wnt signalling pathway. The overexpression of the isolated ICD induces constitutive activation of the canonical Wnt signaling. The ICD domain is rich in proline, serine, and threonine and has a conserved PPSPXS motif critical for its function (Niehrs & Shen, 2010).

Repertoire of the Wnt receptor molecules is broader than that of the Fz and LRP proteins. The Wnt ligands can also signal through alternative receptors, structurally related homologs Ror1 and Ror2 that belong to the receptor tyrosine kinase (RTK) superfamily, and Ryk that is an atypical tyrosine kinase receptor. Transgenic mice with the Ror2 loss-of-function

mutations demonstrate very similar phenotype to mice with the Wnt5a depletion (Oishi et al., 1999). Ror2 contains extracellular Fz-like cysteine-rich domains (CRDs), membrane-proximal Kringle domains and immunoglobulin (Ig)-like domains. While CRDs and the Ig-like domains participate in ligand binding, function of the Kringle domains is still unclear. However, it is assumed that the Kringle domains in Ror act as recognition modules for binding of the Wnt regulatory proteins (Minami et al., 2010). Another receptor molecule Ryk is thought to mediate the Wnt signaling. Unlike to Ror2, Ryk is an atypical Tyr kinase receptor. It lacks kinase activity due to amino acid substitutions in the evolutionarily conserved residues of the intracellular kinase domains (Halford et al., 1999; Hovens et al., 1992). In addition to the intracellular kinase domain, Ryk contains a single transmembrane domain and an extracellular Wnt inhibitory factor (WIF) domain. The last one mediates binding of various Wnt ligands (Halford & Stacker, 2001). The choice of a ligand for interaction is rather controversial. According to different data, Ryk can bind both canonical and noncanonical Wnts. The physical interaction between Ryk and Wnt1 and Wnt3a with subsequent activation of the canonical Wnt signaling was demonstrated in the HEK293T cells (Lu et al., 2004). On the contrary, the noncanonical Wnt5a serves as a binding partner for Ryk in the Wnt5a-mediated axon guidance (Keeble et al., 2006).

To complete the description of the Wnt-interacting partners, a special group of proteins that can bind Wnt ligands but unable to participate in signal transduction should be mentioned. These proteins act as inhibitors blocking the Wnt functional activity. The soluble Frizzled-Related Proteins (sFRPs) represent a typical example of the extracellular Wnt inhibitors. They bind to Wnt proteins preventing them from coupling with their receptors. sFRPs are able to block both canonical and noncanonical Wnt signaling pathway. Another soluble Wnt inhibitor is called WIF-1 (Wnt inhibitory factor). Unlike to sFRPs, it does not contain a CRD domain. However, it can bind to Wnts through a unique domain that is similar to extracellular region of the Ryk receptors (Patthy, 2000).

Another group of the Wnt signaling inhibitors consists of secreted Wnt antagonists. The Dickkopf (Dkk) family of the Wnt antagonists inhibits the Wnt signaling by direct binding to LRP5/6 (Glinka et al., 1998). It is considered that Dkk can form a ternary complex with LRP5/6 and a single-pass transmembrane receptor Kremen-1 in order to promote LRP5/6 internalization with subsequent inactivation of the Wnt signaling (Mao et al., 2002). Binding of LRP by soluble Wnt signaling inhibitors is not a unique feature of Dkk. Some other secreted Wnt inhibitors (Wise and SOST) use the same mechanism (Itasaki et al., 2003; Semenov et al., 2005).

Availability of different receptors, co-receptors and inhibitors create cellular context. An effect of the Wnt signaling pathway depends not on the Wnt ligand itself, but on the Wnt ligand in the cellular context. The same Wnt ligand can activate different Wnt signaling branches, and classification of Wnt ligands based on a type of Wnt signaling activated seems artificial.

2.1 Different branches of the Wnt signaling

The Wnt signaling is commonly considered as a combination of at least three different signaling branches: the β -catenin pathway, the Wnt/ Ca^{2+} pathway and the planar cell polarity (PCP) pathway. There is evidence of significant crosstalk between them.

2.1.1 The canonical Wnt signaling pathway

The canonical Wnt signaling pathway, or the β -catenin pathway, was the first Wnt signaling discovered. It participates in multiple biological processes including embryogenesis, cell

proliferation and differentiation in adults, stem cell renewal in multiple tissues (hematopoietic, epidermal, and intestinal) and tumorigenesis (Clevers, 2006). A lot of growth genes (*c-myc*, *cyclin D1*, *PPAR δ*) are under control of this signaling (He et al., 1998, 1999; Tetsu & McCormick, 1999). Other target genes include the matrix metalloproteinase, fibronectin and the transcription factors AP-1, c-jun and fra1 (Brabletz et al., 1999; Gradl et al., 1999; Mann et al., 1999). The canonical Wnt signaling pathway can be activated by Wnts, such as Wnt1, Wnt3a and Wnt8. In the absence of Wnts, concentration of the cytoplasmic β -catenin is maintained at a low level by a special destruction complex. The destruction complex consists of the scaffolding proteins Axin and APC (adenomatous polyposis coli), GSK3 β (glycogen synthase kinase 3 β), CKI (casein kinase I), PP2A (protein phosphatase 2A) and, probably, ubiquitin ligase β -TrCP. When the destruction complex is active, β -catenin is phosphorylated by GSK3 β . The phosphorylated β -catenin in turn is recognized by the ubiquitin ligase β -TrCP, which targets it for ubiquitin-mediated proteosomal degradation (Weeraratna, 2005). The canonical Wnt signaling is initiated by simultaneous binding of the Wnt ligand both to Fz receptor and LRP-5/6 coreceptor (Tamai et al., 2000). The ternary complex formation recruits a group of proteins including Disheveled (Dvl), Axin and GSK3 β to the plasma membrane, where they form the Lrp6 signalosomes (Bilic et al., 2007). Aggregation causes CK1 γ and GSK3 β -mediated LRP6 phosphorylation. According to the modern view, the cytoplasmic scaffolding protein Dvl is required for the LRP6 aggregation and phosphorylation (Niehrs & Shen, 2010). Wnt-stimulated Dvl becomes hyperphosphorylated and forms polymers that are recruited to the plasma membrane, providing a platform for the Axin-GSK3 β relocation (Cliffe et al., 2003). This relocation in turn results in inhibition of the β -catenin phosphorylation and further signaling transduction (Mao et al., 2001). The β -catenin stabilization is a key event in activation of the canonical Wnt signaling. The stabilized β -catenin accumulates in the cytoplasm, translocates into the nucleus and participates in target gene regulation. β -catenin by itself is unable to bind DNA, but it can induce transcriptional activity of Tcf/Lef1 (Behrens et al., 1996). In the absence of β -catenin, Tcf/Lef1 acts as a transcriptional repressor. It can bind to the consensus motif (A/T)(A/T)CAA(A/T)G on DNA but has no trans-activation domain to induce transcription. In the β -catenin/Tcf/Lef1 complex, Tcf/Lef1 facilitates DNA-binding whereas β -catenin provides transactivation domains (Brantjes et al., 2002).

2.1.2 The noncanonical Wnt signaling pathways

At least two additional signaling pathways activated by Wnt ligands have been identified: the Wnt/Ca²⁺ pathway and the PCP pathway. Both of them are β -catenin-independent and are referred to as the noncanonical signaling pathways. Similar to the β -catenin signaling, the noncanonical pathways are essential for embryogenesis. They regulate multiple morphogenetic processes including gastrulation and neural tube closure (Jenny & Mlodzik, 2006; Kohn & Moon, 2005).

2.1.2.1 The Wnt/Ca²⁺ pathway

The Wnt/Ca²⁺ pathway includes calcium release from the intracellular stores and induction of enzymatic activity of Ca²⁺-dependent protein kinases like calmodulin kinases, protein kinase C (PKC) and calcineurin (Kohn & Moon, 2005). The first ligand identified to induce transient spikes of the intracellular calcium is Wnt5a. In *Xenopus* embryos, it was shown that Wnt5a expression blocked the ability of the canonical Wnt8 to induce axis duplication. These data confirmed an idea of existence of multiple Wnt signaling pathways. The more

precise data came from studies in *Xenopus* and zebrafish models. It was revealed that Wnt5a overexpression phenocopied overexpression of a serpentine receptor that stimulates the intracellular calcium release in a G-protein-dependent manner. Later, an appropriate Fz receptor Rfz-2 was identified (Kuhl et al., 2000). The Wnt coupling to receptor (Fz, Rfz2) leads to dissociation of the $G\alpha$ and $G\beta/\gamma$ subunits. $G\beta/\gamma$ activates phospholipase C (PLC), which translocates to the membrane and hydrolyzes phosphatidylinositol 4,5- biphosphate (PIP₂) into inositol 1,4,5- triphosphate (IP₃) and di-acyl glycerol (DAG). DAG activates PKC, while IP₃ induces Ca^{2+} release from the intracellular stores. Elevation of the intracellular calcium level in turn stimulates the Ca^{2+} -dependent effector molecules. Reminiscent of the canonical Wnt signaling-mediated Dvl translocation, Wnt5a expression results in PKC translocation to the plasma membrane where it is able to interact with its target proteins (Weeraratna, 2005). There is a positive feedback loop between PKC and Wnt5a. Inhibition of PKC can result in decrease of Wnt5a expression, whereas PKC activation leads to Wnt5a upregulation (Jonsson et al., 1998). Signaling cascades activated by PKC affect cytoskeletal organization and cell motility.

2.1.2.2 The Planar Cell Polarity pathway

Another noncanonical Wnt signaling pathway is the Planar Cell Polarity, or PCP, pathway. It was initially discovered in *Drosophila melanogaster*, but later found to control some critical biological processes in vertebrates (Mlodzik, 2002; Wang & Nathans, 2007). Polarity is an important feature in living organisms, underlying the proper performance of many functions. There are several kinds of polarity. Epithelial cells are polarized in two different manners. Besides apical-basal polarity, they also display polarization along the plane of the epithelial layer orthogonal to the apical-basal axis. This kind of polarization is called tissue polarity, or planar cell polarity (PCP). It is notable that, besides the epithelial cell polarity, the PCP proteins also provide mesenchymal cell intercalation during axis elongation process in vertebrates (*Xenopus* and zebrafish). The principal mechanism for tissue elongation is an insertion of mesenchymal cells between their neighbors along one axis. In this case, the mesenchymal cells have no apical-basal polarity but are able to extend polarized protrusions in the same direction. Thus, these cells are oriented with respect to direction of movement (Zallen, 2007). The transmembrane PCP proteins Frizzled, Strabismus, and Flamingo and the cytoplasmic PCP proteins Dvl and Prickle are critical for proper intercalation (Keller, 2002; Myers et al., 2002; Wallingford et al., 2002). Reestablishing of cell polarity after a mitotic cell division is also dependent on the PCP pathway (Nechiporuk & Vasioukhin, 2006). The Wnt/PCP pathway controls activity of the small GTPases Rho and Rac. The Rho signaling branch requires activation of the Dvl-Daam-1 (Dishevelled-associated activator of morphogenesis 1) complex and results in the Rho-associated kinase (ROCK) and myosin activation (Habas et al., 2001; Marlow et al., 2002; Weiser et al., 2007). Daam-1 is a member of the Formin protein family. It mediates an assembly of the Dvl-RhoA complex acting as a scaffolding protein (Habas et al., 2001). The Rac branch of signaling is Daam-1-independent. It requires another domain of Dvl to induce activity of small Rac GTPase. Activated Rac in turn stimulates Jun kinase (JNK) (Habas et al., 2003; Li et al., 1999). The PCP pathway regulates modifications of actin cytoskeleton structures and, as a consequence, provides cytoskeletal rearrangements and directed migration. In addition, it is considered that, unlike to the above mentioned pathways, the PCP cascade appears to function independently of transcription. Actually, the majority of molecular cascades connected with Rho/Rac GTPases and PKC mostly affect cell motility. This observation allows to suggest that the noncanonical Wnt

signaling branches are implicated exactly in these processes. Contrariwise, the canonical Wnt signaling controls predominately proliferation and differentiation acting at a transcriptional level.

