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Differentiation pathways in carcinogenesis and in chemo- and radioresistance

Minireview

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Cancer stem cells (CSCs) share many features with embryonic stem cells (ESCs) such as the ability for self-renewal and differentiation. Signaling pathways that are involved in these processes are also involved in chemo- and radioresistance (e.g. Wnt, Notch and Hedgehog pathways). This review is focused on the influence of three important differentiation pathways on carcinogenesis and on chemo- and radioresistance in ESCs and CSCs.

Key words: Stem cell, Chemoresistance, Radioresistance, Wnt pathway, Notch pathway, Hedgehog pathway

Differentiation signaling pathways (e.g. Wnt, Notch and Hedgehog) are active in stem cells. Nowadays, embryonic stem cells (ESCs) and cancer stem cells (CSCs) are the most studied types of stem cells. They share many common characteristics, e.g. self-renewal, unlimited proliferative potential, ability to differentiate in certain cell types and cell detoxification by special molecules; but there are also some differences between both cell types (Tab. 1). Stem cell hallmarks are termed as markers of "stemness" [1-5].

ESCs have been first time isolated from inner cell masses of late mouse blastocyst cells [6]. Human ESCs have been isolated in 1998. These cell lines have normal karyotypes [unlike embryonic cancer cells (ECCs)], high telomerase activity and surface markers specific for primate ESCs [7].

Tumor cells possessing self-renewal ability, multilineage differentiation and maintenance of malign growth are termed cancer stem cells (CSCs). Tumor cells are surrounded with microenvironment called tumor niche. Factors maintaining the CSCs in self-renewing and undifferentiated state are presented in this niche [8]. ESCs have not stable niche *in vivo*, because during embryo development they are attendant only transiently [9]. Factors in the ESC niche are changed depending on the ESC final destination.

Cancer stem cells (CSCs) have been isolated from tumors of diverse organs, for example blood, breast, brain, lung, prostate, colon, liver, pancreas and skin. [10]. Fluorescence-activated cell sorting (FACS) by flow cytometer is used for isolation of side population (SP) enriched by CSCs on the basis of the ability to exclude Hoechst 33342 stain by ABCG2, or other members of the ABC (ATP-binding cassette)-transporter family, such as ABCB1 (P-glycoprotein or MDR-1), ABCC1 (MRP-1) and ABCA2 [11, 12]. Recently, drug surviving cells (DSCs) were isolated from human cancer cell lines exposed to cisplatine, doxorubicin and ectoposid. These cells share the same main characteristics as CSCs (self-renewal, clonogenic potential, expression of specific markers and the ability to differentiate). From this point of view DSCs can be considered CSCs [13].

Major mechanisms of radio- and chemoresistance in ESC and CSC. Most of the cancer cells are destroyed during the treatment, but some of these (CSCs) survive. Stem cells express many pumps (such as ABC-transporters ABCB1, ABCC1, ABCG2) which are able to exclude chemotherapeutic agents out of the cells and enzymes metabolising drugs [e.g. ALDH1 (aldehyde dehydrogenase 1), GST4 (glutathione S-transferase 4)]. They also produce DNA repair [e.g. Ku80, MGMT (O-6-methylguanine-DNA methyltransferase), BRCA1 (breast

Table 1. ESC and CSC features

Feature	ESC	CSC	References
Self-renewal potential	Yes	Yes	1-4
Differentiation potential	Pluripotent	Multipotent	1-4
Toxic stress response	Yes	Yes	2-4, 14, 15
DNA-repair mechanisms	Active	Active	14, 16
Anti-apoptotic mechanisms	Active	Active	14, 16
Stem cell niche	Changing	Stabile	8, 9
The role of Wnt signaling	Cell-fate determination, anterior-posterior body orientation, formation of primitive streak, mesoderm and endoderm, apoptosis prevention	Cell migration during metastasis formation, apoptosis, chemo- and radioresistance, maintenance of CSCs	16, 27, 40, 41, 44-47, 49, 53, 54,56-58,
The role of Notch signaling	Boundary formation, lateral inhibition, cell-fate assignation, several organs and systems development	Epithelial-mesenchymal transition during progression and metastasing of tumors, pro-/anti-apoptotic effect, chemo- and radioresistance, self-renewal or cell fate decision or terminal differentiation	15, 68, 69, 72, 77-79, 82, 88-92, 102, 103
The role of Hedgehog signaling	Organs development, proliferation	Growth, self-renewal and metastatic potential, apoptosis prevention	15, 110, 112-116, 125, 126,

cancer type 1 susceptibility protein)] and other anti-apoptotic proteins [(e.g. BCL-2, BCL-XL, FLIP (flice inhibitory protein)] preventing the cells against apoptosis. The big problem for targeting stem cells by chemotherapeutic agents is a slow rate of cell turnover because these drugs naturally impair cell cycle in rapidly replicating cells [14-16].

