WNT SIGNALING PATHWAY AND ITS SIGNIFICANCE FOR MELANOMA DEVELOPMENT

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Melanoma is characterized by its high metastatic propensity. Melanoma metastasis is associated with an activation of signaling pathways that are responsible for embryogenesis. Wnt signaling pathway is considered as one of the key signaling cascades, whose aberrant activation results in melanoma development. Wnt signaling includes a complex network of intracellular interactions. Its ligands are able to initiate at least three signal transduction pathways: canonical and two noncanonical. According to modern views, canonical branch of Wnt signaling pathway is involved in cell proliferation and differentiation. Noncanonical Wnt signaling pathways, on the contrary, control cytoskeleton organization and cell motility. Currently, canonical and noncanonical Wnt signaling cascades are supposed to affect different stages of tumor progression. Canonical Wnt signaling pathway contributes to melanoma formation, while noncanonical branches of Wnt signal transduction are involved in metastasis.

Key words: Wnt signaling pathway, melanoma, metastasis, β-catenin.

Ligands and receptors of Wnt signaling pathway

Genes encoding Wnt family proteins are identifies both in vertebrates and invertebrates [1]. In human Wnt family has 19 representatives. All Wnt proteins are highly modified glycolproteins, 40 kDa in size, with the properties typical characteristic of secreting growth factors [2]. Posttranslational lipid modifications are believed to be absolutely essential for their biological activity. Enzyme treatment removing the residual palmic acid decreases hydrophilic property and signal activity of Wnt ligands [3].

For signaling inside a cell, Wnt family proteins should bind an appropriate receptor or a receptor group on a cell surface. Frizzled (Fz) family receptors have been discovered first among a variety of transmembrane molecules acting as receptors for Wnt-ligands. Fz proteins are referred to a large group of receptors bound to G-proteins, or GPCR (G-protein-coupled receptors). They transmit a signal inside a cell by affecting heterotrimeric G-proteins [4].

In addition to Fz-receptors, on a cell surface there are other proteins able to accept Wnt ligands, and among them — a family of LRP receptor molecules (LDL-receptor-related protein). LRP proteins have a single transmembrane domain that provides Wnt-signaling into a cell [5]. In vertebrates there are two LRP family members (LRP5 and LRP6) able to bind Wnt proteins. Phenotypes of knockout by *LRP5/6* mice imitate the effects of silencing some Wnt gene expression. For example, silencing of *LRP6* expression leads to the disturbance of development of middle and posterior brain (imitation of *Wnt1* blocking), displacement of limbs in ventral direction (*Wnt7a*) and nerve tissue volume expansion (*Wnt3a*) [6]. LRP5/6 is considered to be a participant of canonical Wnt signaling pathway [5].

Signaling from Wnr ligands can be performed by alternative receptors Ror1, Ror2 and Ryk. Ror1 and Ror2 are considered as coreceptors of noncanonical Wnt signaling pathway. Being structurally close homologues, they belong to the family of RTK (receptor tyrosine kinase) [7]. Transgenic mice defect in *Ror2* gene demonstrate a phenotype similar to *Wnt5a*-deletion mutants [8].

Ryk protein is atypical receptor tyrosine kinase, deficient in the ability to phosphorylate proteins. The lack of kinase activity is the consequence of amino-acid replacement in recalcitrant areas of intracellular kinase

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domain [9]. According to various researches, Ryk can bind both canonical and noncanonical Wnt ligands. Physical interaction between Ryk, Wnt1 and Wnt3a with further activation of canonical Wnt signaling pathway has been shown in cell line HEK293T [10]. In the process of neural binding formation in the growing brain, Ryk serves as a receptor for a ligand of noncanonical Wnt signaling pathway Wnt5a [11].

Signaling pathways activated by Wnt family proteins

According to classical concepts, the combination of Wnt, its receptor and coreceptor determines the type of signal cascade triggered. Currently, there are distinguished three signal cascades activated by Wnt family proteins, among them: one canonical, or β-catenin signaling pathway, and two noncanonical: Wnt/Ca^{2+}-signaling pathway and pathway of cell polarization (PCP) (See Fig.).