2.2 Wnt signaling in skin biology

In embryogenesis, components of the Wnt cascade are involved in multiple processes including neural crest induction, specification and differentiation (Dorsky et al., 1998). Neural crest cells arise from a region called neural folds at the border of the neural plate and non-neural ectoderm. During neurulation, neural folds converge at the dorsal midline of an embryo to form the neural tube. According to the conventional view, this process is mediated by molecular signals emanating from the ectoderm and receiving by the neuroepithelium. Numerous experiments in amphibian, zebrafish, avian and murine embryos revealed Wnt signaling as a driving force in this process (García-Castro et al., 2002; Huang & Saint-Jeannet, 2004; Lewis et al., 2004). Depletion of the main effector component of the canonical Wnt signaling, β -catenin, abrogates the neural crest induction (Wu et al., 2005), and the promoter region of neural crest-specific gene *Slug* contains a binding site for Lef/ β -catenin complex (Vallin et al., 2001).

The fate of the neural crest cells is also under control of the Wnt signaling pathway. Some reports confirm that Wnt6 and Wnt8 are required both for the neural crest induction and its expansion (Labonne & Bronner-Fraser, 1998; Sakai et al., 2005). After neurulation, neural crest cells from the roof plate of the neural tube undergo an epithelial-to-mesenchymal transition and migrate to the peripheral sites. This migration is an elaborate process, tightly controlled on a molecular level. It is believed that, unlike to the neural crest induction controlled by the canonical Wnt signaling, migration of neural crest cells mostly depends on another kind of the Wnt signaling called the noncanonical or the Planar Cell Polarity (PCP). Inhibition of the PCP factors like Wnt11, Frizzled7 (Fz7) and Dishevelled (Dvl) results in a failure of the neural crest cells migration, indicating that the noncanonical Wnt signaling is essential for the neural crest migration *in vivo* (De Calisto et al., 2005).

The neural crest cells give rise to diverse cell types, including neurons, glia and melanocytes. Neurogenic and melanogenic populations of the neural crest cells have distinct migratory specificities governed by expression of different surface receptors and signaling molecules, including Wnts (Thomas & Erickson, 2008). The Wnt signaling is supposed to be important for the neural crest cells specification. Neuroblasts and glioblasts show strong expression of the Wnt signaling inhibitor protein called Frzb-1. Conversely, the Frzb-1 expression level in melanoblasts is decreased (Jin et al., 2001). Other evidence of the Wnt signaling participation in the neural crest cells specification comes from knockout studies. The Wnt1 and Wnt3a double-knockout mice exhibit defects in the neural crest cell derivatives, including melanocytes (Ikeya et al., 1997). The β -catenin knockout in the neural crest cells leads to loss of both melanocytes and sensory neurons (Hari et al., 2002). In the presence of Wnt3a in conditioned medium, the cultured quail neural crest cells evolve into melanoblasts (Jin et al., 2001). Injection of mRNA encoding cytoplasmic β -catenin into the neural crest cells of a zebrafish embryo targets them to a pigment cell fate at the expense of neurons and glia (Dorsky et al., 1998). In melanocytes, the canonical ligand Wnt3a promotes up-regulation of the microphthalmia-associated transcription factor (MITF), the master regulator of melanogenesis. The MITF expression starts in melanoblasts soon after their migration from the neural tube, and loss of MITF results in the absence of melanocytes

(Thomas & Erickson, 2009). Interestingly, the MITF expression is abolished in the fibroblasts derived from skin treated with the Wnt signaling inhibitor Dkk-1. Along with the MITF inhibition, these samples also show the decreased β -catenin expression level. Dkk-1 is supposed to be a key molecular determinant for a regional specificity in human skin pigmentation. Hands and feet express Dkk-1 at a higher level comparing to a trunk, and are less pigmented due to a lower melanocyte density. It is believed that in epidermis Dkk-1 inhibits both melanocyte differentiation and melanin production (Yamaguchi et al., 2007).

The terminal cell differentiation can be inhibited in a β -catenin-independent manner. A way to keep cells in a precursor state is provided by a member of the Tcf/Lef family, Tcf-3, that acts as a repressor of the Wnt-mediated transcription. Tcf-3 is normally expressed in the hair follicle bulge and basal layer of the outer root sheath. Ectopic expression of Tcf-3 in interfollicular epidermis inhibits terminal keratinocytes differentiation and induces a shift to progenitor-like molecular phenotype (Nguyen et al., 2006). The noncanonical Wnt signaling activated by Wnt5a also can antagonize the canonical signaling and inhibit expression of melanogenic antigens (Dissanayake et al., 2008). This observation leads to an idea that, whereas the canonical Wnt signaling is important for melanoblasts positioning and differentiation, the other Wnt signaling type serves for maintenance of the de-differentiated cell state. The idea is supported by data from the hematopoietic stem cells (HSCs). These cells are maintained in a quiescent state by Wnt5a, and the canonical Wnt signaling, contrariwise, makes them differentiate (Nemeth et al., 2007). The melanocyte stem cells express receptors for the Wnt signaling pathway on their surfaces (Yamada et al., 2010). Taking into account these data and the data from melanocytes regulation, it was speculated that Wnt5a could be involved in the quiescence maintenance of the melanocyte stem cells (MSCs), keeping them in their niche environment (Nishikawa & Osawa, 2007; O'Connell & Weeraratna, 2009). Stem cells committed to the melanocyte lineage reside in the bulge area of hair follicles (Nishimura et al., 2002). Being a source of melanoblasts and melanocytes, in adults they are most likely to be related to hyperpigmentation and age-associated hair graying (Yamada et al., 2010).

Mammalian skin serves many critical biological functions to maintain homeostasis. Evidence indicates that the Wnt signaling is implicated in this process. The protective function of skin can be disturbed by wounding. The only way to restore skin integrity and, as a result, its protective function is healing of the wound. Unfortunately, wound healing and true regeneration are not the same. Cutaneous repair after the loss of full-thickness skin usually leads to scarring. The healed tissue contains a collagen-rich dermal matrix with a stratified epithelial covering. It is less elastic, has a lower tensile strength level and unable to form skin appendages. Interestingly, skin actually has a potential to regenerate, since it contains the multipotent epidermal stem cells in hair follicles and the undifferentiated mesenchymal cells in the dermis. Thus, it is postulated that scar repairing is more favorable than regeneration due to molecular context in the affected area. It is considered that the Wnt signaling pathway contributes significantly to this molecular context. To elucidate the role of the Wnt signaling in wound healing, the Wnt expression is examined at various times after wounding. Wnt-4 is expressed early in the process, while Wnt-5a and Wnt-11 expression peaks are at the time of wound remodeling. The "TOPGAL" mice experiments reveal that the canonical Wnt signaling activity is increased in the hair follicles adjacent to the lesion, but not within the wound or overlying epithelium. Furthermore, the stabilized β -catenin expression results in epithelial appendages formation like hair follicles and sebaceous

glands within the wound (Fathke et al., 2006). The rate of wound closure is not affected by the β -catenin expression. Interestingly, the TGF β -induced wound healing is partially regulated by β -catenin. Expression of *Mmp-3* and *Mmp-14* stimulated by TGF β requires the β -catenin expression (Cheon et al., 2005). The noncanonical Wnt signaling is also able to direct adult skin progenitor cells toward regeneration. Wound transduction with the retrovirus expressing the typical noncanonical Wnt ligand Wnt-5a leads to even more abundant epithelial appendage formation in the wound, as compared with the stabilized β -catenin expression. Moreover, the authors report that the noncanonical Wnt signaling activation in epidermis is not associated with epithelial tumors, in contrast to the β -catenin-dependent signaling whose activation sometimes results in tumor formation (Fathke et al., 2006).

Skin development and homeostasis are considerably dependent on regulation by the Wnt signaling. It is implicated in the neural crest induction and specification, determining of epidermal and melanocyte stem cells fate, hair follicle establishment and entry of its cells into the active growth phase and wound healing. Considering significance of the Wnt signaling in skin biology, it is not surprising that its malfunctions are often seen in different pathological conditions. Expression pattern of many Wnts is affected in malignant melanoma.

2.3 Wnt signaling in melanoma

Aberrant activation of the Wnt signaling pathways is often observed in melanoma. The cutaneous melanoma is considered as a neural crest-derived malignancy. It originates from the melanocyte progenitor cells or from the pigment-producing melanocytes. Taking into account that the Wnt signaling cascades play a crucial role in the neural crest induction, specification and melanocyte differentiation, implication of the different Wnt signaling branches in melanoma pathogenesis seems quite natural.

The cutaneous melanoma is a common skin cancer characterized by high aggressiveness, morbidity and mortality. The melanoma development is a multistep process. It includes a congenital or acquired nevus, a dysplastic atypical nevus, a radial growth phase (RGP) melanoma, a vertical growth phase (VGP) melanoma and a metastatic melanoma (Larue & Beermann, 2007). Uncontrolled cell proliferation leads to the mole formation. It is considered that further immortalization is required for melanoma induction (Larue et al., 2009). A fraction of atypical nevi undergoes malignant transformation into a RGP melanoma with low probability to metastasize. Unlike to RGP, a VGP melanoma has high invasive and metastatic capabilities. A metastatic melanoma is usually resistant to chemotherapy and radiation (Govindarajan et al., 2007). It is believed that components of the Wnt signaling cascades can contribute to all stages of melanoma progression. There are several hypotheses describing potential roles of the canonical and noncanonical Wnt signaling branches in melanomagenesis. Thus, according to one of them, aberrations in the canonical signaling lead to melanoma formation, whereas the noncanonical Wnt signaling malfunctions are associated with metastatic progression. Furthermore, during melanoma progression, the β -catenin signaling serves as a negative regulator of tumor growth. Numerous data confirms this idea. However, it is worth mentioning that some researchers argue against this conception. They consider the canonical Wnt signaling as a fully oncogenic. Anyway, the Wnt signaling is somehow implicated in melanoma progression. In the case of melanoma, Wnt1, Wnt3a and Wnt5a are best described. Wnt1 and Wnt3a are considered as the canonical ligands and Wnt5a as an activator of the Wnt/Ca²⁺ pathway.