Another mechanism how stem cells protect themselves against anti-cancer therapy is that these cells produce growth and angiogenic factors encouraging tumor recurrence. Angiogenesis and metastasis formation are at least in part managed by the niche, that might protect CSCs from radio- and chemotherapies [8, 17, 18]. The radiation therapy modifies the tumor niche. It leads to the enhanced expression or activation of many anti-apoptotic proteins or pro-inflamatory cytokines, or tumor promoting factors [19]. VEGF (vascular endothelial growth factor) receptors activation stimulates CSCs proliferation and protects them from drug-induced apoptosis [13].

Similarly, HIF1 (hypoxia inducible factor 1) is responsible for tumor radiotherapy-response. Depending on other factors in the niche, HIF1 either induces ATP (adenosine-5'-triphosphate) metabolism, proliferation or apoptosis by p53 activation (cell sensitization to the therapy), or it allows CSCs to survive (tumor growth maintenance) [20]. Therefore, the use of drugs that inhibit HIF1 may have a double effect.

Under hypoxic conditions the expression of transcription factor (TF) OCT4 is activated due to the HIF [21]. Recently, it was observed in lung cancer that increased level of OCT4 may provide tumor resistance to the chemo-radiation therapy. It seems that OCT4 is linked with P-glycoprotein, member of the ABC-transporter family, and thus it may participate in the emergence of multidrug resistance (MDR) [22].

Other downstream targets of TFs OCT4 and SOX2 are responsible for the drug resistance of ESCs. TF ZFP206 (zinc finger protein 206) provides them the resistance to the retinoic acid-induced differentiation [23], TF ZIC3 (zinc finger protein of the cerebellum 3) prevents endodermal specification in ESCs [24] and ESG1 regulates the pluripotency and the development of primordial germ cells (PGCs) [25].

Differentiation pathways involved in carcinogenesis and in radio- and chemoresistance. For maintaining stem cells characteristics, the specific pathways supporting these processes must be active. These pathways are responsible for the cell-fate determination of ESCs, since they downregulate the expression of certain target genes affecting cell differentiation. Among these constitutive mechanisms with this ability are Wnt, Hedgehog and Notch signaling pathways [26, 27].

Wnt signaling pathway. The Wnt signaling pathway is activated by secreted WNT glycoproteins (a gene family consisting of 19 members) binding to the 7-transmembrane (TM) receptor of the Frizzled (FZD) family. Human ESCs express all WNTs and most FZDs, while in multipotent cells, for example human mesenchymal cells (MSCs) and in the ECCs, the expression of certain WNTs and FZDs is missing [28, 29]. Depending on associated co-receptor, signals are transmitted in two different ways. When FZD cooperates with LRP5/6 (low-density lipoprotein receptor-related proteins 5 and 6) [30], the so-called canonical pathway participation in β-catenin stabilisation is activated (Fig. 1a) [31]. For this reason it is also called a Wnt/β-catenin signaling pathway [32]. Target genes of this pathway participate in self-renewal, pluripotency, proliferation and cell-fate determination [(*c-Myc*, *Cyclin D1, MMPs (*matrix metalloproteinase*), VEGF, NOS2* (nitric oxide synthase 2), *PPARδ* (peroxisome proliferator-activated receptor-δ), *COX-2* (cyclooxygenase-2), *JAG1, CER1* (cerberus 1)][33-36]. When FZD collaborates with ROR2/RYK co-receptors, the non-canonical pathway is triggered. This in turn depends on Ca^{2+} and G-proteins that activate kinase cascades (Fig. 1b) [37].