Canonical Wnt signaling pathway

Canonical Wnt signaling pathway among signal cascades activated by Wnt family proteins is studied comprehensively. It is used in a wide range of biological processes beginning with embryogenesis and ending with tissue regeneration in adults. On cellular level, canonical Wnt signaling pathway is involved in the regulation of proliferation and differentiation, as well as in the maintenance of stem cell population [12].

The key event in the activation of canonical Wnt signal cascade is β-catenin stabilization, so this signaling pathway is also called β-catenin signaling pathway. In the absence of an activating signal, β-catenin concentration in a nucleus and cytoplasm is supported on a relatively low level. It is achieved by a special protein complex of "destruction" including proteins Axin and APC (adenomatous polyposis coli) and protein kinase GSK-3β (glycogen synthase kinase 3β). As a part of the complex, β-catenin is phosphorylated that is a signal for its further degradation [13].

The activation of canonical Wnt signaling pathway is associated with the formation of a triple complex consisting of Wnt-ligand, Fz family receptor, and LRP5/6 coreceptor [5]. The triple complex formation results in the translocation of a number of proteins on membrane, among which there are Dishevelled (Dvl), Axin and GSK-3β, the degradation of "destruction" complex, and β-catenin phosphorylation inhibition [14]. Stabilized β-catenin is accumulated in cytoplasm, then is translocated in a nucleus, where acts as coactivator of TCF/LEF-depended genes transcription [13] (See Fig.).

Wnt/Ca^{2+}-signaling pathway

Ca^{2+}-dependent signaling pathway activated by Wnt proteins includes a chain of events related to the release of Ca^{2+} ions from intracellular storage. The recognition of the receptor by Wnt ligand results in the dissociation of heterotrimeric G-protein into Gα- and Gβ/γ-subunits. Free Gβ/γ complex is able to activate phospholipase C (PLC) that is translocated on membrane and hydrolyzes phosphotidilinositol(4,5)-biphosphates (PIP2) up to inisitol(1,4,5)-triphosphates (IP3) and diacylglicerol (DAG). DAG activates PKC kinase, while IP3 induces the release of Ca^{2+} ions from intracellular depots. The increase in Ca^{2+}cytoplasm level, in its turn, promotes Ca^{2+}-dependent effector molecules6 among them: Ca^{2+}/calmodulin-dependent kinase II (CaMKII), nuclear factor associated with T-cells (NFAT), and calcineurin [15] (See Fig.). Wnt/Ca^{2+}-pathway is supposed to be used mainly in the regulation of the organization of cytoskeleton and cell motility [16].