2.3.1 Implication of the canonical Wnt signaling in melanoma cell fate

In normal skin, melanocytes are interspersed among keratinocytes at the epidermal-dermal border. Physical and functional interaction with keratinocytes provides control of melanocyte proliferation and differentiation. In the absence of this control, melanocytes tend to rapid proliferation and expression of cell surface molecules normally associated with melanoma (McGary et al., 2002). A transmembrane protein E-cadherin is considered as the major mediator of human melanocyte adhesion to keratinocytes. Loss of E-cadherin is often associated with tumorigenesis including melanoma (Haass et al., 2005). Interestingly, E-cadherin does not only act as a cell-cell adhesion molecule, but also mediates intracellular signaling through β -catenin. Indeed, β -catenin was originally identified as an interlink between E-cadherin and α -catenin in the adherens junctions (Cowin, 1994; Gumbiner & Neuron, 1993; Nagafuchi & Takeichi, 1989; Ozawa et al., 1989). Downregulation of E-cadherin leads to release of β -catenin from the membrane-associated pool and to increase of its transcriptional activity. The activated canonical Wnt signaling is found in approximately one third of melanomas (Larue et al., 2009). Keeping in mind that the β -catenin signaling promotes expression of many "growth" genes, including *cyclin D* and *c-myc*, constitutive activation of this cascade can potentially stimulate tumor formation. For example, *c-myc* is a well-known oncogene. Its overexpression is found in a variety of human cancers including colorectal cancer, breast cancer, leukemia and melanoma (Dang, 1999). Inhibition of *c-myc* is supposed to be an important step in tumor growth restriction. Supporting this idea, the *c-myc* antisense oligonucleotides have been shown to decrease proliferation of different cancer cells (Iversen et al., 2003).

Expression of the canonical Wnt signaling negative regulators is often reduced in melanoma. Thus, Dkk-1, -2 and -3 are downregulated or lost both in melanoma cell lines and tumor samples (Kuphal et al., 2006). Dkk-1 and Dkk-2 inhibit the β -catenin signaling by binding to LRP5/6 coreceptor. It has been shown that Dkk-1 can suppress melanocyte growth and melanogenesis (Yamaguchi et al., 2004). Activation of the Dkk-1 expression results in inhibition of tumorigenicity and induction of apoptosis in melanoma cells during *in vivo* growth in the athymic nude mice (Mikheev et al., 2007). Another Wnt signaling inhibitor repressed in melanoma is WIF-1 (Wnt inhibitory factor-1) (Haqq et al., 2005). Unlike to Dkk, WIF-1 binds directly to the Wnt ligand blocking its signaling activity. Unfortunately, it remains unknown, which type of the Wnt signaling is inhibited by WIF-1. Anyway, the WIF-1 silencing may be a critical event in constitutive activation of the canonical Wnt pathway in melanoma cells. It is reported that melanoma cell growth is suppressed by WIF-1 overexpression, and the suppression is related to transcriptional and translational inhibition of the canonical Wnt signaling components, including β -catenin, Dvl-3, and cyclin D1 (Lin et al., 2007).

Besides extracellular inhibitors of the canonical Wnt signaling, like Dkk and WIF-1, expression of the cytoplasmic CK1 α in melanoma is frequently decreased or completely lost. CK1 α is responsible for initial phosphorylation of β -catenin that is required for the GSK3 β -mediated β -catenin degradation (Liu et al., 2002). CK1 α expression in invasive melanoma cells decreases growth rate and induces cell cycle arrest and apoptosis, whereas suppression of CK1 α in primary melanomas has an opposite effect. Evidence confirming β -catenin implication in this effect comes from the β -catenin downregulation experiments. Inhibition of the β -catenin expression in the nonmetastatic melanoma cell lines results in inhibition of invasive growth (Sinnberg et al., 2010).

As mentioned above, β -catenin is a multifunctional protein, and its function is supposed to depend on cellular localization. At the membrane, β -catenin is a component of the cadherin adherens junctions and in the nucleus it acts as a transcriptional activator of the canonical Wnt signaling target genes. There are several reports that β -catenin is accumulated in the cytoplasm or nucleus of human melanoma cell lines and original tumors (Rubinfeld et al., 1997). But unlike to other cancers, the elevated level of β -catenin in melanoma is rarely associated with mutations (Giles et al., 2003). Melanoma is very heterogeneous. The activity and regulation of the canonical Wnt signaling vary significantly among different patients. Nuclear β -catenin localization is not always sufficient for the canonical Wnt signaling activation. Several cell lines with high nuclear β -catenin level are unable to activate this signaling (Kulikova et al., in press).

According to some notions, β -catenin can induce melanocyte immortalization by bypassing the senescence barrier (Delmas et al., 2007). A growth arrest after a limited number of divisions represents a good way to protect cells from oncogenic transformation (Campisi, 2005). Uncontrolled proliferation and delayed senescence are considered to be enough for melanoma transformation. Activating mutations in N-Ras and B-Raf (the MAP-kinase signaling pathway) can provide signals for proliferation, whereas β -catenin is believed to contribute to senescence escape (Delmas et al., 2007; Gray-Schopfer et al., 2005; Tsao et al., 2000). Senescence is associated with the G₀/G₁-like cell cycle arrest induced by the tumor suppressor Rb1 that in turn is controlled by p16^{INK4a} (Narita et al., 2003). In melanoma, the p16^{INK4a} expression is often silenced by genetic and epigenetic mechanisms. It has been shown that in transgenic mice, the stabilized β -catenin decreases a number of melanoblasts and stimulates immortalization of primary skin melanocytes by silencing the p16^{INK4a} promoter (Delmas et al., 2007). Transgenic animals expressing the stabilized form of β -catenin demonstrate an elevated β -catenin level in the nucleus of target cells that mimics the constitutively active β -catenin in melanomas. Moreover, in human melanoma cells, this activated β -catenin represses p16^{INK4a} directly in a TCF4-dependent manner. It should be noted that β -catenin by itself is unable to induce either melanocyte proliferation or melanoma formation. However, the double transgenic animals carrying both N-Ras and β -catenin mutations show a high rate of melanoma incidences. Moreover, these double mutants are more subject to melanomagenesis than the single N-Ras transgenic mice. Thus, it has been postulated that the constitutively activated canonical Wnt signaling acts synergistically with the MAP kinase pathways in order to induce melanoma in the absence of the p16^{INK4a} mutation (Delmas et al., 2007).

The MAP kinase and β -catenin signaling cascades regulate activity of the master regulator of melanogenesis, MITF-M. In melanoma characterized by simultaneous activation of N-Ras and β -catenin, the MITF level is higher than in N-Ras-driven tumors (Larue et al., 2009). MITF-M regulates a wide range of biological processes including cell proliferation and differentiation (Palmieri et al., 2009). According to the current hypothesis, the β -catenin capacity to activate MITF-M underlies its ability to regulate the melanocyte number (Widlund et al., 2002; Larue & Delmas, 2006; Schepsky et al., 2006). A high level of MITF activity restricts cell division, whereas a low level is associated with proliferation (Carreira et al., 2005, 2006; Loercher et al., 2005; Wellbrock & Marais, 2005). Thus, β -catenin, being a direct regulator of the MITF-M expression, can affect melanocyte proliferation. In case of the β -catenin transgenic mice, the overactivated canonical Wnt signaling can lead to MITF activation and restriction of melanoblast proliferation.

In spite of numerous studies confirming the β -catenin implication in malignant transformation of melanocytes, the exact role of the Wnt/ β -catenin signaling in melanoma and especially in melanoma metastasis remains quite controversial. Whereas a lot of papers claim that β -catenin is not associated with melanoma progression (not transformation), there is a report revealing a form of β -catenin that does correlate to the disease stage. Surprisingly, this phosphorylated form of β -catenin in human melanoma bioptic samples is accumulated in nucleus. However, as far as we know, the phosphorylated β -catenin is usually targeted for degradation in the cytoplasm (Kielhorn et al., 2003). Moreover, in contrast to the results from transgenic mice revealing no activation of proliferation in response to the stabilized β -catenin, in human melanoma cell lines β -catenin induced melanoma growth mediated by the MITF upregulation (Widlund et al., 2002). On the other hand, a lot of data demonstrate that the β -catenin signaling actually acts as a negative regulator of melanoma progression. Almost all benign nevi are positive for the nuclear β -catenin, but the rate of nevi transformation into melanoma is very low (Tsao et al., 2003). Metastatic melanoma progression is associated with a loss of the nuclear β -catenin. Contrariwise, the nuclear accumulation of β -catenin is a good sign for patients (Bachmann et al., 2005; Chien et al., 2009; Kageshita et al., 2001; Maelandsmo et al., 2003). The canonical Wnt signaling plays a crucial role in the pigment cell biology (Fang D et al., 2006). Wnt3a and Wnt1 are factors required for differentiation of pluripotent stem cells into functional melanocytes (Dorsky et al., 1998). Treatment of melanoma cells with Wnt3a results in increased pigmentation, transcriptional upregulation of melanogenic antigens and decreased metastatic ability (Chien et al., 2009). The Wnt3a-upregulated melanogenic antigens, including Trpm1, Kit, Met, and Mlana, are normally associated with melanocyte differentiation. They are frequently lost during metastatic tumor progression (Ryu et al., 2007). Transfection of the B16 melanoma cells with Wnt3a leads to decreased proliferation and induces differentiation (Chien et al., 2009). Moreover, silencing of β -catenin in this cell line actually promotes metastasis (Takahashi et al., 2008). Inhibition of negative regulators of the canonical Wnt signaling has a similar effect. The GSK3 β suppression increases melanogenesis both in the B16 cells and melanocytes and decreases proliferation of the cultured B16 and human melanoma cells (Bellei et al., 2008; Chien et al., 2009).

Thus, the canonical Wnt signaling pathway seems to play a dual role in melanomagenesis, depending on the context. On the one hand, it can somehow contribute to melanocyte transformation, and on the other, it can restrain tumor progression. Taking into consideration all these observations, it is possible that the Wnt/ β -catenin signaling may be required to maintain a homeostasis (Lucero et al., 2010). The dysregulation of specific transcriptional programs in melanocytes or nevi can lead to early melanoma transformation.

2.3.2 Contribution of the noncanonical Wnt signaling to melanoma metastasis

Regarding the noncanonical Wnt cascades in melanoma, almost all data available at the moment is related to the Wnt5a and Wnt/ Ca^{2+} -signaling pathway. It is likely that Wnt/ Ca^{2+} -pathway is not implicated in melanocyte transformation, but rather contributes to melanoma progression. The Wnt5a overexpression is frequently observed in highly aggressive melanomas (Bittner et al., 2000; Dissanayake et al., 2007; Weeraratna et al., 2002). There is a positive correlation between its expression and a tumour stage (Weeraratna et al., 2002). Moreover, Wnt5a was identified as a good criterion for melanoma division into highly aggressive tumors and less invasive counterparts (Bittner et al., 2000). Transfection of

less aggressive melanoma cells with Wnt5a converts them into more metastatic derivatives. Moreover, in melanoma, Wnt5a is predominantly presented at the leading edge of invasion. It is considered that the conversion is connected with the PKC activation (Weeraratna et al., 2002). The PKC signaling is often associated with alterations in cell motility, invasion and metastasis. Wnt5a can promote melanoma progression via the PKC-mediated mechanisms (Mapelli et al., 1994; Weeraratna et al., 2002). Another possible mechanism that can underlie the Wnt5a-dependent metastasis is the PCP pathway. Wnt5a, as well as Wnt1 and Wnt11, has been shown to activate the PCP pathway. Key components of this cascade, Rac, Rho and Cdc42, participate in melanoma metastasis (Choi & Han, 2002; Clark et al., 2000; Nakahara et al., 2003). But, unfortunately, there is no direct evidence supporting this idea.

