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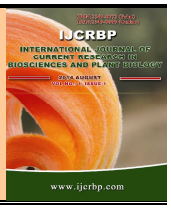

Review Article

Stem Cells Development Using WNT Signaling Pathways and Regenerative Medicine against Carcinogenesis

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Abstract

Stem cells have crucial role in human life cycle and ability to proliferate any types of specialized cells. Many signals are involved in stem cells development cycle, especially Wnt signals are crucial impact. Since 1982, Wnt gene was identified. The function of Wnt gene was extensively discovered in stem cell progress and cancer. Wnt signal is observed from invertebrate to vertebrate and regulate early embryonic development. Wnt signal is enhanced the regulation of embryonic stem cells (ESCs), cancer stem cells (CSCs) and induced pluripotent stem cells (iPSCs). Despite, deregulation of Wnt signal is associated with many human cancers and degenerative disorders. In this review disclose the detail of expression of Wnt signaling and related to cancer proliferations. Full constructed Wnt signaling pathways are developed such as, Wnt/ β-catenin signaling, Wnt/Planer Cell Polarity pathway (PCP), Wnt/Ca2+ pathway and explain the detailed functions of all Wnt signal proteins involvement in stem cell development and cancer. In cancer, the details are revealing such as deregulation of Wnt signaling pathway, abnormality or mutations of Wnt proteins and failure details about drugs designing against cancer.

Keywords

Cancer

Deregulation

Drugs

Mutations

Stem cells

Wnt signaling pathway

Introduction

Stem Cells have ability to grow different specialized organs. An adult mature cell does not have the ability. Wnt signals are widely express in normal and cancer cells. Although Wnt signal has a unique character and function compare than other signaling pathway known as Nanog, Hedgehog. The int-1 gene was first identified by activation of mouse mammary tumor virus (MMTV) in mammary carcinomas. The int-1 gene encodes a glycoprotein as same as in *Drosophila melanogaster* wingless (Wg) gene. Those genes were finally named as Wnt gene (Wingless int gene) (Nusse and Varmus, 1982; Nusse et al., 1991; Nusse and Varmus, 1992).

Stem cells Niche

The stem cells were enhanced from zygote fusion of egg and sperm cell. Zygote has ability

to form whole species called totipotent stem cells. Differentiation of zygote to form blastomeres, such blastomere contains embryonic stem cells called pluripotent stem cell. Further, Embryonic stem cells (ESCs) have four types of differentiation such as mesoderm, ectoderm, endoderm and germ cells. These divisions are finally differentiate to accomplished the formation of organogenesis (Thomson et al., 1998; Intawicha et al., 2013) The Wnt gene has crucial play key role in formation of mature adult cells from embryonic stem cells. Moreover, Wnt gene plays major role in mesoderm and endoderm development and differentiation. Unfortunately, abnormality of Wnt signaling leads to stimulate the cancer formation and other disorders in the life cycle (Nusse, 2008; Sui et al., 2013).

Wnt Signaling

The Wnt signaling has crucial role in stem cell development and regenerative medicine. In stem cell development, Wnt play major role in many type of aspects such as, stem cell maintenance and self-renewal, placental differentiation, trophoblast invasion and differentiation, germ layer formation, maintenance of pluripotency, cell survival and migration, cell-fate specification and adhesion, primitive streak formation. In organogenesis, osteoclastogenesis, osteoblastogenesis, cardiomyogenesis, tissue morphogenesis, neurogenesis, spermatogenesis, myogenesis, wound healing and regeneration like head formation and limb patterning. (Nusse et al., 2008; Knofler and Pollheimer, 2013). In cancer, Wnt signal act as a carcinogenesis when it is an abnormal state and other degenerative disorders.