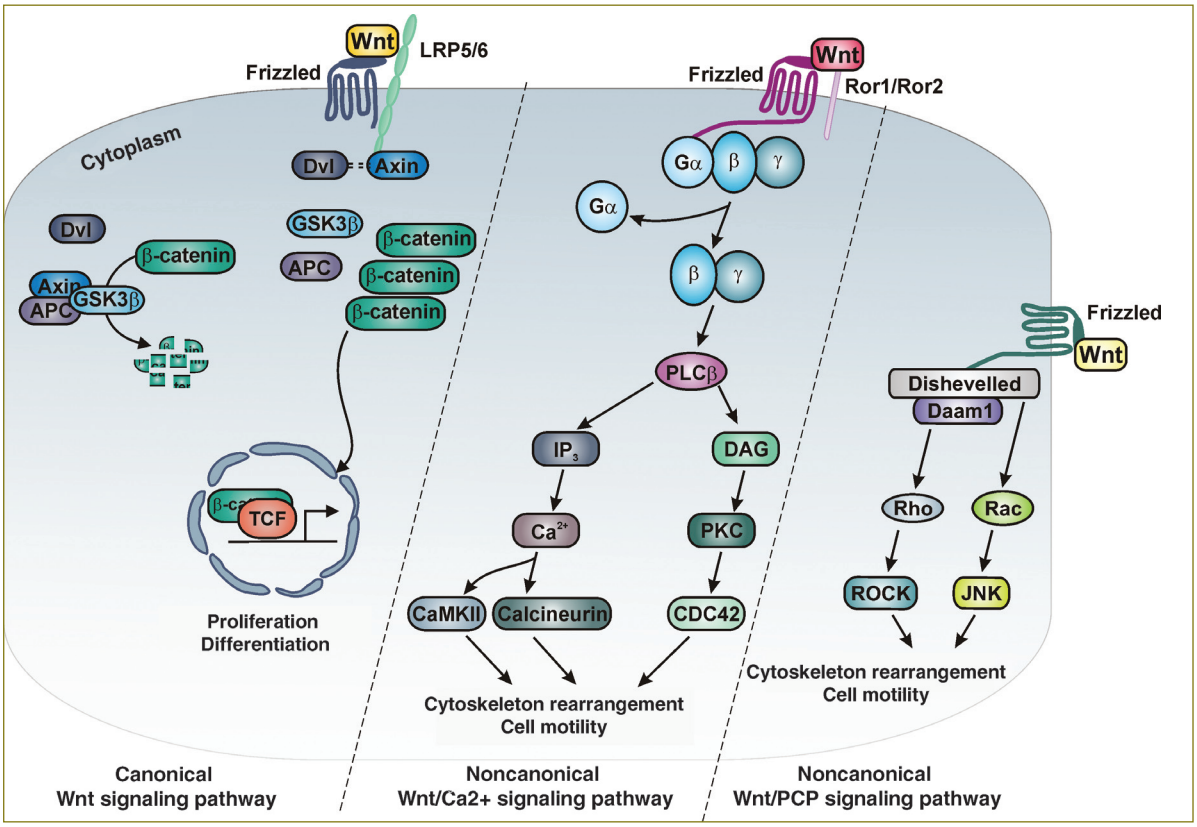
Pathway of cell polarization

Polarity is considered to be an important feature of living organisms. Asymmetric organization of components underlies the correct functioning of many cell systems. Several polarity types are known. In addition to the polarization of cells in apical basolateral direction, there is also polarization in simple epithelium plane. This type of cell organization was originally called tissue polarity, and later was renamed as planar cell polarity (PCP). The example of PCP in insects, such as *Drosophila melanogaster*, is a certain direction of hair inclination on wings [17]. PCP in mammals controls the orientation of stereocilia bundles located on apical side of hair cells of auditory sensory organs [18].

To provide and maintain planar cell polarity, noncanonical Wnt/PCP-signaling pathway is used. It is stimulated under the effect of ligands Wnt7a and Wnt11. Wnt/PCP-signaling pathway controls the activity of small GTP-ases: Rac and Rho [19]. Rho-dependent branch of signal cascade is related to the activation of myosin and Rho-associated kinase ROCK (Rho-associated kinase). The activation results from the formation of Dvl-Daam-1 (Dishevelled-associated activator of morphogenesis 1) complex. Daam-1 enters the family of phormin proteins participating in actin polymerization [20]. Acting as structural protein, it mediates the formation of Dvl with RhoA complex [21]. Rac-dependent signal cascade is associated with induction of kinase activity of JNK [22, 23]. In contrast to Rho-dependent, it does not require the participation of Daam-1, as small GTP-ase, Rac is able to interact with Dvl directly [24] (See Fig.). The path of cell polarization is involved in the regulation of modification of actin cytoskeleton structures, and thereby exercises a significant influence on polarization and motility of cells [13].