The Wnt5a signaling can antagonize the β -catenin signaling (Topol et al., 2003). As it was discussed before, silencing of the β -catenin signaling in melanoma cells actually promotes metastasis (Takahashi et al., 2008). Taken together, these observations provide a model where melanoma metastasis is at least partially associated with the Wnt5a-mediated repression of the β -catenin signaling. Notably, treatment of melanocytes with Wnt5a can induce their apoptosis but not transformation (O'Connell & Weeraratna, 2009). However, in melanoma, the Wnt5a-mediated increase in CamKII phosphorylation has been shown to protect cells against the Trail-induced apoptosis (Dissanayake et al., 2007; Xiao et al., 2005). Considering the tumor suppressor role of Wnt5a in other cancers, it is easy to speculate that a cellular context may be very important for the Wnt5a response determination. Moreover, balance between the canonical and noncanonical Wnt signaling pathways may be crucial for homeostasis maintenance (O'Connell & Weeraratna, 2009). In benign nevi, Wnt5a may prevent the β -catenin-dependent melanocyte transformation; in melanoma progression, the canonical Wnt signaling may inhibit the Wnt5a-driven metastasis. The opposite is true for melanomagenesis. The aberrantly activated canonical Wnt signaling is important for melanoma establishment, and, during cancer progression, it should be inhibited by the Wnt5a signaling in order to keep melanoma cells undifferentiated.

Expression of Wnt5a in the nevi is quite controversial. While some researchers argue against the Wnt5a expression in the majority of benign nevi, others report the relatively strong Wnt5a expression (Da Forno et al., 2008; Mapelli et al., 1994; Pham et al., 2003). However, this disagreement can be explained by different sensitivity of methods used for Wnt5a detection. Considering high motility of the nevus cells and association of Wnt5a with decreased proliferation, the observation of a high Wnt5a level in the nevi is quite conforming to the behavior of these cells.

Multiple data confirms that Wnt5a mediates its effects via the PKC activation (Dissanayake et al., 2007). In order to activate the noncanonical Wnt signaling in melanoma cells, Wnt5a must bind to a specific cohort of receptors, including Fz2, Fz5 and Ror2 (Billiard et al., 2005; Weeraratna et al., 2002). Treatment of melanoma cells with an antibody against Fz5 leads to inhibition of the PKC activation, reduced motility and invasion of melanoma cells (Sen et al., 2001, Weeraratna et al., 2002). But the major Wnt5a receptor implicated in melanoma metastasis is supposed to be Ror2 (O'Connell et al., 2010). Like Wnt5a, it is upregulated predominantly in metastatic melanomas and has a negative correlation with melanoma patients survival (O'Connell et al., 2010). A knockdown of this receptor abrogates ability of Wnt5a to signal and mediate metastasis. Interestingly, overexpression or silencing of Wnt5a increases or decreases level of Ror2, respectively. However, downregulation of Ror2 has no effect on the Wnt5a expression (O'Connell et al., 2010).

Wnt5a can promote melanoma metastasis via different cellular mechanisms. First of all, it can induce morphological changes of melanoma cells. There is a report showing that transfection of melanoma cells (derived from an axillary lymph node) with the low endogenous Wnt5a expression with the Wnt5a-coding plasmid leads to shift from compact and roughly triangular cell shape into thin and spreading morphology. Cell shape changes were accompanied by increase in a number of contacts with the substrate (increased adhesion), actin cytoskeleton reorganization and PKC activation. Moreover, the transfected cells were more motile, as compared with the parent cells. Interestingly, the most affected PKC isoforms in the Wnt5a-transfected cells were PKC μ and PKC β II, that are considered to participate in cytoskeletal organization and invasion, respectively (Weeraratna et al., 2002). Morphological changes induced by the Wnt5a overexpression remind characteristics of the mesenchymal phenotype. Actually, cells with similar features are observed in many cancers. Mesenchymal motility is characterized by polarized and elongated cell morphology. It requires degradation of extracellular matrix (ECM) components in order to generate a "path" for moving cells (Parri et al., 2009). Wnt5a is considered to promote exactly this form of invasion. Thus, the Wnt5a overexpression stimulates production of matrix metalloproteinases like MMP-2 and MMP7 that degrade ECM (O'Connell et al., 2008; Pukrop et al., 2006). It also induces a shift in expression of cadherins, from E-cadherins to N-cadherins (Hsu et al., 2002). This shift decreases cell-to-cell contacts and facilitates cell-to-ECM interactions. Notably, Wnt5a also increases a level of an intermediate filament protein called vimentin and up-regulates expression of the transcriptional factor Snail, which are typical components of the mesenchymal cells. Wnt5a mediates its action on vimentin and Snail levels via the PKC activation (Dissanayake et al., 2007). Snail is a transcription factor capable of reducing the E-cadherin expression by binding to E-box elements in the cadherin promoter (Batlle et al., 2000). There is a positive feedback between Wnt5a and PKC. Wnt5a induces production of PKC, and vice versa (Jonsson et al., 1998). Thus, on the one hand, Wnt5a is able to support its own expression level, and on the other, it can affect cell motility through induction of the Snail-mediated E-cadherin repression.

Besides the N-cadherin and vimentin expression, Wnt5a also stimulates expression of the glycosaminoglycan hyaluronan receptor CD44, a tumor cell homing and metastasis-associated gene, and inhibits expression of the metastasis suppressor of melanoma KISS-1 (Dissanayake et al., 2007). CD44 is a promigratory factor. *In vitro* study reveals its ability to enhance melanoma cell invasion in collagen gel (Albini, 1998). Inhibition of the Wnt5a-induced PKC activation significantly reduces the CD44 expression (Dissanayake et al., 2007). Recently it has been shown that Wnt5a can increase the calpain protease-mediated cleavage of the cytoskeletal protein filamin. In the migratory osteoblast cells, filamin can induce filopodia formation. Overexpression of filamin is frequently observed in highly metastatic melanomas. In these cells filamin is distributed in a diffuse manner. Its cleavage is associated with enhanced cell motility (Nomachi et al., 2008; O'Connell et al., 2009). Inhibition of filamin in melanoma decreases cell migration. Calpain is a Ca²⁺-sensitive protein. Thus, Wnt5a promotes melanoma metastasis by induction of Ca²⁺ release and subsequent activation of calpain and calpain-mediated filamin cleavage (O'Connell et al., 2009).

Another mechanism underlying melanoma metastasis is associated with alterations in cell adhesion. In the case of melanoma, adhesion predicts a less favorable outcome for patients. Many cell adhesion molecules are upregulated during tumor progression (O'Connell & Weeraratna, 2009). There is a notion explaining a positive correlation between enhanced adhesion and invasion. It is considered that for efficient tumor dissemination cancer cells

must bind to endothelial cells (Albini, 1998; Cardones et al., 2003). Activated endothelial cells expose numerous adhesion molecules (VCAM-1, ICAM-1, E-selectin) on their surfaces (Chirivi et al., 1996). These adhesion molecules present good binding partners for cancer cell receptors. Cancer cells can both promote endothelial cells activation by production of IL-1 α and enhance expression of its receptors for interaction with endothelial cells (Albini, 1998). Thus, melanoma is pushed towards invasion by enhanced adhesion. β 1 integrins promote the CXCR4-mediated interaction between tumor and endothelial cells (Cardones et al., 2003). And α 3 β 1 integrin has been shown to provide migration and invasion of several melanoma cell lines (Melchiori et al., 1995). Wnt5a overexpression can lead to increase in substrate adhesion (Weeraratna et al., 2002). Moreover, melanoma cells exposed to the CXCL12 chemokine gradient demonstrate redistribution of the melanoma cell adhesion molecules (MCAM) into a polarized structure. And this process is controlled by the Wnt5a signaling (Witze et al., 2008).

At last, the Wnt5a signaling has been shown to modulate immunogenicity of melanoma cells. The melanoma-associated antigens important for the cytotoxic T lymphocyte (CTL) response include MART-1, DCT, TYRP-1 (GP75) and GP100 (SILV). All of them are regulated by the transcription factors Sox10, Pax3 and MITF. Wnt5a can decrease melanosomal antigen expression by activation of PKC and STAT3 (Dissanayake et al., 2008). It is believed that PKC stimulated by Wnt5a is implicated in the STAT3 phosphorylation (Gartsbein et al., 2006; Sheldahl et al., 1999). Phosphorylated STAT3 is active and can reduce the Pax3 expression (Kamaraju et al., 2002). Inhibition of the Pax3 expression in turn results in the MITF repression and subsequent MART-1 silencing. Treatment of melanoma cells with phorbol ester has the same effect as the Wnt5a stimulation, and in the presence of STAT3 or PKC inhibitors, Wnt5a loses its ability to decrease the MART-1 expression (Dissanayake et al., 2008). Ratio of the Wnt5a-positive to the MART-1-negative tumors is increasing dramatically in metastasis (Dissanayake et al., 2008). Initially, it was demonstrated that melanoma patients could be separated into several cohorts according to their Wnt5a and MITF status (Hoek et al., 2006). A cohort with the high Wnt5a and low MITF expression has a weak proliferative, but a high metastatic potential. And cohort with the low Wnt5a and high MITF demonstrates opposite features: a high ability to proliferate and a low ability to metastasize. It is useful to remember that MITF is considered as a target of the Wnt/ β -catenin signaling. And loss of the nuclear transcriptionally active β -catenin is frequently observed at the advanced stages of melanoma. Thus, an opposite correlation between the canonical and noncanonical Wnt signaling functions in melanomas can be revealed. During metastasis, melanoma cells prefer to downregulate their antigen expression in order to escape from immune surveillance of the tumors. The evidence for the notion comes from the experiment with MART-1 positive/negative cells presented to cytotoxic T-cells. The cytotoxic T-lymphocytes could be activated by melanoma cells expressing MART-1. Treatment of these cells with the recombinant Wnt5a abrogates their ability to activate the cytotoxic T-cells and makes them more resistant to cytolysis. The opposite is also true. Silencing of Wnt5a by siRNA in Wnt5a high-leveled cells enhances the MART-1 expression and susceptibility to the T-cells-mediated cytolysis (Dissanayake et al., 2008).

3. Conclusion

The Wnt signaling pathways compose a really complex network both in melanocytes and in melanoma. Multiple receptors, coreceptors, inhibitors and agonists create a cellular context

that can vary significantly in benign nevi and tumors. The context determines a kind of a response to the Wnt ligand stimulation and gives a key to understanding why the canonical and noncanonical Wnt signaling cascades can act both as oncogenic and tumor suppressor factors. The Wnt5a and β -catenin signaling cascades play opposite roles in melanomagenesis. The canonical Wnt signaling is critical at the early stages of tumor development, but in advanced melanoma it serves as a tumor suppressor by promoting more differentiated phenotype. The Wnt5a signaling pathway does not participate in melanoma formation. On the contrary, it can control the canonical signaling preventing its aberrant activation. However, overexpression of Wnt5a is often associated with aggressive melanoma phenotype and is believed to promote metastasis. Thus, normally melanocyte homeostasis is maintained by tightly regulated system, and aberrations in the system regulation result in melanomagenesis.