Wnt Signaling Pathway and regulations

The Wnt gene family secreted various Wnt proteins by using multiple Wnt signaling pathways. In vertebrate, Wnt gene family represents large number of signaling molecules involved in Wnt signaling pathways. Up to now, 19 Wnt ligands interact with secreted and membrane associated proteins to initiate signaling process, 10 transmembrane, frizzed heterodimeric receptor (FZD), 2 low density lipoprotein receptor-related protein co-repressor (LRP-5 and 6) have been identified in mammals Wnt signaling. Depending on the type of Wnt-ligand receptor interaction, intracellular signaling molecules, specific target transcriptional activations, Wnt signaling pathways have been defined. Wnt gene family regulated three different signaling pathways known as canonical pathway. Other two pathways are commonly called as non-canonical pathways such as Wnt/planer cell polarity (PCP) pathway and Wnt/Ca^{2+} pathway. These canonic and non-canonic pathways involved crucial role in different aspects. In canonical pathway, Wnt signal transduced for cell fate determination and non-canonical pathway regulated the purpose of controlling cell movement and tissue polarity (Sonderegger et al., 2007).

The Canonical Wnt/β-catenin Signaling Pathway

The canonical pathway is stimulated by Wnt protein family. β-catenin has major role in the canonical pathway. β-catenin has a dual function such act as a intracellular contacts and intracellular messanger. In canonical pathway, β-catenin acts as a transcriptional transactivator. In stem cell development, β-catenin requires the development of dual function of embryonic stem cell self-renewal and germ layer formation. Wnt signal, the number of extracellular Wnt- modulating protein such as DKK1, Crescent, FrzB, SFRP, WIF, Cer, Kremlin, Norrin. Dickkopf 1 (DKK1), most secreted molecules of Wnt antagonist, bind to the low density lipoprotein receptor such as LRP5/6 to inhibit Wnt signaling. Other extra cellular molecules like Crescent, FrzB, SFRP, WIF, Cer are inhibited Wnt ligands to bind frizzed heterodimeric receptor (FZD) (Zhang et al., 2013).

Absence of Wnt ligand (off-state), stimulate the phosphorylation of β-catenin. In cytoplasm, β-catenin is intricate with APC (adenomatous polyposis coli), WTX, Axin (axis-inhibitor 1) both stimulated the phosphorylation of the β-catenin protein by casein kinase I (CKI) and glycogen synthase kinase 3 (GSK-3). Phosphorylation of β-catenin, achieved via

ubiquitin-dependent/ proteasome pathway. This provokes phosphorylation or degradation of β-catenin protein through the b-TrCP (b-transducin repeat-containing protein) resulting in low β-catenin levels in cytoplasm (Fig.1) (Chen et al., 2008).

Presence of Wnt ligand (on-state), bind to the cysteine rich domine CRD of frizzed heterodimeric receptor (FZD) triggering the demolition of Axin/APC/GSK-3/CKI complex (Fig. 2). Wnt ligands stimulate the requirement of Dishevelled (Dsh) bind to FZD, called as signalosome. Destruction of the β-catenin with Axin compelx and activation of Dsh/Dvl result achieved in impaired degradation and accumulation of enormous amount of β-catenin in cytosol. High level of active cytosolic β-catenin then translocates into the nucleus when β-catenin act as a transcriptional co-regulator (Zhang et al., 2013).

Many transcription inhibitors are involved to prevent the transcriptional activation of T cell-specific factor (TCF)/lymphoid enhancing factor (LEF), such as ICAT, Chibby, NLK, CtBP, Groucho and HDAC. Transcriptional regulation stimulated when high level expression of β-catenin in cytosol. Activation of β-catenin, demolition the complex of Groucho and HDAC then binded to the TCF/ LEF Transcription factor. The active complex of β-catenin has many protein molecules known as BCL9/Lgs, Brg-1, Pygo, Tsh, CBP/p300 leads stimulation of many required target genes, such as c-Myc, c-Jun, Cyclin D1, MMP-7, CD-44, WISP1, FRA-1, Nanog, Oct-4, Sox-2, Cdx 1, PPAR, LBH, Jagged. Other Wnt target genes are summarized at the Wnt homepage (http://www.stanford.edu/wrnusse/wntwindow.html) (Sonderegger et al., 2007; Chen et al., 2008; Zhang et al., 2013).