Wnt signaling pathway: the significance for embryogenesis

In the process of embryogenesis the components of Wnt signaling pathway provide the regulation of formation and differentiation of neural crest cells [25]. Neural crest cells originate from neural folds located in the border of neural plate and non-neural ectoderm. In the course of neurulation, neural folds drift shut in the area of dorsal medial line of embryo giving rise to neural tube. The removal of β-catenin, the main effector of canonical Wnt signaling pathway, disturbs the process of neuron crest induction [26]. Promote site of genetic marker of neural crest *Slug* has the binding site for a complex LEF/β-catenin [27]. The fate of



Signaling pathways activated by Wnt family proteins. Explanatory notes to a figure are in the text

neural crest cells is also controlled by the components of Wnt signaling pathway. A number of studies [28, 29] show Wnt6 and Wnt8 to be important both for induction, and for expansion of neural crest. After neurulation, a part of neural crest cells located on dorsal side of neural tube migrates into peripheral sites. This migration is strictly controlled on molecular level. Neural crest induction is believed to be under control of canonical Wnt signaling pathway, while noncanonical Wnt/PCP-signaling pathway participates predominantly in the migration process. Activity inhibition of such components of this signaling pathway as Wnt11, Fz7 and Dvl leads to the failure of neural crest cells motility indicating the importance of noncanonical Wnt-signaling pathway for migration of these cells *in vivo* [30].

Neural crest cells give rise to a variety of various derivatives including neurons, glia, and melanocytes. Wnt signaling pathway is considered to be used in the specification of neural crest cells. Double *Wnt1* and *Wnt3a* knockout mice are deprived of a number of derivative neural crest cells including melanocytes [31].

Wnt signaling pathway and tissue regeneration

Mammalian skin performs a wide variety of functions, and one of them is the protection of environment. Barrier function can be damaged if there are any skin disorders of any character. Therefore, early healing of wounds and restoration of skin integrity is of great importance for body homeostasis maintenance as a whole. Some factors indicate Wnt signal cascade to be involved in this process.

The study of gene expression of Wnt ligands at various intervals after wounding has shown *Wnt4* to start expressing among the first ones, while the maximum of gene transcription activity of *Wnt5a* and *Wnt11* fall within the period of wound remodeling [32]. Experiments on transgenic mice have shown the activity of canonical Wnt signaling pathway to be increased in hair follicles adjacent to the damage area but not in the wound itself. Moreover, the production of stabilized form of β-catenin promotes the formation of epithelial processes including hair follicles and oil glands in the damage area. The treatment of the wound by retroviral vector having gene of Wnt5a ligand results in more effective formation of skin processes in the wound than in the case of β-catenin stabilization [32].

Thus, both canonical, and noncanonical Wnt signaling pathway is able to direct progenitor cells in skin of adult organism to regenerate tissues.

Wnt signaling pathway and melanoma

Formation of melanoma. Skin melanoma is considered as malignancy originating from neural crest cells or transformed pigment-producing cells — melanocytes. Melanoma development is a multistage process [33]. The components of Wnt signal cascades to participate in all the stages of melanoma progressing.

Canonical Wnt signaling pathway is activated approximately in one third of cases of melanoma [34]. Taking into account that signal cascade with β-catenin controls the transcription of genes the products of which are used in the

processes of growth and development (e.g., cyclin D and c-myc), constitutive activation of the cascade potentially can promote tumour development. For example, *c-myc* is well known oncogene. Its superexpression is characteristic of many types of oncological diseases including bowel cancer, breast cancer, leukemia, and melanoma [35].

In melanoma, the expression of genes of negative regulators of canonical Wnt signaling pathway is frequently inhibited. For instance, the production of Dkk-1, 2 and 3 that inhibit β-catenin signal cascade by binding with coreceptor LRP5/6 is strongly reduced or lost both in melanoma cell lines, and tumour samples [36]. Dkk-1 can suppress melanocytes growth [37]. Active suppression of *Dkk-1* gene results in oncogenicity reduction and the induction of apoptosis of melanoma cells in nude mice *in vivo* [38]. Another inhibitor of Wnt signaling pathway is WIF-1 (Wnt inhibitory factor-1), its production is suppressed in melanomas [39]. In contrast to Dkk, WIF-1 is bound directly with Wnt ligands blocking their signal activity. Melanoma cells growth is known to be inhibited in *WIF-1* gene superexpression. Moreover, the suppression of tumour cells growth is accompanied by inhibition of transcription and translation of the components of canonical Wnt signaling pathway [40].