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5. References

- Albini, A. (1998). Tumor and endothelial cell invasion of basement membranes. *Pathol Oncol Res*, Vol. 4, No. 3, (n.d.), pp. (230-241), ISSN 1532-2807
- Angers, S., & Moon, R.T. (2009). Proximal events in Wnt signal transduction. *Nature*, Vol. 10, No. 7, (July 2009), pp. (468-477), ISSN 0028-0836
- Bachmann, I.M., Straume, O., Puntervoll, H.E., Kalvenes, M.B., & Akslen, L.A. (2005). Importance of P-cadherin, beta-catenin, and Wnt5a/frizzled for progression of melanocytic tumors and prognosis in cutaneous melanoma. *Clin Cancer Res*, Vol. 11, No. 24, (December 2005), pp. (8606-8614), ISSN 1557-3265
- Batlle, E., Sancho, E., Francí, C., Domínguez, D., Monfar, M., Baulida, J., & García De Herreros, A. (2000). The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol*, Vol. 2, No. 2, (February 2000), pp. (84-89), ISSN 1465-7392
- Behrens, J., von Kries, J. P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R., & Birchmeier, W. (1996). Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature*, Vol. 382, No. 6592, (August 1996), pp. (638-642), ISSN 0028-0836
- Bellei, B., Flori, E., Izzo, E., Maresca, V., & Picardo, M. (2008). GSK3beta inhibition promotes melanogenesis in mouse B16 melanoma cells and normal human melanocytes. *Cell Signal*, Vol. 20, No. 10, (October 2008), pp. (1750-1761), ISSN 1873-3913
- Bilic, J., Huang, Y.L., Davidson, G., Zimmermann, T., Cruciat, C.M., Bienz, M., & Niehrs, C. (2007). Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. *Science*, Vol. 316, No. 5831, (June 2007), pp. (1619-1622), ISSN 0036-8075
- Billiard, J., Way, D.S., Seestaller-Wehr, L.M., Moran, R.A., Mangine, A., & Bodine, P.V. (2005). The orphan receptor tyrosine kinase Ror2 modulates canonical Wnt signaling in osteoblastic cells. *Mol Endocrinol*, Vol. 19, No. 1, (January 2005), pp. (90-101), ISSN 0888-8809

- Bittner, M., Meltzer, P., Chen, Y., Jiang, Y., Seftor, E., Hendrix, M., Radmacher, M., Simon, R., Yakhini, Z., Ben-Dor, A., Sampas, N., Dougherty, E., Wang, E., Marincola, F., Gooden, C., Lueders, J., Glatfelter, A., Pollock, P., Carpten, J., Gillanders, E., Leja, D., Dietrich, K., Beaudry, C., Berens, M., Alberts, D., & Sondak, V. (2000). Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature*, Vol. 406, No. 6795, (August 2000), pp. (536–540), ISSN 0028-0836
- Brabletz, T., Jung, A., Dag, S., Hlubek, F., & Kirchner, T. (1999). β -catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer, *Am J Pathol*, Vol. 155, No. 4, (October 1999), pp. (1033–1038), ISSN 1525-2191
- Brantjes, H., Barker, N., van Es, J., & Clevers, H. (2002). TCF: Lady Justice casting the final verdict on the outcome of Wnt signalling. *Biol Chem*, Vol. 383, No. 2, (February 2002), pp. (255–261), ISSN 0021-9258
- Cadigan, K.M., & Peifer, M. (2009). Wnt signaling from development to disease: insights from model systems. *Cold Spring Harb Perspect Biol*, Vol. 1, No. 2, (August 2009), a002881, ISSN 1943-0264
- Campisi, J. (2005). Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell*, Vol. 120, No. 4, (February 2005), pp. (513–522), ISSN 0092-8674
- Cardones, A.R., Murakami, T., & Hwang, S.T. (2003). CXCR4 enhances adhesion of B16 tumor cells to endothelial cells in vitro and in vivo via beta(1) integrin. *Cancer Res*, Vol. 63, No. 20, (October 2003), pp. (6751–6757)
- Carreira, S., Goodall, J., Aksan, I., La Rocca, S.A., Galibert, M.D., Denat, L., Larue, L., & Goding, C.R. (2005). Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression. *Nature*, Vol. 433, No. 7027, (February 2005), pp. (764–769), ISSN 0028-0836
- Carreira, S., Goodall, J., Denat, L., Rodriguez, M., Nuciforo, P., Hoek, K.S., Testori, A., Larue, L., & Goding, C.R. (2006). Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. *Genes & Dev*, Vol. 20, No. 24, (December 2006), pp. (3426–3439), ISSN 0890-9369
- Cheon, S., Poon, R., Yu, C., Khoury, M., Shenker, R., Fish, J., & Alman, B.A. (2005). Prolonged beta-catenin stabilization and tcf-dependent transcriptional activation in hyperplastic cutaneous wounds. *Lab Invest*, Vol. 85, No. 3, (March 2005), pp. (416–425), ISSN 0023-6837
- Chien, A.J., Moore, E.C., Lonsdorf, A.S., Kulikaukas, R.M., Rothberg, B.G., Berger, A.J., Major, M.B., Hwang, S.T., Rimm, D.L., & Moon, R.T. (2009). Activated Wnt/beta-catenin signaling in melanoma is associated with decreased proliferation in patient tumors and a murine melanoma model. *Proc Natl Acad Sci USA*, Vol. 106, No. 4, (January 2009), pp. (1193–1198)
- Chirivi, R.G., Chiodoni, C., Musiani, P., Garofalo, A., Bernasconi, S., Colombo, M.P., & Giavazzi, R. (1996). IL-1 α genetransfected human melanoma cells increase tumor-cell adhesion to endothelial cells and their retention in the lung of nude mice, *Int J Cancer*, Vol. 67, No. 6, (September), pp. (856–863), ISSN 0020-7136
- Choi, S.C., & Han, J.K. (2002). Xenopus Cdc42 regulates convergent extension movements during gastrulation through Wnt/Ca²⁺ -signaling pathway. *Dev Biol*, Vol. 244, No. 2, (April 2002), pp. (342–357), ISSN 0012-1606

- Clark, E.A., Golub, T.R., Lander, E.S., & Hynes, R.O. (2000). Genomic analysis of metastasis reveals an essential role for RhoC. *Nature*, Vol. 406, No. 6795, (August 2000), pp. (532–535), ISSN 0028-0836
- Clevers, H. (2006). Wnt/ β -catenin signaling in development and disease. *Cell*, Vol. 127, No. 3, (November 2006), pp. (469–480), ISSN 0092-8674
- Cliffe, A., Hamada, F., & Bienz, M. (2003). A role of Dishevelled in relocating Axin to the plasma membrane during wingless signaling. *Curr Biol*, Vol. 13, No. 11, (May 2003), pp. (960–966), ISSN 0960-9822
- Cowin, P. (1994). Unraveling the cytoplasmic interactions of the cadherin superfamily. *Proc. Natl Acad Sci USA*, Vol. 91, No. 23, (November 1994), pp. (10759–10761)
- Da Forno, P.D., Pringle, J.H., Hutchinson, P., Osborn, J., Huang, Q., Potter, L., Hancox, R.A., Fletcher, A., & Saldanha, G.S. (2008). WNT5A expression increases during melanoma progression and correlates with outcome. *Clin Cancer Res*, Vol. 14, No. 18, (September 2008), pp. (5825–5832), ISSN 1557-3265
- Dang, C.V. (1999). c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol Cell Biol*, Vol. 19, No. 1, (January 1999), pp. (1–11), ISSN 0270-7306
- De Calisto, J., Araya, C., Marchant, L., Riaz, C.F., & Mayor, R. (2005). Essential role of non-canonical Wnt signalling in neural crest migration. *Development*, Vol. 132, No. 11, (June 2005), pp. (2587–2597), ISSN 0950-1991
- Delmas, V., Beermann, F., Martinozzi, S., Carreira, S., Ackermann, J., Kumasaka, M., Denat, L., Goodall, J., Luciani, F., Viros, A., Demirkan, N., Bastian, B.C., Goding, C.R., & Larue, L. (2007). Beta-catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma development. *Genes Dev*, Vol. 21, No. 22, (November 2007), pp. (2923–2935), ISSN 0890-9369
- Dissanayake, S.K., Olkhanud, P.B., O'Connell, M.P., Carter, A., French, A.D., Camilli, T.C., Emeche, C.D., Hewitt, K.J., Rosenthal, D.T., Leotlela, P.D., Wade, M.S., Yang, S.W., Brant, L., Nickoloff, B.J., Messina, J.L., Biragyn, A., Hoek, K.S., Taub, D.D., Longo, D.L., Sondak, V.K., Hewitt, S.M., & Weeraratna, A.T. (2008). Wnt5A regulates expression of tumor-associated antigens in melanoma via changes in signal transducers and activators of transcription 3 phosphorylation. *Cancer Res*, Vol. 68, No. 24, (December 2008), pp. (10205–10214)
- Dissanayake, S.K., Wade, M., Johnson, C.E., O'Connell, M.P., Leotlela, P.D., French, A.D., Shah, K.V., Hewitt, K.J., Rosenthal, D.T., Indig, F.E., Jiang, Y., Nickoloff, B.J., Taub, D.D., Trent, J.M., Moon, R.T., Bittner, M., & Weeraratna, A.T. (2007). The Wnt5A/protein kinase C pathway mediates motility in melanoma cells via the inhibition of metastasis suppressors and initiation of an epithelial to mesenchymal transition. *J Biol Chem*, Vol. 282, No. 23, (June 2007), pp. (17259–17271), ISSN 0021-9258
- Dorsky, R., Moon, R., & Raible, D. (1998). Control of neural crest cell fate by the Wnt signalling pathway. *Nature*, Vol. 396, No. 6709, (November 1998), pp. (370–373), 9845073
- Fang, D., Leishear, K., Nguyen, T.K., Finko, R., Cai, K., Fukunaga, M., Li, L., Brafford, P.A., Kulp, A.N., Xu, X., Smalley, K.S., & Herlyn, M. (2006). Defining the conditions for the generation of melanocytes from human embryonic stem cells. *Stem Cells*, Vol. 24, No. 7, (July 2006), pp. (1668–1677)