The Noncanonical-Wnt/Planer Cell Polarity Pathway

The non-canonical Planer Cell Polarity pathway (PCP) is one of the most important pathways in Wnt signaling. PCP pathway has specialized role in Wnt signaling such as cell polarity, adhesion, shape and cell movement (Fig. 3). The PCP pathway originally identified in Drosophila. Regulation of PCP pathway not require or without β-catenin, it is independent pathway of β-catenin, perhaps even inhibit β-catenin-nuclear activity (Zhang et al., 2013). In stimulation of PCP pathway not requiring LRP as a co-receptors. Wnt signaling pathway, PCP pathway is always activated when Wnt molecules bind to the FZD and its co-receptors. The complex of Wnt-FZD recruits Dvl/Dsh for enhances the signaling pathway which is used to form a complex with Dishevelled-associated activator of morphogenesis 1 (DAAM1). Activation of G-protein Rho through guanine exchange factor (GTP) by DAAM1, then Rho stimulate Rho-associated kinase (ROCK), which is one of the important regulators of the cytoskeleton. Dsh also make another complex such as Dsh-Rac1 and mediates binding of profilin and actin. This profilin-actin complex mediates the restructuring of the cytoskeleton and gastrulation. Rac1 to stimulate the activation of JNK, which lead to the actin polymerization and also active JNK involved in the activation of gene transcription such as c-Jun, AP1. PCP pathway shows the crucial role in stem cell development such, angiogenesis, bone morphogenesis, gastrulation (Sonderegger et al., 2007; Zhang et al., 2013).

The Noncanonical-Wnt/Ca^{2+} Pathway

Noncanonical Wnt/Ca^{2+} pathway cascade, Wnt5a ligand induced signaling pathway which mediates the synthesis of secondary messenger such as calcium. Later, this secondary messenger signaling pathway was discovered as Wnt/Ca^{2+} pathway. The Wnt/Ca^{2+} signaling pathway is another noncanonical pathway, which does not accumulate the β-catenin in cytosol (Fig.4). Wnt/Ca^{2+} pathway was discovered by the stimulation with specific Wnt ligands such as Wnt5a, which leads to release calcium from endoplasmic reticulum (ER) in order to control intracellular Ca^{2+} levels. Wnt5a ligand binds to the FZD to stimulate the signaling mechanism. Active FZD receptor directly recruits Dsh and mediates specific Dsh protein domains. Unlikely other Wnt signaling pathway, the Wnt/Ca^{2+} pathway mediates FZD receptors directly interact with a trimeric G-protein.

Fig. 1: Schematic representation of Off-State of Wnt/β-catenin signaling pathway.