Uncontrolled proliferation and retardation of ageing are considered to be sufficient condition for melanoma development. Activating mutations in genes *N-Ras* and *B-Raf* (participants of MAP-kinase signaling pathway) are considered as the signals for proliferation enhancement, and β-catenin function is associated with ageing avoidance. Ageing is associated with cell cycle arrest at the stage of G_0/G_1 under the effect of tumour suppressor Rb1 that is controlled by $p16^{INK4a}$. In human melanoma cells, activated β-catenin is able to inhibit directly $p16^{INK4a}$ expression by the effect on transcription factor TCF-4 [41]. It is worth noting that β-catenin itself is able to induce neither melanocyte proliferation, nor melanoma formation. However, for double transgenic animals carrying mutations both in β-catenin (*CTNNB1*), and in *N-Ras* is characteristic of high incidence of melanoma. Moreover, such double mutants are more susceptible to tumour formation than those carrying mutation in gene *N-Ras* only.

Thus, it seems certain that constitutively activated canonical Wnt signaling pathway acts synergisticly with MAP-kinase cascade. Furthermore, both signaling pathway are aimed at the induction of melanoma formation [41].

Melanoma metastasis. Despite numerous studies proving the participation of β-catenin in malignant transformation of melanocytes, an exact role of canonical Wnt signaling pathway in melanoma metastasis is still questionable.

There is evidence that β-catenin may be negative regulator of melanoma progression. The cells of practically all benign nevi have sufficient β-catenin in the nucleus. However, the frequency of malignant transformation of nevi in melanoma is very low [42]. Moreover, melanoma metastatic progression is associated with the loss of nuclear β-catenin. The accumulation of β-catenin in cell nuclei of both primary tumour, and metastases, on the contrary, is a good prognostic for patients [43]. The transfection of murine melanoma cell line by B16 plasmid encoding Wnt3a results in blocking proliferation and stimulation of differentiation [43]. But inhibition of β-catenin gene expression in this cell line contributes to the increase of cell metastatic activity [44].

Unlike canonical Wnt signaling pathway, the role of noncanonical cascade in melanoma progression is more determined. Noncanonical Wnt/Ca^{2+}-dependent signaling pathway activated under the effect of Wnt5a protein is believed to contribute to tumour metastasis. *Wnt5a* gene superexpression is frequently associated with high grade melanomas [16]. In addition, there is correlation between the gene expression of the ligand and the stage of the disease [45]. In melanoma *Wnt5a* are predominantly expressed by the cells situated on invasive tumour margin [46]. Transfection of melanoma cells with low metastatic activity by plasmid having *Wnt5a* gene promotes their transformation in more aggressive derivatives [45]. The main Wnt5a receptor involved in melanoma metastasis is Ror2. Increased *Ror2* gene expression is also the characteristic of metastatic melanoma. The suppression of this receptor production leads to the loss of Wnt5a ability to activate signaling pathway in a cell and promote metastasis [47].

Conclusion. Wnt signaling pathway is a complex chain of intracellular interactions, it is of prime importance both for formation and maturation of melanocytes, and for melanoma development. Canonical and noncanonical signal cascades have different roles in melanoma development. β-catenin signaling pathway is used in malignant transformation of melanocytes. In advanced melanoma, the activation of this signaling pathway, on the contrary, causes tumour growth inhibition by promoting cell differentiation. Noncanonical signaling pathway does not participate in melanoma conditions. Nevertheless, increased expression of *Wnt5a* gene is the characteristic of metastatic melanoma. There is a hypothesis that this signaling pathway can participate in the control of activity of canonical Wnt signaling pathway in order not to concede the superactivation of the latter. Thus, normally, homeostasis in melanocytes is maintained by well coordinated, complex system of signaling pathways, and the disturbances in the system result in tumour origination and development.

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