During carcinogenesis, activation of Wnt/β-catenin signaling is often involved through upregulation of Wnt ligands or

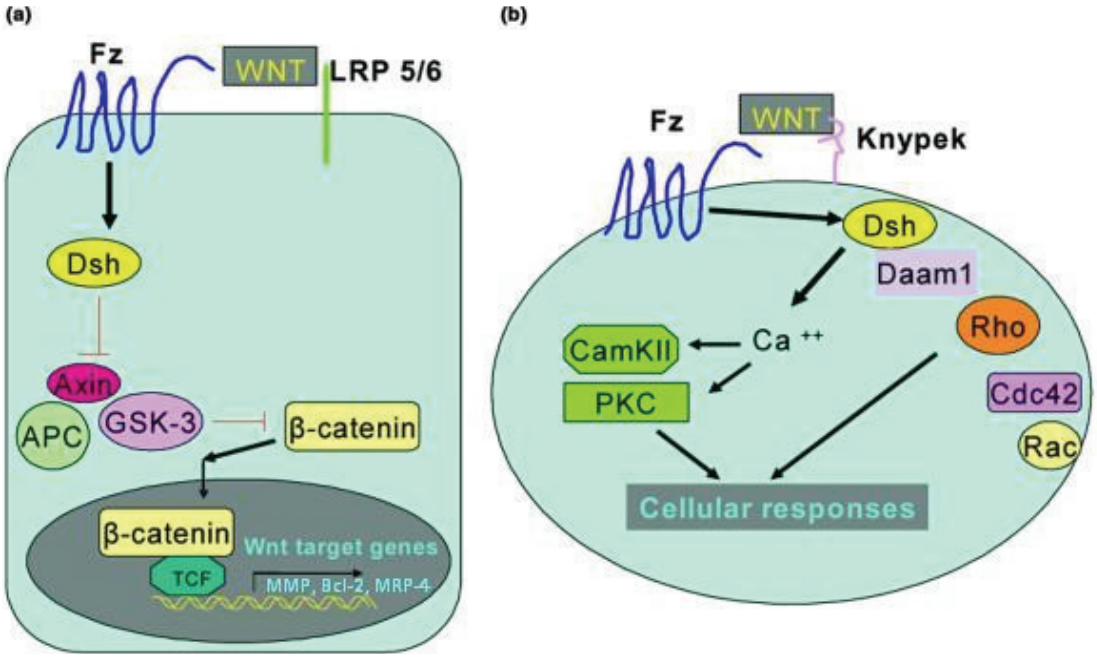

Figure 1. Wnt signaling

a) Canonical pathway activated in the presence of certain Wnt ligands through Dishevelled (DVL) phosphorylation leads to the inactivation of β-catenin destruction complex consisting of APC (adenomatous polyposis coli), scaffold protein AXIN and GSK3β (glycogen synthase kinase 3β). β-catenin is translocated to the nucleus where it is complexed with TCF/LEF (T-cell factor/lymphoid enhancer factor) family transcription factor. In the absence of the activating Wnt signals, GSK3β phosphorylates β-catenin. Thus, GSK3β predestinates β-catenin for the ubiquitination and subsequent degradation in proteasomes.

b) Wnt signals of non-canonical pathway either phosphorylate DVL activating small G-proteins (RHO, RAC, CDC42) and JNK, that through MAP-kinase cascade transmit the signal up to the nucleus, or they release Ca^{2+} affecting NLK (Nemo-like kinase) or NFAT (nuclear factor of activated T-cells). This inhibits the canonical Wnt signaling pathway and facilitates cytoskeletal reorganization during invasion and metastasis. [31].

FZD receptors. Parallelly, β-catenin degradation is stopped due to failure of some upstream members of the signaling pathway, and/or the β-catenin is mutated [38, 39]. Intracellular accumulation of β-catenin and its translocation into the nucleus regulates the expression of target genes, or it forms complexes with E-cadherin in the cytoplasmatic membrane, thereby it becomes a part of cell-cell contacts. In this manner, β-catenin may be involved in cell migration during metastasis [40]. Negative regulators, such as sFRP (secreted Frizzled-related protein), AXIN and PPARγ (peroxisome proliferator-activated receptor γ), can be silenced or mutated [37, 41, 42]. As Wnt signaling is connected to the chromosome orientation during mitosis, its perturbations may lead to a mitotic disjunction typical of many cancer cells [43].

Wnt signaling is active in both CSCs and ESCs. In ESCs it has been shown, that Wnt signaling manages the undifferentiated cell proliferation depending on the type of Wnt ligand and others factors. The Wnt pathway itself fails to maintain these cells in the pluripotent state. Its activity increases at the stage when the cell makes the decision which direction of differentiation will develop. In quiescent ESCs, the OCT4/SOX2 pathway is first activated and during cell-fate determination Wnt signaling is enhanced [44, 45, 27]. At that time, the Wnt pathway regulates the anterior-posterior body orientation [46] and formation of primitive streak, mesoderm and endoderm [47]. Wnt signaling is also necessary for the maintenance of the progenitor cells pool [26].