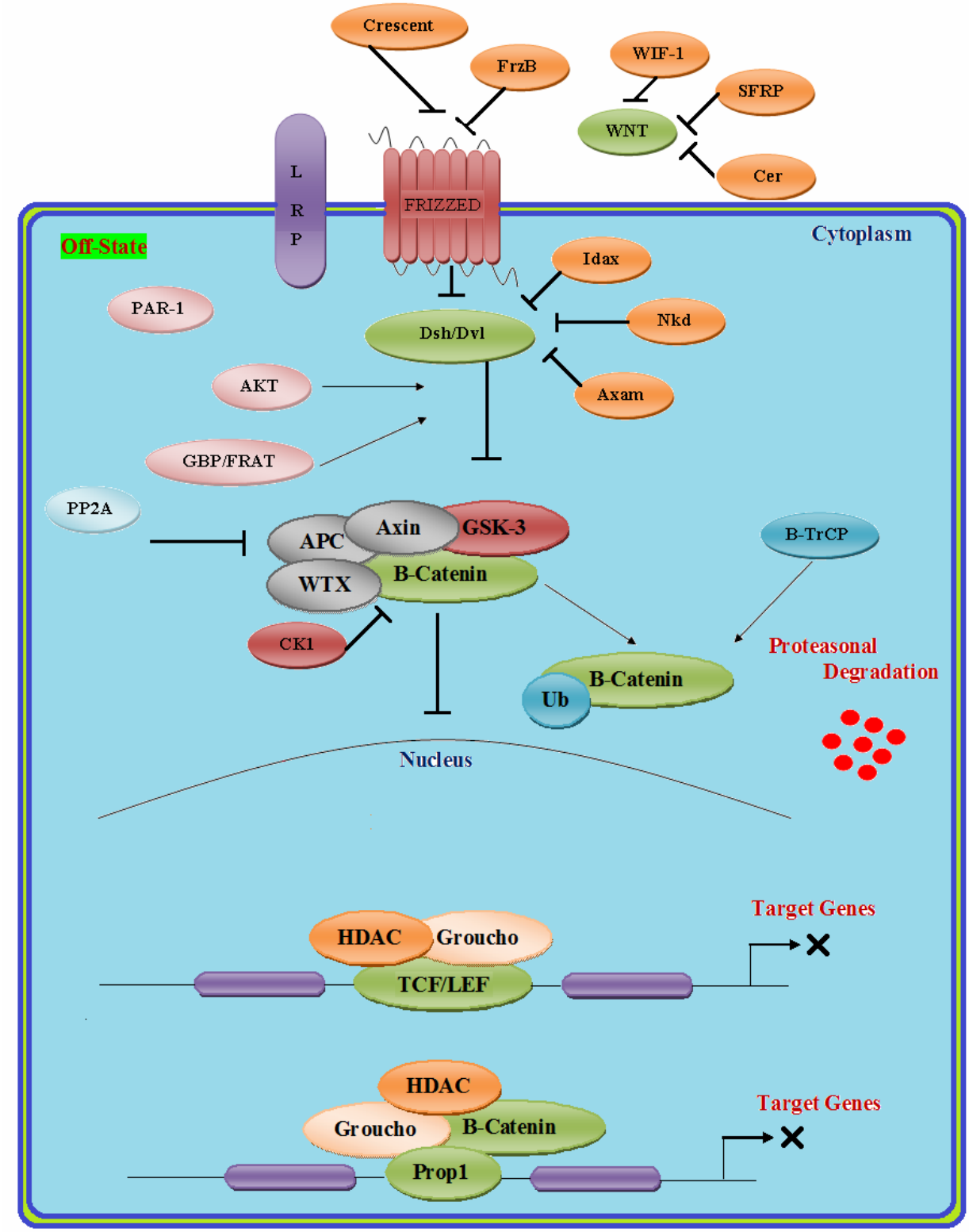


Fig. 2: Schematic representation of On-State of Wnt/β-catenin signaling pathway.