- Fathke, C., Wilson, L., Shah, K., Kim, B., Hocking, A., Moon, R., & Isik, F. (2006). Wnt signaling induces epithelial differentiation during cutaneous wound healing. *BMC Cell Biology*, 7:4, (January 2006), ISSN 1471-2121
- García-Castro, M.I., Marcelle, C., & Bronner-Fraser, M. (2002). Ectodermal Wnt function as a neural crest inducer. *Science*, Vol. 297, No. 5582, (August 2002), pp. (848-851), ISSN 0036-8075
- Gartsbein, M., Alt, A., Hashimoto, K., Nakajima, K., Kuroki, T., & Tennenbaum, T. (2006). The role of protein kinase C delta activation and STAT3 Ser727 phosphorylation in insulin-induced keratinocyte proliferation. *J Cell Sci*, Vol. 119, No. Pt3, (February 2006), pp. (470-481), ISSN 0021-9533
- Giles, R.H., van Es, J.H., & Clevers, H. (2003). Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim Biophys Acta*, Vol. 1653, No. 1, (June 2003), pp. (1-24)
- Glinka, A., Wu, W., Delius, H., Monaghan, A.P., Blumenstock, C., & Niehrs, C. (1998). Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature*, Vol. 391, No. 6665, (January 1998), pp. (357-362), ISSN 0028-0836
- Govindarajan, B., Sligh, J.E., Vincent, B.J., Li, M., Canter, J.A., Nickoloff, B.J., Rodenburg, R.J., Smeitink, J.A., Oberley, L., Zhang, Y., Slingerland, J., Arnold, R.S., Lambeth, J.D., Cohen, C., Hilenski, L., Griendling, K., Martínez-Diez, M., Cuezva, J.M., & Arbiser, J.L. (2007). Overexpression of Akt converts radial growth melanoma to vertical growth melanoma. *Clin Invest*, Vol. 117, No. 3, (March 2007), pp. (719-729), ISSN 0021-9738
- Gradl, D., Kühl, M. & Wedlich, D. (1999). The Wnt/Wg signal transducer beta-catenin controls fibronectin expression. *Mol Cell Biol*, Vol. 19, No. 8, (August 1999), pp. (5576-5587), ISSN 0270-7306
- Gray-Schopfer, V.C., da Rocha Dias, S., & Marais, R.. (2005). The role of B-RAF in melanoma. *Cancer Metastasis Rev*, Vol. 24, No. 1, (January 2005), pp. (165-183), ISSN 0167-7659
- Gumbiner, B.M. (1993). Proteins associated with the cytoplasmic surface of adhesion molecules. *Neuron*, Vol. 11, No. 4, (October 1993), pp. (551-564), ISSN 0896-6273
- Haass, N.K., Smalley, K.S., Li, L., & Herlyn, M. (2005). Adhesion, migration and communication in melanocytes and melanoma. *Pigment Cell Res*, Vol. 18, No. 3, (June 2005), pp. (150-159), ISSN 0893-5785
- Habas, R., Dawid, I.B., & He, X. (2003). Coactivation of Rac and Rho by Wnt/Frizzled signaling is required for vertebrate gastrulation. *Genes Dev*, Vol. 17, No. 2, (January 2003), pp. (295-309), ISSN 0890-9369
- Habas, R., Kato, Y., & He, X. (2001). Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1. *Cell*, Vol. 107, No. 7, (December 2001), pp. (843-854), ISSN 0092-8674
- Halford, M.M., & S.A. Stacker. (2001). Revelations of the RYK receptor. *Bioessays*, Vol. 23, No. 1, (January 2001), pp. (34-45), ISSN 0265-9247
- Halford, M.M., Oates, A.C., Hibbs, M.L., Wilks, A.F. & Stacker, S.A. (1999). Genomic structure and expression of the mouse growth factor receptor related to tyrosine kinases (Ryk). *J Biol Chem*, Vol. 274, No. 11, (March 1999), pp. (7379-7390), ISSN 0021-9258
- Haqq, C., Nosrati, M., Sudilovsky, D., Crothers, J., Khodabakhsh, D., Pulliam, B.L., Federman, S., Miller, J.R. 3rd, Allen, R.E., Singer, M.I., Leong, S.P., Ljung, B.M., Sagebiel, R.W., & Kashani-Sabet, M. (2005). The gene expression signatures of

- melanoma progression. *Proc Natl Acad Sci USA*, Vol. 102, No. 17, (April 2005), pp. (6092–6097)
- Hari, L., Brault, V., Kleber, M., Lee, H.Y., Ille, F., Leimeroth, R., Paratore, C., Suter, U., Kemler, R., & Sommer, L. (2002). Lineage-specific requirements of beta-catenin in neural crest development. *J Cell Biol*, Vol. 159, No. 5, (December 2002), pp. (867–880), ISSN 0021-9525
- He, T.C., Chan, T.A., Vogelstein, B., & Kinzler, K.W. (1999). PPARdelta is an APC-regulated target of nonsteroidal anti-inflammatory drugs. *Cell*, Vol. 99, No. 3, (October 1999), pp. (335–345), ISSN 0092-8674
- Hoek, K.S., Schlegel, N.C., Brafford, P., Sucker, A., Ugurel, S., Kumar, R., Weber, B.L., Nathanson, K.L., Phillips, D.J., Herlyn, M., Schadendorf, D., & Dummer, R. (2006). Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. *Pigment Cell Res*, Vol. 19, No. 4, (August 2006), pp. (290–302), ISSN 0893-5785
- Hovens, C.M., Stacker, S.A., Andres, A.C., Harpur, A.G., Ziemiecki, A. & Wilks, A. F. (1992). RYK, a receptor tyrosine kinase-related molecule with unusual kinase domain motifs. *Proc Natl Acad Sci USA*, Vol. 89, No. 24, (December 1992), pp. (11818–11822)
- Hsu, M.Y., Meier, F., & Herlyn, M. (2002). Melanoma development and progression: a conspiracy between tumor and host. *Differentiation*, Vol. 70, No. 9-10, (December 2002), pp. (522–536), ISSN 0301-4681
- Huang, X., & Saint-Jeannet, J.P. (2004). Induction of the neural crest and the opportunities of life on the edge. *Dev Biol*, Vol. 275, No. 1, (November 2004), pp. (1–11), ISSN 0012-1606
- Ikeya, M., Lee, S.M., Johnson, J.E., McMahon, A.P., & Takada, S. (1997). Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature*, Vol. 389, No. 6654, (October 1997), pp. (966–970), ISSN 0028-0836
- Itasaki, N., Jones, C.M., Mercurio, S., Rowe, A., Domingos, P.M., Smith, J.C., & Krumlauf, R. (2003). Wise, a context-dependent activator and inhibitor of Wnt signaling. *Development*, Vol. 130, No. 18, (September 2003), pp. (4295–4305), ISSN 0950-1991
- Iversen, P.L., Arora, V., Acker, A.J., Mason, D.H., & Devi, G.R. (2003). Efficacy of antisense morpholino oligomer targeted to c-myc in prostate cancer xenograft murine model and a Phase I safety study in humans. *Clin Cancer Res*, Vol. 9, No. 7, (July 2003), pp. (2510–2519), ISSN 1557-3265
- Jenny, A., & Mlodzik, M. (2006). Planar cell polarity signaling: a common mechanism for cellular polarization. *Mt Sinai J Med*, Vol. 73, No. 5, (September 2006), pp. (738–750)
- Jin, E.J., Erickson, C.A., Takada, S., & Burrus, L.W. (2001). Wnt and BMP signaling govern lineage segregation of melanocytes in the avian embryo. *Dev Biol*, Vol. 233, No. 1, (May 2001), pp. (22–37), ISSN 0012-1606
- Jönsson, M., Smith, K., & Harris, A.L. (1998). Regulation of Wnt5a expression in human mammary cells by protein kinase C activity and the cytoskeleton. *Br J Cancer*, Vol. 78, No. 4, (August 1998), pp. (430–438), ISSN 0007-0920
- Kageshita, T., Hamby, C.V., Ishihara, T., Matsumoto, K., Saida, T., & Ono, T. (2001). Loss of beta-catenin expression associated with disease progression in malignant melanoma. *Br J Dermatol*, Vol. 145, No. 2, (August 2001), pp. (210–216), ISSN 0007-0963

- Kamaraju, A.K., Bertolotto, C., Chebath, J., & Revel, M. (2002). Pax3 down-regulation and shutoff of melanogenesis in melanoma B16/F10.9 by interleukin-6 receptor signaling. *J Biol Chem*, Vol. 277, No. 17, (April 2002), pp. (15132-15141), ISSN 0021-9258
- Keeble, T.R., Halford, M.M., Seaman, C., Kee, N., Macheda, M., Anderson, R.B., Stacker, S.A., & Cooper, H.M. (2006). The Wnt receptor Ryk is required for Wnt5a-mediated axon guidance on the contralateral side of the corpus callosum. *J Neurosci*, Vol. 26, No. 21, (May 2006), pp. (5840-5848), ISSN 0270-6474
- Keller, R. (2002). Shaping the vertebrate body plan by polarized embryonic cell movements. *Science*, Vol. 298, No. 5600, (December 2002), pp. (1950-1954), ISSN 0036-8075
- Kielhorn, E., Provost, E., Olsen, D., D'Aquila, T.G., Smith, B.L., Camp, R.L., & Rimm, D.L. (2003). Tissue microarray-based analysis shows phospho-beta-catenin expression in malignant melanoma is associated with poor outcome. *Int J Cancer*, Vol. 103, No. 5, (February 2003), pp. (652-656)
- Kohn, A.D., & Moon, R.T. (2005). Wnt and calcium signaling: beta-catenin- independent pathways. *Cell Calcium*, Vol. 38, No. 3-4, (September-October 2005), pp. (439- 446), 16099039
- Kühl, M., Sheldahl, L.C., Park, M., Miller, J.R., & Moon, R.T. (2000). The Wnt/Ca²⁺- pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet*, Vol. 16, No. 7, (June 2000), pp. (279-283), ISSN 0168-9525
- Kulikova, K., Posvyatenko, A., Gnuchev, N., Georgiev, G., Kibardin, & Larin, S. Nuclear β -catenin localization is not sufficient for canonical Wnt signaling activation in human melanoma cell lines. *Molecular Biology*, in press., ISSN 0026-8933
- Kuphal, S., Lodermeier, S., Bataille, F., Schuierer, M., Hoang, B.H., & Bosserhoff, A.K. (2006). Expression of Dickkopf genes is strongly reduced in malignant melanoma. *Oncogene*, Vol. 25, No. 36, (August 2006), pp. (5027-5036), ISSN 0950-9232
- LaBonne, C., & Bronner-Fraser, M. (1998). Neural crest induction in *Xenopus*: evidence for a two-signal model. *Development*, Vol.125, No. 13, (July 1998), pp. (2403-2414), ISSN 0950-1991
- Larue, L. & Delmas, V. (2006). The WNT/ β -catenin pathway in melanoma. *Front. Biosci*, Vol. 11, (January 2006), pp. (733-742), ISSN 1945-0516
- Larue, L., & Beermann, F. (2007). Cutaneous melanoma in genetically modified animals. *Pigment Cell Res*, Vol. 20, No. 6, (December 2007), pp. (485-497), ISSN 0893-5785
- Larue, L., Luciani, F., Kumasaka, M., Champeval, D., Demirkan, N., Bonaventure, J., & Delmas, V. (2009). Bypassing melanocyte senescence by β -catenin: A novel way to promote melanoma. *Pathol Biol (Paris)*, Vol. 57, No.7-8, (November-December 2009), pp. (543-547)
- Lewis, J.L., Bonner, J., Modrell, M., Ragland, J.W., Moon, R.T., Dorsky, R.I., & Raible, D.W. (2004). Reiterated Wnt signaling during zebrafish neural crest development. *Development*, Vol. 131, No. 6, (March 2004), pp. (1299-1308), ISSN 0950-1991
- Li, L., Yuan, H., Xie, W., Mao, J., Caruso, A.M., McMahon, A., Sussman, D.J., & Wu, D. (1999) Dishevelled proteins lead to two signaling pathways. Regulation of LEF-1 and c-Jun N-terminal kinase in mammalian cells. *J Biol Chem*, Vol. 274, No. 1, (January 1999), pp. (129-134), ISSN 0021-9258
- Lin, Y.C., You, L., Xu, Z., He, B., Yang, C.T., Chen, J.K., Mikami, I., Clément, G., Shi, Y., Kuchenbecker, K., Okamoto, J., Kashani-Sabet, M., & Jablons, D.M. (2007). Wnt