Wnt signaling is not a single unit but depending on the cell type it collaborates with other cell factors. It cooperates with several signaling pathways (e.g. Hedgehog, Notch, TGFβ, PI3K/AKT, Src/ERK, Activin/Nodal, FGF) [31, 48-50], and also with many nuclear receptors (NRs), such as the androgen receptor (AR), the retinoic acid receptor (RAR), the vitamin D receptor (VDR), the progesterone receptor (PR) and PPAR. β-catenin acts in their transcription as a co-activator. NRs serve as transcription factors, but they also affect posttranslational events, such as phosphorylation. Thus they can interact with other signaling pathways, including the Wnt [51]. Further, NRs (AR, RAR amd VDR) act as repressors of Wnt/β-catenin signaling [52], since they can increase β-catenin accumulation in the plasma membrane in conjunction with E-cadherin. In this way its nuclear levels decline and stabilisation of the adherent junction is increased [51].

During both embryogenesis and carcinogenesis, Wnt ligands prevent apoptosis in progenitor cells. When DNA is damaged, the β-catenin affinity to TCF (T-cell factor) is in-

creased due to PARP-1, and for this reason, β-catenin target genes expression is found [53]. Some of these genes may be responsible for the emergence of resistance to therapy, for example *MMPs, Livin, BCL-2* and MRP4. MMPs through EGFR (epidermal growth factor receptor) activate the Src/ERK signaling pathway which may contribute to the emergence of endocrine therapy resistance [41]. Livin, a member of the IAP (inhibitor of apoptosis) family, prevents cell apoptosis and thus takes part in the development of tumor cell chemoresistance [54]. PI3K/AKT signaling is activated by the non-canonical Wnt pathway. Both Wnt pathways (canonical and non-canonical) regulate the expression of anti-apoptotic protein BCL-2 [49]. This is one of the anti-apoptotic mechanisms in stem cells. For this reason, the Wnt/β-catenin signaling pathway activation may be involved in tumorigenesis by promoting cell survival [55]. Radiotherapy leads to breaks in the DNA molecule, but since apoptosis is blocked, the cell switches on reparation mechanisms and survives. In this way, Wnt signaling is also involved in CSC radioresistance [56]. *ABCC4*, known as *MRP4* (multidrug resistance protein 4), is also the target gene of Wnt signaling. It is an ABC-transporter and participates in the transport of cyclic nucleotides and some nucleoside monophosphate analogs [57]. Therefore, treatment with these chemotherapeutic agents fails in the presence of MRP4 and the cells become chemoresistant.

Recently, it has been found that Wnt/β-catenin signaling is one of the key pathways in the maintenance of CSCs (for example in lung, colon, liver, leukemia, melanoma, breast and skin cancer) [58]. Surprisingly, this pivotal role of Wnt/β-catenin signaling is not so evident in human ESCs because inhibition of this pathway alone does not influence their self-renewal potential. It depends on collaboration with other signaling pathways and especially, on the origin of ESCs (e.g. mouse, rat or human ESCs) [44].

"The stemness" of colon CSCs depends not only on the presence of mutations in APC or β-catenin but also on myofibroblast-secreted factors which are able to restore the CSC phenotype in more differentiated tumor cells [59]. Other examples of molecules presented in the microenvironment during carcinogenesis and cancer are the inflammatory tissue products, interleukins (ILs). These stimulate the signaling pathways that regulate expression of Wnt ligands. IL6 induces through JAK/STAT3 signaling in CSCs, the activity of both canonical and non-canonical Wnt pathways and therefore it takes part in self-renewal and also metastasis. Under physiological conditions this activation maintains tissue homeostasis [60]. In the presence of different inflammatory cytokines, *NOS2* expression is increased through TBE (Tcf-binding element) [34].

A number of substances have been found recently to inhibit the Wnt signaling pathway. Some of these, such as non-steroidal anti-inflammatory drugs (NSAIDs) (for example aspirin and indomethacin) and vitamins (retinoids and vitamin D), are used in the treatment of inflammatory diseases [61-63]. Others are developed for the treatment of cancer, such as the tyrosine-kinase inhibitors (e.g. imatinib) [64] and some substances are chemical compounds, like lithium, curcumin and flavonoids [65]. Many small molecules [for example 6-bromoindirubin-3′-oxime (BIO), deoxycholic acid and pyrimidine derivates] inhibit Wnt signaling, but there are also agents that support Wnt activation (e.g. GS11 – increases the level of free β-catenin by dissociation of binding with E-cadherin) [66]. In mouse ESCs, the small molecule IQ-1 can support long-term pluripotency. The destiny of ESC has been reported to depend on β-catenin co-activators. If CBP (CREB binding protein) is present, the expression of transcription factors maintaining pluripotency (OCT4, SOX2) occurs. After CBP switching by p300, the cell initiates differentiation (e.g. c-MYC expression). IQ-1 blocks the switching by p300 co-activator and guarantees mouse ESCs their pluripotency [67].