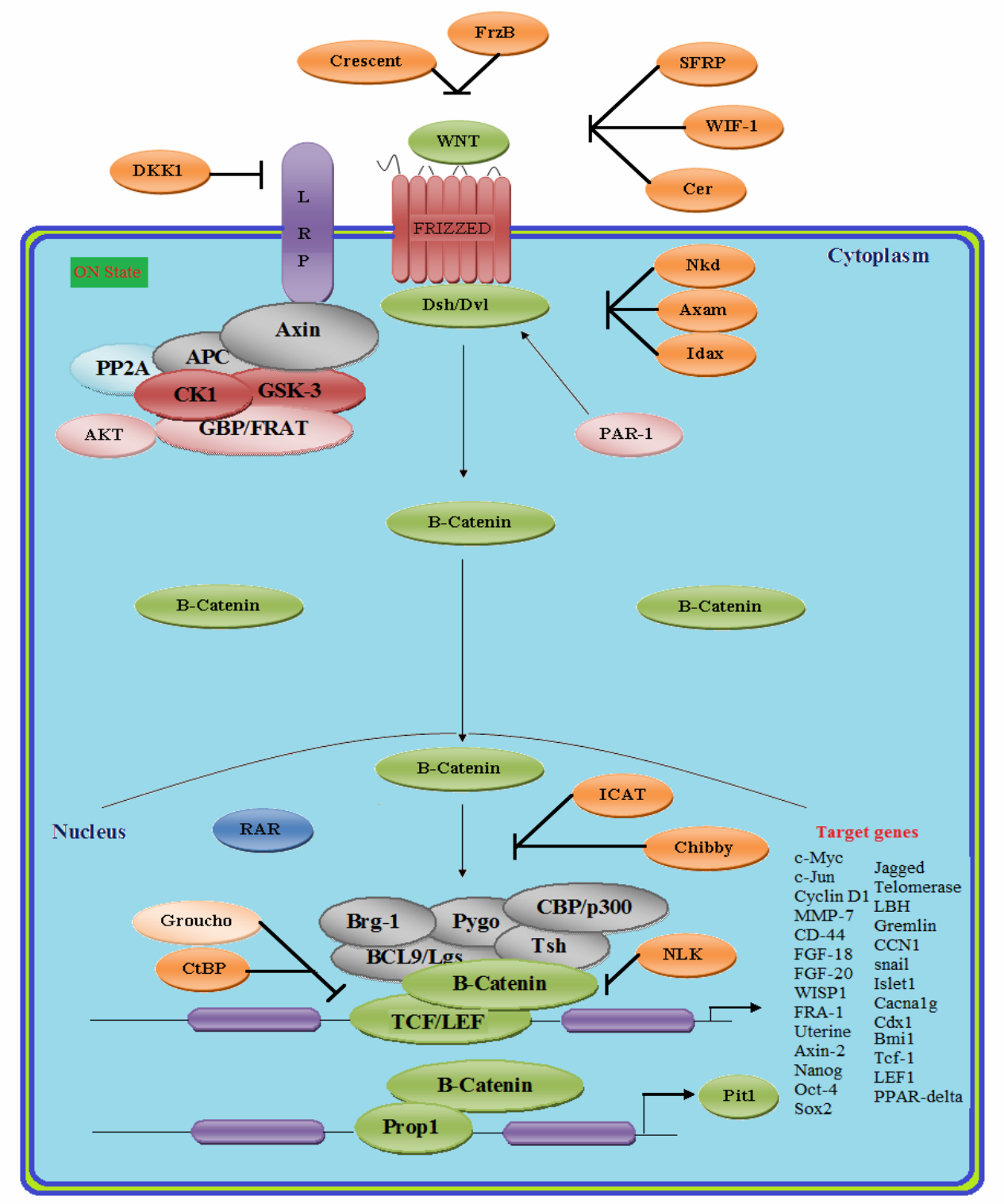


Fig. 3: Wnt/PCP signaling pathway in stem cells development.