- inhibitory factor-1 gene transfer inhibits melanoma cell growth. *Hum Gene Ther*, Vol. 18, No. 4, (April 2007), pp. (379–386), ISSN 1043-0342
- Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G.H., Tan, Y., Zhang, Z., Lin, X., & He, X. (2002). Control of β -catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell*, Vol. 108, No. 6, (March 2002), pp. (837–847), ISSN 0092-8674
- Liu, T., Liu, X., Wang, H., Moon, R.T., & Malbon, C.C. (1999). Activation of rat frizzled-1 promotes Wnt signaling and differentiation of mouse F9 teratocarcinoma cells via pathways that require Galpha(q) and Galpha(o) function, *J Biol Chem*, Vol. 274, No. 47, (November 1999), pp. (33539–33544), ISSN 0021-9258
- Loercher, A.E., Tank, E.M., Delston, R.B., & Harbour, J.W. (2005). MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. *J Cell Biol*, Vol. 168, No. 1, (January 2005), pp.(35–40), ISSN 0021-9525.
- Lu, W., Yamamoto, V., Ortega, B. & Baltimore, D. (2004). Mammalian Ryk is a Wnt coreceptor required for stimulation of neurite outgrowth. *Cell*, Vol. 119, No. 1, (October 2004), pp. (97–108), ISSN 0092-8674
- Lucero, O.M., Dawson, D.W., Moon, R.T., & Chien, A.J. (2010). A re-evaluation of the "oncogenic" nature of Wnt/beta-catenin signaling in melanoma and other cancers. *Curr Oncol Rep*, Vol. 12, No. 5, (September 2010), pp. (314–318), ISSN 1534-6269
- Maelandsmo, G.M., Holm, R., Nesland, J.M., Fodstad, O., & Florenes, V.A. (2003). Reduced betacatenin expression in the cytoplasm of advanced-stage superficial spreading malignant melanoma. *Clin Cancer Res*, Vol. 9, No. 9, (August 2003), pp. (3383–3388), ISSN 1557-3265
- Malbon, C.C., Wang, H., & Moon, R.T. (2001). Wnt signaling and heterotrimeric G-proteins: strange bedfellows or a classic romance? *Biochem Biophys Res Commun*, Vol. 287, No. 3, (September 2001), pp. (589–593), 11563835
- Mann, B., Gelos, M., Siedow, A., Hanski, M.L., Gratchev, A., Ilyas, M., Bodmer, W.F., Moyer, M.P., Riecken, E.O., Buhr, H.J., & Hanski, C. (1999). Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. *Proc Natl Acad Sci USA*, Vol. 96, No. 4, (February 1999), pp. (1603–1608)
- Mao, B., Wu, W., Davidson, G., Marhold, J., Li, M., Mechler, B.M., Delius, H., Hoppe, D., Stannek, P., Walter, C., Glinka, A., & Niehrs, C. (2002). Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. *Nature*, Vol. 417, No. 6889, (June 2002), pp. (664–667), ISSN 0028-0836
- Mao, B., Wu, W., Li, Y., Hoppe, D., Stannek, P., Glinka, A. & Niehrs, C. (2001). LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. *Nature*, Vol. 411, No. 6835, (May 2001), pp. (321–325), ISSN 0028-0836
- Mapelli, E., Banfi, P., Sala, E., Sensi, M., Supino, R., Zunino, F., & Gambetta, R.A. (1994). Effect of protein kinase C inhibitors on invasiveness of human melanoma clones expressing different levels of protein kinase C isoenzymes. *Int J Cancer*, Vol. 57, No. 2, (April 1994), pp. (281–286), ISSN 0020-7136
- Marlow, F., Topczewski, J., Sepich, D., & Solnica-Krezel, L. (2002). Zebrafish Rho kinase 2 acts downstream of Wnt11 to mediate cell polarity and effective convergence and extension movements. *Curr Biol*, Vol. 12, No. 11, (June 2002), pp. (876–884), ISSN 0960-9822

- McGary, E.C., Lev, D.C., & Bar-Eli, M. (2002). Cellular adhesion pathways and metastatic potential of human melanoma.. *Cancer Biol Ther*, Vol. 1, No. 5, (September-October 2002), pp. (459-465), ISSN 1538-4047
- McMahon, A.P. (1992). The Wnt family of developmental regulators, *Trends Genet*, Vol. 8, No. 7, (July 1992), pp. (236-242), ISSN 0168-9525
- Melchiori A., Mortarini R., Carlone S. et al. (1995), The $\alpha 3 \beta 1$ integrin is involved in melanoma cell migration and invasion, *Exp Cell Res*, Vol. 218, No. 1, (July 1995), pp. (233-242), ISSN 0014-4827
- Mikheev, A.M., Mikheeva, S.A., Rostomily, R., & Zarbl, H. (2007). Dickkopf-1 activates cell death in MDA-MB435 melanoma cells. *Biochem Biophys Res Commun*, Vol. 352, No. 3, (January 2007), pp. (675-680), ISSN 0006-291X
- Miller, J.R. (2001). The Wnts. *Genome Biol*, Vol. 3, No. 1, (n. d.), ISSN 474-7596
- Minami, Y., Oishi, I., Endo, M., & Nishita, M. (2010). Ror-family receptor tyrosine kinases in noncanonical Wnt signaling: their implications in developmental morphogenesis and human diseases. *Dev Dyn*, Vol. 239, No. 1, (January 2010), pp. (1-15), ISSN 1058-8388
- Mlodzik, M. (2002). Planar cell polarization: do the same mechanisms regulate *Drosophila* tissue polarity and vertebrate gastrulation? *Trends Genet*, Vol. 18, No. 11, (November 2002), pp. (564-571), ISSN 0168-9525
- Myers, D.C., Sepich, D.S., & Solnica-Krezel, L. (2002). Convergence and extension in vertebrate gastrulae: cell movements according to or in search of identity? *Trends Genet*, Vol. 18, No. 9, (September 2002), pp. (447-455), ISSN 0168-9525
- Nagafuchi, A., & Takeichi, M., (1989). Transmembrane control of cadherin-mediated cell adhesion: a 94 kDa protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. *Cell Regul*, Vol. 1, No. 1, (November 1989), pp. (37-44), ISSN 1044-2030
- Nakahara, H., Otani, T., Sasaki, T., Miura, Y., Takai, Y., & Kogo, M. (2003). Involvement of Cdc42 and Rac small G proteins in invadopodia formation of RPMI7951 cells. *Genes Cells*, Vol. 8, No. 12, (December 2003), pp. (1019-1027), ISSN 1356-9597
- Narita, M., Núñez, S., Heard, E., Narita, M., Lin, A.W., Hearn, S.A., Spector, D.L., Hannon, G.J., & Lowe, S.W. (2003). Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell*, Vol. 113, No. 6, (June 2003), pp. (703-716), ISSN 0092-8674
- Nechiporuk, T. & Vasioukhin, V. (2006). Planar cell polarity planes the inconveniences of cell division into a smooth morphogenetic process. *Dev Cell*, Vol. 10, No. 2, (February 2006), pp. (153-154), ISSN 1534-5807
- Nemeth, M.J., Topol, L., Anderson, S.M., Yang, Y., & Bodine, D.M. (2007). Wnt5a inhibits canonical Wnt signaling in hematopoietic stem cells and enhances repopulation. *Proc Natl Acad Sci USA*, Vol. 104, No. 39, (September 2007), pp. (15436-15441)
- Niehrs, C., & Shen, J. (2010). Regulation of Lrp6 phosphorylation. *Cell Mol Life Sci*, Vol. 67, No. 15, (August 2010), pp. (2551-2562), ISSN 1420-682X
- Nishikawa, S., & Osawa, M. (2007). Generating quiescent stem cells. *Pigment Cell Res*, Vol. 20, No. 4, (August 2007), pp. (263-270), ISSN 0893-5785
- Nishimura, E.K., Jordan, S.A., Oshima, H., Yoshida, H., Osawa, M., Moriyama, M., Jackson, I.J., Barrandon, Y., Miyachi, Y., & Nishikawa, S. (2002). Dominant role of the niche

- in melanocyte stem-cell fate determination, *Nature*, Vol. 416, No. 6883, (April 2002), pp. (854–860), ISSN 0028-0836
- Nomachi, A., Nishita, M., Inaba, D., Enomoto, M., Hamasaki, M., & Minami, Y. (2008). Receptor tyrosine kinase Ror2 mediates Wnt5a-induced polarized cell migration by activating c-Jun N-terminal kinase via actin-binding protein Filamin A, *J Biol Chem*, Vol. 283, No. 41, (October 2008), pp. (27973–27981), ISSN 0021-9258
- O'Connell, M.P., French, A.D., Leotlela, P.D., & Weeraratna, A.T. (2008). Assaying Wnt5A-mediated invasion in melanoma cells. *Methods Mol Biol*, Vol. 468, (n.d.), pp. (243–253), ISSN 1064-3745
- O'Connell, M.P., & Weeraratna, A.T. (2009). Hear the Wnt Ror: how melanoma cells adjust to changes in Wnt. *Pigment Cell Melanoma Res*, Vol. 22, No. 6, (December 2009), pp. (724–739), ISSN 1755-1471
- O'Connell, M.P., Fiori, J.L., Baugher, K.M., Indig, F.E., French, A.D., Camilli, T.C., Frank, B.P., Earley, R., Hoek, K.S., Hasskamp, J.H., Elias, E.G., Taub, D.D., Bernier, M., & Weeraratna, A.T. (2009). Wnt5A activates the calpain-mediated cleavage of Filamin A. *J Invest Dermatol*, Vol. 129, No. 7, (July 2009), pp. (1782–1789), ISSN 0022-202X
- O'Connell, M.P., Fiori, J.L., Xu, M., Carter, A.D., Frank, B.P., Camilli, T.C., French, A.D., Dissanayake, S.K., Indig, F.E., Bernier, M., Taub, D.D., Hewitt, S.M., & Weeraratna, A.T. (2010). The orphan tyrosine kinase receptor, ROR2, mediates Wnt5A signaling in metastatic melanoma. *Oncogene*, Vol. 29, No. 1, (October 2009), pp. (34–44), ISSN 0950-9232
- Oishi, I., Takeuchi, S., Hashimoto, R., Nagabukuro, A., Ueda, T., Liu, Z.J., Hatta, T., Akira, S., Matsuda, Y., Yamamura, H., Otani, H., & Minami, Y. (1999). Spatio-temporally regulated expression of receptor tyrosine kinases, mRor1, mRor2, during mouse development: implications in development and function of the nervous system. *Genes Cells*, Vol. 4, No. 1, (January 1999), pp. (41–56), ISSN 1356-9597
- Ozawa, M., Baribault, H., & Kemler, R. (1989). The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J*, Vol. 8, No. 6, (June 1989), pp. (1711–1717)
- Palmieri, G., Capone, M., Ascierto, M.L., Gentile, G., Stroncek, D.F., Casula, M., Sini, M.C., Palla, M., Mozzillo, N., & Ascierto, P.A. (2009). Main roads to melanoma *J Transl Med*, Vol. 7:86, (October 2009), ISSN 1479-5876
- Parri, M., Taddei, M.L., Bianchini, F., Calorini, L., & Chiarugi, P. (2009). EphA2 reexpression prompts invasion of melanoma cells shifting from mesenchymal to amoeboid-like motility style. *Cancer Res*, Vol. 69, No. 5, (March 2009), pp. (2072–2081)
- Patthy, L (2000). The WIF module. *Trends Biochem Sci*, Vol. 25, No. 1, (January 2000), pp. (12–3), ISSN 0968-0004
- Pham, K., Milovanovic, T., Barr, R.J., Truong, T., & Holcombe, R.F. (2003). Wnt ligand expression in malignant melanoma: Pilot study indicating correlation with histopathological features. *Mol Pathol*, Vol. 56, No. 5, (October 2003), pp. (280–285)
- Pinson, K.I., Brennan, J., Monkley, S., Avery, B.J., & Skarnes, W.C. (2000). An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature*, Vol. 407, No. 6803, (September 2000), pp. 535–538, ISSN 0028-0836
- Pukrop, T., Klemm, F., Hagemann, T., Gradl, D., Schulz, M., Siemes, S., Trümper, L., & Binder, C. (2006). Wnt 5a signaling is critical for macrophage-induced invasion of