Apparently, Wnt signaling takes place in different processes during embryogenesis and carcinogenesis, but it cooperates with many other signaling pathways in a species-specific manner. These facts markedly complicate any definition of the exact function of the Wnt pathway.

Notch signaling pathway. Notch signaling differs from other signaling pathways mainly in activation by cell-cell contact. The Notch receptor is a single transmembrane protein composed of intra- and extracellular regions with distinct domains responsible for its specific functions. Notch ligands are also single transmembrane proteins. In mammals, four types of Notch receptors (Notch1 – 4) and five types of Notch ligands [Delta-like1, 3, 4 (DLL1, 3, 4) and Jagged1 and 2 (JAG1, 2)] have been described [68]. When the ligand binds to the receptor, the transfer of signal is activated (Fig. 2) [69]. However, the high levels of ligand may have, conversely, an inhibitory effect [70, 71]. Target genes (transcription factors *HES* (hairy/enhancer of split) and *HERP* (HES-related repressor protein family) are bHLH (basic helix-loop-helix) transcriptional repressors maintaining the self-renewal potential in the stem cells. During development they govern boundary formation, lateral inhibition and cell-fate assignation (differentiation, proliferation or apoptosis). Likewise, they contribute to the emergence of developmental diseases and tumors, as well as to EMT (epithelial-mesenchymal transition) during tumor metastasis [68, 69, 72].

Boundary formation is a necessary event in development. For example, in vertebrates it is involved in somite formation. The oscillation of Notch signaling is controlled by WNT3a, which is evidence of Notch with Wnt signaling cooperation [73, 74]. Lateral inhibition is a process by which the cells over the Notch signaling inhibit differentiation potential in neighbouring cells [e.g. the selection for sensory organ precursor (SOP) in *Drosophila* neurogenesis, or in mice development of hair cell in the inner ear] [75, 76]. The cell-fate assignation is a determination of the fate of two daughter cells. These acquire a variable differentiation potential and therefore are able to differentiate into distinct cell types. For instance, different regulators (Numb and Neuralized) are involved in asymmetric cell division during SOP. These regulators are asymmetrically distributed among the daughter cells. After