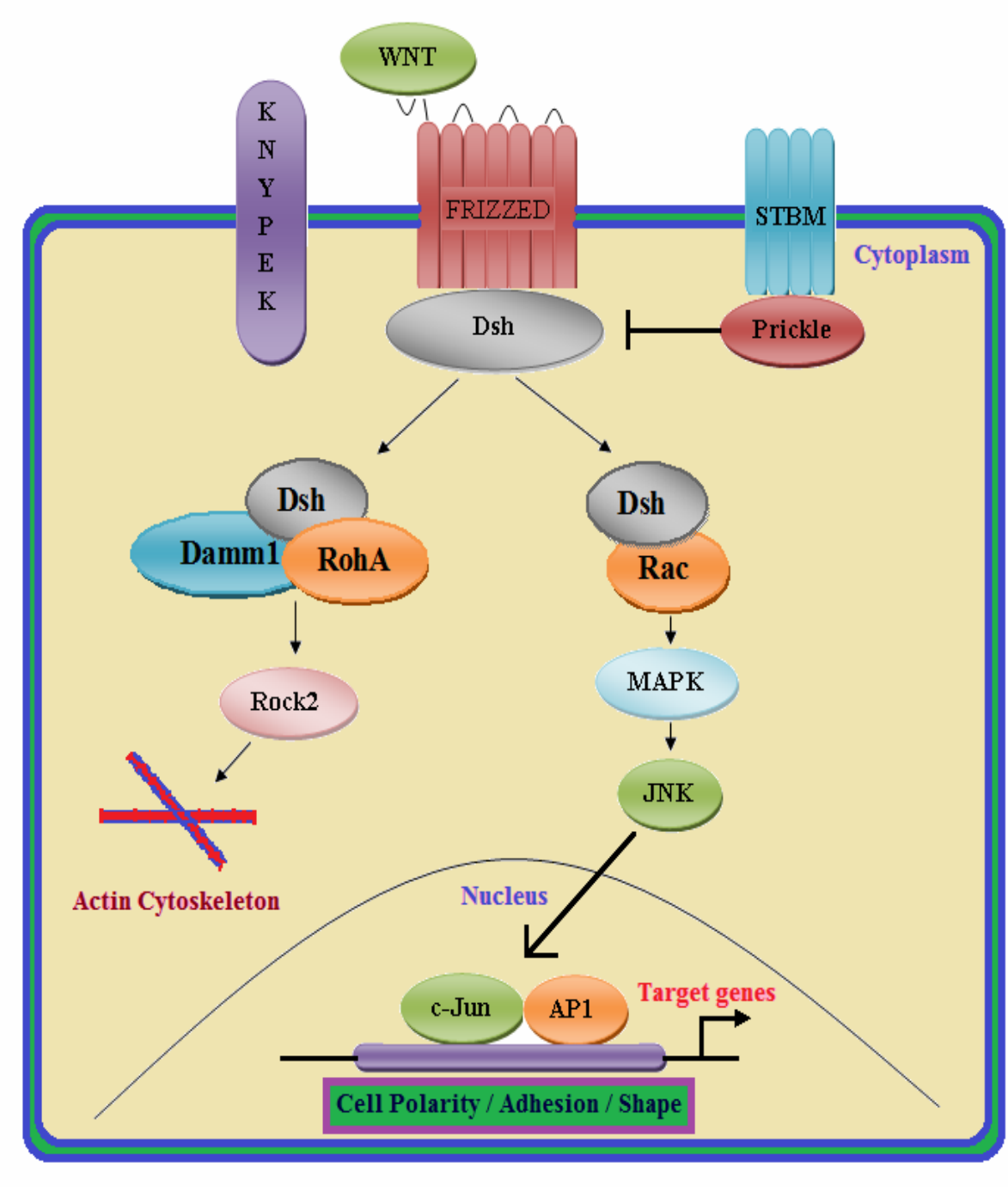
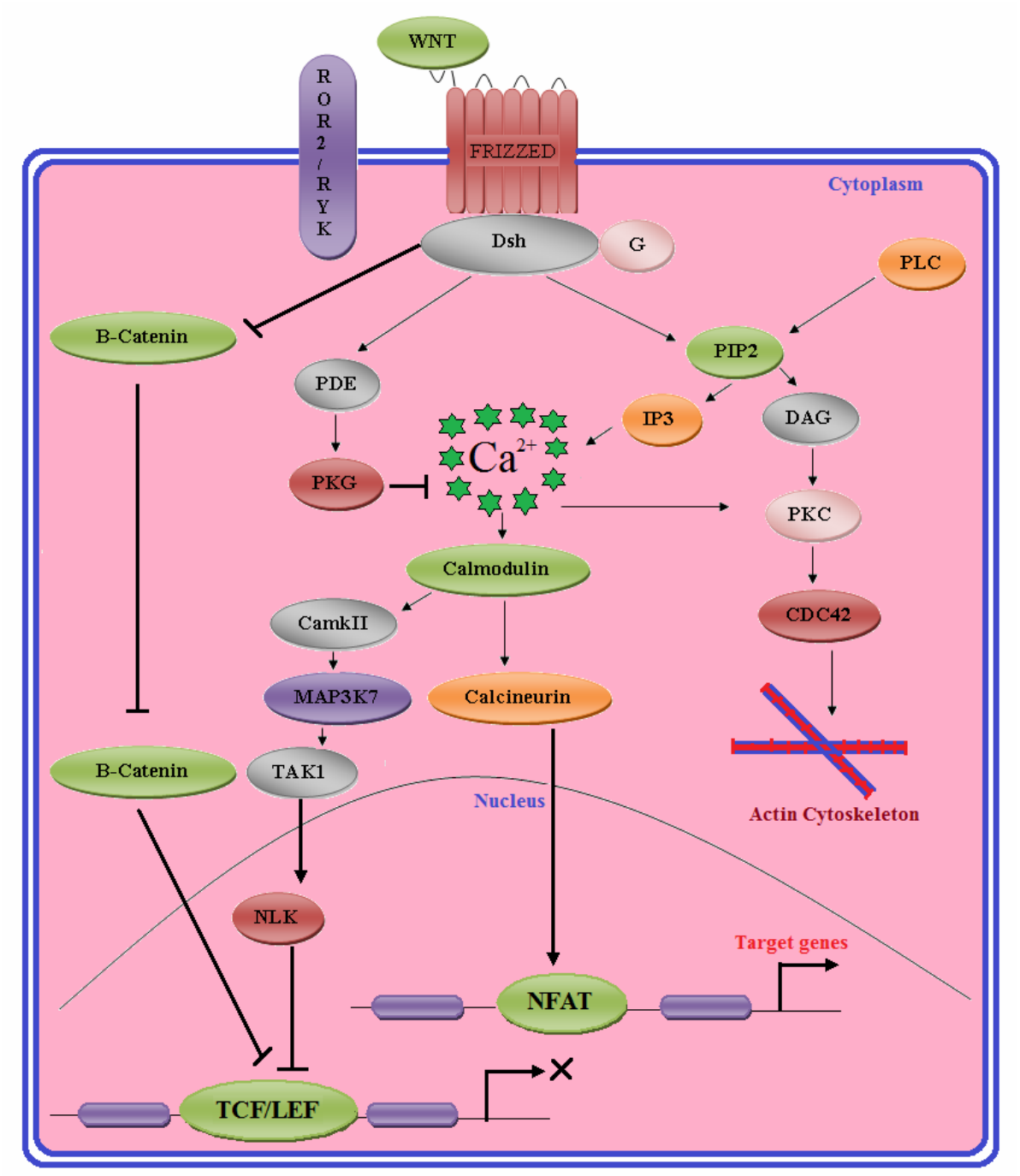