- breast cancer cell lines. *Proc Natl Acad Sci USA*, Vol. 103, No. 14, (April 2006), pp. (5454–5459)
- Rubinfeld, B., Robbins, P., El-Gamil, M., Albert, I., Porfiri, E., & Polakis, P. (1997). Stabilization of beta-catenin by genetic defects in melanoma cell lines. *Science*, Vol. 275, No. 5307, (March 1997), pp. (1790–1792), ISSN 0036-8075
- Ryu, B., Kim, D.S., Deluca, A.M., & Alani, R.M. (2007). Comprehensive expression profiling of tumor cell lines identifies molecular signatures of melanoma progression. *PLoS ONE*, Vol. 2, No. 7, (July 2007), pp. (e594)
- Sakai, D., Tanaka, Y., Endo, Y., Osumi, N., Okamoto, H., & Wakamatsu, Y. (2005). Regulation of *Slug* transcription in embryonic ectoderm by β -catenin-Lef/Tcf and BMP-Smad signaling. *Dev Growth Differ*, Vol. 47, No. 7, (September 2005), pp. (471–482)
- Schepsky, A., Bruser, K., Gunnarsson, G.J., Goodall, J., Hallsson, J.H., Goding, C.R., Steingrimsson, E., & Hecht, A. (2006). The microphthalmia-associated transcription factor Mitf interacts with β -catenin to determine target gene expression. *Mol Cell Biol*, Vol. 26, No. 23, (December 2006), pp. (8914–8927), ISSN 0270-7306
- Semenov, M., Tamai, K., & He, X. (2005). SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J Biol Chem*, Vol. 280, No. 29, (July 2005), pp. (26770–26775), ISSN 0021-9258
- Sen, M., Chamorro, M., Reifert, J., Corr, M., & Carson, D.A. (2001). Blockade of Wnt-5A/frizzled 5 signaling inhibits rheumatoid synoviocyte activation. *Arthritis Rheum*, Vol. 44, No. 4, (April 2001), pp. (772–781)
- Sheldahl, L.C., Park, M., Malbon, C.C., & Moon, R.T. (1999). Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. *Curr Biol*, Vol. 9, No. 13, (July 1999), pp. (695–698), ISSN 0960-9822
- Sinnberg, T., Menzel, M., Kaesler, S., Biedermann, T., Sauer, B., Nahnsen, S., Schwarz, M., Garbe, C., & Schitteck, B. (2010). Suppression of casein kinase 1 α in melanoma cells induces a switch in beta-catenin signaling to promote metastasis. *Cancer Res*, Vol. 70, No. 17, (September 2010), pp. (6999–7009)
- Takahashi, Y., Nishikawa, M., Suehara, T., Takiguchi, N., & Takakura, Y. (2008). Gene silencing of b-catenin in melanoma cells retards their growth but promotes the formation of pulmonary metastasis in mice. *Int J Cancer*, Vol. 123, No. 10, (November 2008), pp. (2315–2320)
- Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J.P., & He, X. (2000). LDL-receptor-related proteins in Wnt signal transduction. *Nature*, Vol. 407, No. 6803, (September 2000), pp. (530–535), ISSN 0028-0836
- Tetsu, O., & McCormick, F. (1999). Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature*, Vol. 398, No. 6726, (April 1999), pp. (422–426), ISSN 0028-0836
- Thomas, A.J., & Erickson, C.A. (2009). FOXD3 regulates the lineage switch between neural crest-derived glial cells and pigment cells by repressing MITF through a non-canonical mechanism. *Development*, Vol. 136, No. 11, (June 2009), pp. (1849–1858), ISSN 0950-1991
- Topol, L., Jiang, X., Choi, H., Garrett-Beal, L., Carolan, P.J., & Yang, Y. (2003). Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent b-catenin

- degradation. *J Cell Biol*, Vol. 162, No. 5, (September 2003), pp. (899–908), ISSN 0021-9525
- Tsao, H., Bevona, C., Goggins, W., & Quinn, T. (2003). The transformation rate of moles (melanocytic nevi) into cutaneous melanoma: a population-based estimate. *Arch Dermatol*, Vol. 139, No. 3, (March 2003), pp. (282–288)
- Tsao, H., Zhang, X., Fowlkes, K., & Haluska, F.G. (2000). Relative reciprocity of NRAS and PTEN/MMAC1 alterations in cutaneous melanoma cell lines. *Cancer Res*, Vol. 60, No. 7, (April 2000), pp. (1800–1804)
- Vallin, J., Thuret, R., Giacomello, E., Faraldo, M.M., Thiery, J.P., & Broders, F. (2001). Cloning and characterization of three *Xenopus* slug promoters reveal direct regulation by Lef/ β -catenin signaling. *J Biol Chem*, Vol. 276, No. 32, (August 2001), pp. (30350–30358), ISSN 0021-9258
- Wallingford, J.B., Fraser, S.E., & Harland, R.M. (2002). Convergent extension: the molecular control of polarized cell movement during embryonic development. *Dev Cell*, Vol. 2, No. 6, (June 2002), pp. (695–706), ISSN 1534-5807
- Wang, Y., & Nathans, J. (2007). Tissue/planar cell polarity in vertebrates: new insights and new questions. *Development*, Vol. 134, No. 4, (February 2007), pp. (647–658), ISSN 0950-1991
- Weeraratna, A.T. (2005). A Wnt-er Wonderland - The complexity of Wnt signaling in melanoma. *Cancer and Metastasis Rev*, Vol. 24, No. 2, (June 2005), pp. (237–250), ISSN 0167-7659
- Weeraratna, A.T., Jiang, Y., Hostetter, G., Rosenblatt, K., Duray, P., Bittner, M., & Trent, J.M. (2002). Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell*, Vol. 1, No. 3, (April 2002), pp. (279–288)
- Weiser, D.C., Pyati, U.J., & Kimelman, D. (2007). Gravin regulates mesodermal cell behavior changes required for axis elongation during zebrafish gastrulation. *Genes Dev*, Vol. 21, No. 12, (June 2007), pp. (1559–1571), ISSN 0890-9369
- Wellbrock, C. & Marais, R. (2005). Elevated expression of MITF counteracts B-RAF-stimulated melanocyte and melanoma cell proliferation. *J Cell Biol*, Vol. 170, No. 5, (August 2005), pp. (703–708), ISSN 0021-9525
- Widlund, H.R., Horstmann, M.A., Price, E.R., Cui, J., Lessnick, S.L., Wu, M., He, X., & Fisher, D.E. (2002). Beta-catenin-induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. *J Cell Biol*, Vol. 158, No. 6, (September 2002), pp. (1079–1087), ISSN 0021-9525
- Willert, K., Brown, J.D., Danenberg, E., Duncan, A.W., Weissman, I.L., Reya, T., Yates, J.R. 3rd, & Nusse, R. (2003). Wnt proteins are lipid modified and can act as stem cell growth factors. *Nature*, Vol. 423, No. 6938, (May 2003), pp. (448–452), ISSN 0028-0836
- Witze, E.S., Litman, E.S., Argast, G.M., Moon, R.T., & Ahn, N.G. (2008). Wnt5a control of cell polarity and directional movement by polarized redistribution of adhesion receptors. *Science*, Vol. 320, No. 5874, (April 2008), pp. (365–369), ISSN 0036-8075
- Wnt homepage: <http://www.stanford.edu/group/nusselab/cgi-bin/wnt>
- Wu, J., Yang, J., & Klein, P.S. (2005). Neural crest induction by the canonical Wnt pathway can be dissociated from anterior-posterior neural patterning in *Xenopus*. *Dev Biol*, Vol. 279, No. 1, (March 2005), pp. (220–232)

- Xiao, C., Yang, B.F., Song, J.H., Schulman, H., Li, L., & Hao, C. (2005). Inhibition of CaMKII-mediated c-FLIP expression sensitizes malignant melanoma cells to TRAIL-induced apoptosis. *Exp Cell Res*, Vol. 304, No. 1, (March 2005), pp. (244-255), ISSN 0014-4827
- Yamada, T., Akamatsu, H., Hasegawa, S., Inoue, Y., Date, Y., Mizutani, H., Yamamoto, N., Matsunaga, K., & Nakata, S. (2010). Melanocyte stem cells express receptors for canonical Wnt-signaling pathway on their surface. *Biochem Biophys Res Commun*, Vol. 396, No. 4, (June 2010), pp. (837-842), ISSN 0006-291X
- Yamaguchi, Y., Brenner, M., & Hearing, V.J. (2007). The regulation of skin pigmentation. *J Biol Chem*, Vol. 282, No. 38, (September 2007), pp. (27557-27561), ISSN 0021-9258
- Yamaguchi, Y., Morita, A., Maeda, A., & Hearing, V.J. (2009). Regulation of skin pigmentation and thickness by Dickkopf 1 (DKK1). *J Invest Dermatol Symp Proc*, Vol. 14, No. 1, (August 2009), pp. (73-75), 19675559
- Zallen, J.A. (2007). Planar Polarity and Tissue Morphogenesis. *Cell*, Vol. 129, No. 6, (June 2007), pp. (1051-1063), ISSN 0092-8674

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The book *Research on Melanoma: A Glimpse into Current Directions and Future Trends*, is divided into sections to represent the most cutting-edge topics in melanoma from around the world. The emerging epigenetics of disease, novel therapeutics under development and the molecular signaling aberrations are explained in detail. Since there are a number of areas in which unknowns exist surrounding the complex development of melanoma and its response to therapy, this book illuminates and comprehensively discusses such aspects. It is relevant for teaching the novice researcher who wants to initiate projects in melanoma and the more senior researcher seeking to polish their existing knowledge in this area. Many chapters include visuals and illustrations designed to easily guide the reader through the ideas presented.

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