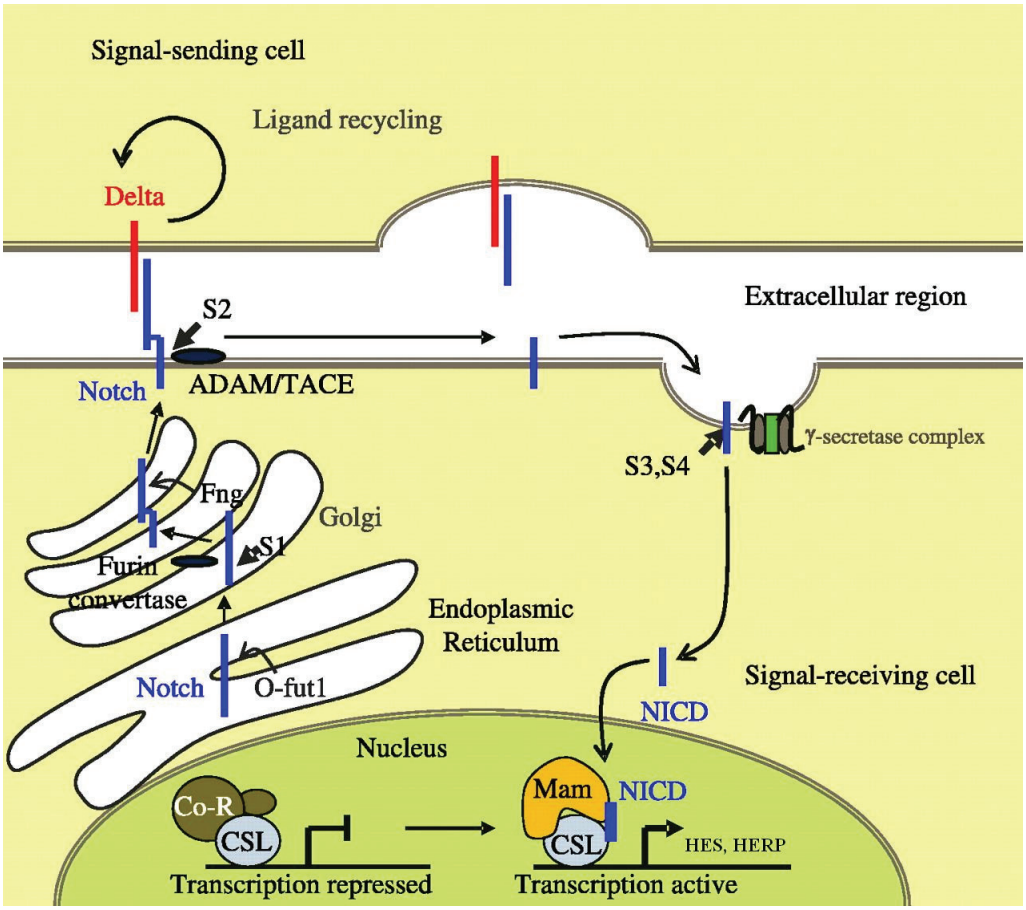

Figure 2. Notch signaling
The first step after the Notch receptor interaction with Notch ligand is the Notch receptor splicing in the site S2 by ADAM/TACE (a desintegrin and metallopeptidase/tumor necrosis factor α converting enzyme) family. It is followed by the cleavage at transmembrane site S3/S4 by γ-secretase. NICD (Notch intracellular domain) is chipped and transported by endocytosis to the nucleus. There it displaces a repressor (co-R) linked with histone deacetylase (HDAC) and associates with transcription factor CSL (C-promoter binding factor1, RBP-jk/Su(H)/Lag-1) and co-activator mastermind (MAM). They together generate a ternary complex and support the activation of target genes [69].

four cycles of division, five cell types arise (socket, shaft, sheath, neuron and glial cells) [69]. In this way the stem cell population can be maintained [77]. Notch signaling may also be involved in the development of several organs and systems, such as the pituitary gland, gut, nervous and vascular system [77-79].

During very early embryogenesis, Notch signaling activity is not required. Even in human ESCs Notch signaling is inactive. In pluripotent ESCs, the transcription factors NANOG, OCT4 and SOX2 are necessary for self-renewal whereas NOTCH1 expression is very low. However, the level of these transcription factors decreases after cell-fate determination (in multipotent stem cells and in ECCs) and the NOTCH1 expression increases [80, 81, 29]. Notch signaling activation does not disturb the ESC differentiation and in some cases it is necessary (e.g. T-cell differentiation) [82]. The cells with active Notch pathway cannot differentiate into nervous system cells, but they maintain a neural stem/progenitor cell pool. They can also differentiate into other developing lines. The lineage commitment depends on the ligand. Recently, it was reported that Notch signaling activated by JAG1 promotes neural commitment in contrast to activation by DLL4 which promotes mesodermal commitment [83-85]. Notch signaling has to be completely switched off before terminal differentiation [86]. The differentiation and maintenance of the stem/progenitor cell pool by Notch signaling in mammary gland or hair formation is regulated by the same inhibition mechanism [87, 76]. The regulation of apoptosis by the Notch pathway is quite controversial. It has pro-apoptotic, as well as anti-apoptotic effects. For example, in endothelial cells or keratinocytes apoptosis is promoted by inactivation of Wnt, respectively PI3K pathways. However, in tumors, where the PI3K pathway is activated, the cells are protected against the upcoming apoptosis [88-92].