Fig. 4: Wnt/Ca^{2+} signaling pathway in stem cells development.



The complex of Dsh and G-protein leads to the activation of PIP2 through PLC/ cGMP specific PDE. Activated PLC, stimulate the cleavage of plasma membrane component PIP2 into DAG and inositol 1,4,5-trisphosphate (IP3). IP3 was bound to the ER then release the calcium ions, increase intracellular calcium level in cytosol. Another way, inhibition of PKG through PDE or activation of PLC and elevation of IP3 can stimulate the calcium release from ER (Sonderegger et al., 2007; Zhang et al., 2013). DAG activated stimulation of cdc42 through

PKC. Cdc42 is part of the major regulators of ventral patterning. Increased intracellular calcium ions level was also stimulated activation of calcineurin and CaMKII through calmodulin.

Calcineurin mediated the activation of nucler factor such as nuclear factor of activated T cells (NFAT), which regulate ventral patterning. NFAT was also regulated many functions known as regulation of cell adhesion, migration and tissues separation. CamkII activated the MAP signaling pathway, which stimulated the activation of TGF-b-activated kinase (TAK1) and Nemo-like kinase (NEMO). Activated TAK1 induces the NLK expression, which prevents the action of β-catenin and TCF/LEF transcriptional factor complex. NLK act as an antagonist of Wnt/ β-catenin signaling pathway by inactivation and phosphorylation of TCF. Despite, if PDE is activated, inhibition of calcium release is occurred from ER. Activated PDE was mediated the activation of PKG, which subsequently cause the inhibition of intracellular calcium release from ER (Zhang et al., 2013; De, 2011).