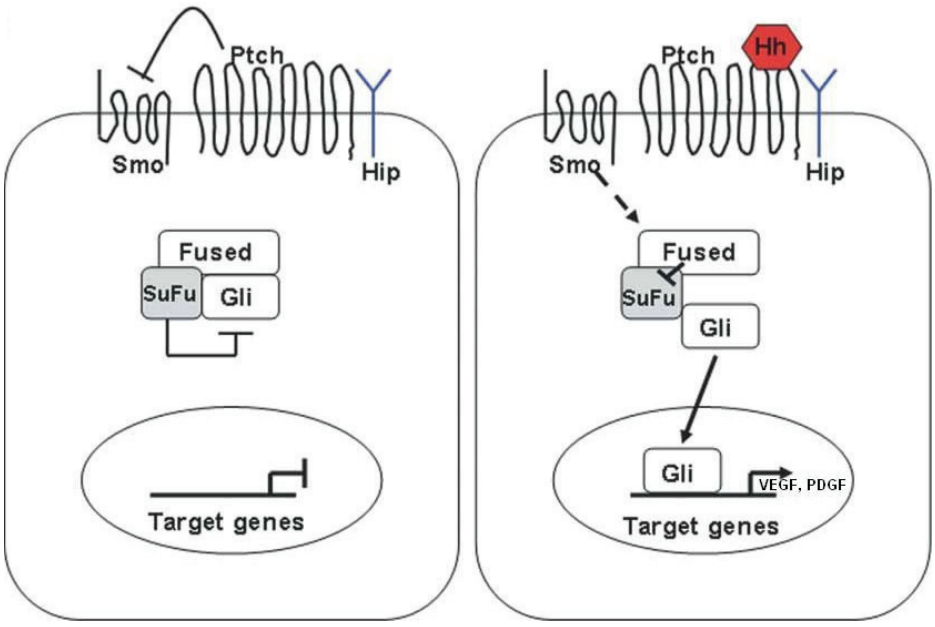

Figure 3. Hedgehog signaling
PTCH represses the SMO activity. After activation of signalisation by Hedgehog ligand the SMO inhibition by PTCH is stopped and SMO governs the suppression of Supressor of Fused (SuFu) in protein complex with Fused (Fu). Then the members of GLI family transcription factors are released from the zinc-finger type protein complex Fu/SuFu. It leads to their translocation into the nucleus and subsequent transcription of target genes [106].

Endocytosis does not operate only in NICD (Notch intracellular domain) transport into the nucleus. It also participates in NICD degradation and recycling and, in activation of Notch receptors. In this way it prevents the continual activation of Notch signaling. NOTCH monoubiquitination with subsequent endocytosis is required before the cleavage of NOTCH by γ-secretase [93 94]. NICD directs to the nucleus after Notch signaling activation and there it guides target genes transcription. In contrast, when NICD associates with a lysosome by ESCRT (endosomal sorting complex required for transport) it leads to NICD degradation. When NICD is ubiquitinylated by E3 ubiquitin ligase Deltex (DX), it is transported back to the membrane for NOTCH recyclation [95-98].

The Notch signaling pathway may bypass transcription factor CSL (C-promoter binding factor1, RBP-jk/Su(H)/Lag-1) and thus activates genes expression in other ways. For example, shaggy-dependent (Su(H)-independent) transcription is driven by a type of *Notch* allele called *Abruptex* (*Ax*) in *Drosophila*. Shaggy encodes the *Drosophila* homologue of GSK3β, which is how Notch and Wnt signaling are connected [99, 100]. There is also evidence on the effect of Notch signaling on β-catenin in vertebrates. For example, Notch1 supresses β-catenin-mediated signaling in mouse skin and therefore may be considered a tumor suppressor [101]. A conserved sequence of double TCF/LEF-binding sites in the JAG1 promoter is another example of Notch and β-catenin cooperation. JAG1 expression by the complex of β-catenin with the TCF/LEF transcription factor may be the first step in Notch signaling activation in progenitor cells for maintaining their homeostasis [35].

Notch1 signaling contributes to p53 inhibition through the mTOR (mammalian target of rapamycin)-dependent PI3K-Akt/PKB (protein kinase B) pathway. When mTOR is mutated or NOTCH1 signaling is aberrantly activated, the cells can avoid apoptosis [92]. Another way of aberrant NOTCH1 activation is through oncogenic H-RAS. This route is associated with the emergence of chemoresistance [102]. JAG1 expression arises in CSCs and activates signalization via NOTCH1 during radiotherapy. As a result the genes supporting proliferation are transcribed (*Cyclin D1*, *CDK2*) and the tumor gains the potential for radioresistance [103]. This can explain why radiotherapy may have similar effect on both Notch signaling and Wnt/β-catenin pathway activation.

In breast cancer, gastrointestinal and certain haematological tumors, Notch signaling governs the stem cell self-renewal and cell-fate decisions and it may act as an oncogene. However, in keratinocyte-derived tumors, the Notch signaling supports terminal differentiation [68] and in some types of lung tumor subtypes [adenocarcinoma, small cell lung cancer (SCLC)], the activation of Notch1 inhibits the growth of tumors [104, 105]. Therefore it can serve either as protooncogen or tumor suppressor depending on the context.

Hedgehog signaling pathway. In mammals, there are 3 types of secreted glycoprotein ligands: Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH). These bind to the 7-TM receptor Patched (PTCH) associated with

the second TM receptor Smoothened (SMO) and activate the signaling (Fig. 3) [106]. Target genes (*GLI1, PTCH1, Cyclin D, Cyclin E, MYC, VEGF, PDGF*, etc.) are able to control tissue patterning, differentiation, proliferation and migration [31, 107, 108]. Hedgehog signaling activation is attenuated by the presence of Hedgehog-interacting protein (HIP) near PTCH, that uptakes Hedgehog ligands [109]. Transcription factors GLI1 and GLI2 serve as positive regulators of Hedgehog signaling pathways, but GLI3 is a negative regulator [110]. Most involved genes are oncogenes but for example PTCH1 is a tumor suppressor [111].

Hedgehog signaling is involved in the development of many organs including mammary gland, pancreas, lung, skin and hair follicle and central nervous system [112-116]. Signaling through SHH facilitates the differentiation of human ESCs, but under other conditions Hedgehog signaling supports their proliferation [117]. In tissue stem/progenitor cells, Hedgehog activity is high, but it is downregulated during the cell differentiation [118]. Thus, Hedgehog signaling promotes survival and proliferation of stem/progenitor cells, rather than the management of cell-fate determination and differentiation [116]. It all depends on the cooperation with other signaling pathways. Among these signaling pathways are Wnt, Notch and PI3K/Akt pathways [86, 119, 120]. In adults, the activity of Hedgehog signaling has to be exactly defined in time and space and there is an unlimited capacity for self-renewal [31, 108]. This means that Hedgehog signaling plays a crucial role in carcinogenesis and metastasis [111].

The disturbances in Hedgehog pathway are commonly observed in a large number of tumors, e.g. medulloblastoma, basal cell carcinoma, stomach, colon, pancreatic, prostate and breast carcinomas [100, 111, 112, 121-123]. The Hedgehog-GLI1 pathway has an essential role in promoting growth, self-renewal and the metastatic potential of CSCs in colon cancer. In mice it operates in paracrine fashion, whereas in humans in autocrine fashion [124, 125].

PTCH loses its tumor suppressor effect either through germline mutations or through silencing of *PTCH* after methylation in the medulloblastoma. This signaling can be stopped with cyclopamine, the SMO inhibitor downstream PTCH. This leads to reduced proliferation and induction of differentiation [126, 127]. The SHH and mTOR signaling may be essential for CSCs self-renewal in pancreatic cancer. On the other hand, the targeting of these pathways by cyclopamine and paramycine is not enough to eliminate pancreatic CSCs. The CSCs were eliminated only after adding gemcitabine, as a standard chemotherapy agent. This means that the combination of targeted therapy and standard chemotherapy could be effective in the elimination of CSCs [128]. The Hedgehog signaling may be also inhibited by treatment with forkoskolin that activates the protein kinase A (PKA). The level of cell cAMP (cyclic adenosine monophosphate) is increased after stimulation of adenylyl cyclase [129], which is commonly a sign of energy depletion in the cell. Then the cell enters apoptosis. Conversely, certain agents are known to amplify the activation of Hedgehog signaling pathway. These are, for example the co-conjugates of chondroitin-6-sulfate with dermatan sulfate. These together increase the expression of IHH [130].

Since Hedgehog signaling promotes stem cells proliferation and prevents their differentiation or entry into apoptosis, it creates appropriate conditions for the accumulation of various genetic events [110]. This is an excellent basis for further detailed research to clarify the involvement of this pathway in chemo- and radioresistance.

Conclusion

The CSCs and ESCs have the ability for self-renewal and differentiation, and they are also able to exclude "toxic" substances from the cell through certain membrane transporters. CSCs in solid tumors are more similar to normal tissue stem cells than to ESCs because CSCs have determined the direction of differentiation and thus they are using competent signaling pathways. ESCs from the inner cell mass of blastocyst are just deciding about the direction of further development. Therefore the signaling pathways in ECSs are only involved in self-renewal and proliferation. The Wnt, Notch and Hedgehog signaling pathways are activated in ESCs only during their differentiation. These pathways can also be actived during carcinogenesis and are also involved in the emergence of drug resistance by regulating their target genes such as ABCC4 (known as MRP4) and antiapoptotic BCL-2. The detailed understanding of the role of these pathways in radio- and chemoresistance however requires further research.

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