Wnt Signal in Cancer and therapeutics

Since its initial discovery, Wnt has associated with cancer development from normal cells. When Wnt1 was first discovered, Wnt1 (Wingless int-1) was first identified proto-ongogene in mouse mammary tumor virus (MMTV) in mammary carcinomas (Nusse and Varmus, 1982). In normal conditions, Wnt1 has crucial role in embryonic stem cell development. Unfortunately, deregulation of Wnt1 leads to unwanted cell growth and movement. APC was initially defined as a tumor suppressor gene in colon cancer. The complex of β-catenin and APC, activating mutation was found in β-catenin gene in human colon cancer. Another mutation was identified in human Axin1 gene, where reported in human hepatocellular carcinoma. In TCF1 act as s tumor suppressor gene, mutated TCF1 in mice enhanced adenoma in the gut and mammary glands, so finalize the TCF1 act as a feedback repressor of target gene (Nusse and Varmus, 2012). Cancers have major play role in human death rise in the World. Wnt signals are involved in many human cancer types. Breast cancer was found in human majority of female in worldwide. Expressions of Wnt/canonical pathway was identified in the development of begin and malignant breast cancer. Increased level of β-catenin in cytosol or nucleus is involved in breast cancer. Presence of β-catenin level in the cytoplasm or nucleus was identified by using immunohistochemical staining and Western blotting. The accumulation of β-catenin due to several impact factor such as lacs of β-catenin destruction, mutation in β-catenin, mutation in APC, loss of inhibitors, over expression of Wnt ligands and decreased activity of Wnt/Ca^{2+} pathway (Howe and Brown, 2004; Clevers and Nusse, 2012).

Wnt signaling was also involved in the epithelial-mesenchymal transition (EMT) to enhance metastasize in breast cancer. Repression of Wnt/β-catenin signaling in breast cancer can prevent EMT and inhibited metastasize (DiMeo et al., 2009). CTNNB1 gene encodes β-catenin, changes in CTNNB1 gene expression measured not only in breast cancer, but also lung cancer, melanoma, prostate cancer, colorectal cancer, colon cancer and other several types. Wnt ligands proteins such as, Win1, Win2 and Wnt7a increased expression have been identified in the development of ovarian cancer, glioblastoma and oesophageal cancer. Absence of other Wnt protein functions in Wnt signaling cause different cancer types such proteins as, ROR1, ROR 2, WIF1, Dsh, Wnt5a and other TCF/LEF family (Anastas Jamie and Moon Randall, 2012; DiMeo et al., 2009).

A variety of compounds are discovered for the purpose of cancer treatment based on Wnt signal expressions. Imatinib, which originally identified to inhibit β-catenin activity in cancer (Rao et al., 2006). Celecoxib, (cyclooxygenase-2 inhibitor), which inhibit β-catenin activity in human colon carcinoma (Maier et al., 2005). Antisence inhibitors have been developed to decrease β-catenin activity against several cancer types such as colon, leukemia, lymphoma cells and esophageal (Phillips et al., 2002). NASAIDs also inhibit the Wnt/β-catenin signaling pathway (Dihlmann et al., 2001). Thiazolidinedione was completely inhibited

metastasize in colon cancer (Yoshizumi et al., 2004). Many compounds are identified against cancer including these compounds.

Future works

Wnt signaling is regulated stem cell development and cancer in normal and abnormal conditions. Therapeutic system is developed a variety of drugs against cancer based on deregulation of Wnt signaling pathways. Unfortunately, many details are not described about Wnt signaling such as, where is the evolution origin of Wnt signaling?, How Wnt signals are activated and which molecules or hormones are needed for this wingless activation?, how Wnt signals are coordinated cell fate changes?, how Wnt signals are involved in self-renewal and differentiation both stem cells and cancer cells at a similar time?, many Wnt inhibitors are present inside the cells and why these inhibitors are not active in cancer cells proliferation?. These details are not evaluated and undetermined up to now. These characters are very significant for drugs design against cancer.

In the current research deals cancer drugs are directly developed based on the deregulation of signaling pathway such as Wnt signaling but many drugs are destroying the cancer cells not cancer stem cells (CSCs). These CSCs are ability to produce lots of cancer cells, another way CSCs are called as mother of cancer cells. In drug designing, lots of complexities lead to failure the action of drugs. A Variety of factors are leads to complicate the synthesis of cancer drugs and each cancer type has specific characters and causing agents. So many scientists are developing new drugs for each cancer type. In future, development of a new drug for all types of cancer caused by deregulation of the Wnt signaling pathway.

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