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Wnt pathway and breast cancer

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1. ABSTRACT

Breast cancer is one of the most debilitating human carcinomas with second highest mortality rate after lung cancer in women. Recent advancement in genetic and biochemical analyses has deciphered the molecular pathways involved in breast cancer development. Wnt signal has long been established to play a critical role in normal development as well as in tumorigenesis. In this review, we summarize the role of Wnt signal in the development of mammary carcinoma, the molecular mechanism via which Wnt signal exerts its malignant potential and various nodal points in the Wnt cascade that can be targeted for drug development and cancer treatment.

2. INTRODUCTION

The combination of Drosophila segment polarity gene *Wingless* (1) and mouse proto-oncogene *Int-1* (2, 3) led to the development of the term 'Wnt'. At present 19 Wnt genes are identified and these proteins constitute the key factors in the regulation of signal transduction in the embryonic development of the metazoan (4, 5). The origin of the pathway can be traced back to show that it is evolutionarily conserved from primitive diploblast hydra (6) to higher order mammals and even in plants (7, 8). Over the past 20 years, numerous data revealing the importance of the Wnt pathway has been generated not only in the context of development but also in cancer pathogenesis and therefore redefining cancer as a result of dysregulation of

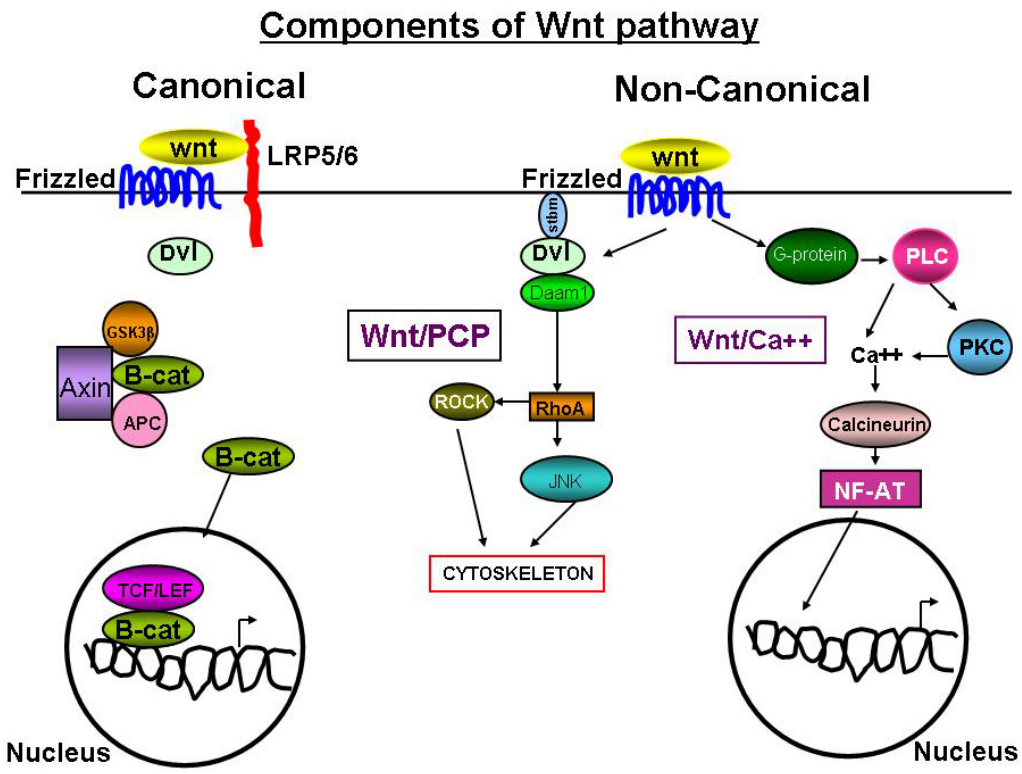

Figure 1. Components of Wnt pathway.

developmental processes. There has been an explosion of essential information regarding the Wnt pathway in recent years which on one hand increases the complexity of the Wnt pathway to an unfathomable extent and, at the same time, leads to the emergence of potential players in the development of human disease. Recently, a genome-wide RNAi (interference RNA) screening of the Drosophila cells led to the identification of 238 new regulators of the Wnt pathway. Fifty percent of them had human orthologs and out of which 18% were associated with human disease, thus adding a higher degree to the already existing intricacy of the Wnt pathway (9).

The Wnt pathway has been implicated in the specification of cell and tissue polarity, mitogenic stimulation and differentiation and also adult tissue homeostasis (10-12). Wnt proteins are palmitoylated on conserved cysteines which are essential for signal transduction (13). The Wnt proteins are also glycosylated on conserved N-linked glycosylation sites (14). A number of degenerative human diseases arise due to the dysregulation of the Wnt pathway. For example, a mutation of LRP5 causes increased bone density (15, 16), vascular defects in the eye called OPPG (osteoperosis-pseudogliioma syndrome) (17) and FEVR (familial exudative vitreoretinopathy) (18). A mutation in Axin2 leads to the development of tooth defects (19), predisposition to colon (19, 20) and liver cancer (21). Wnt signaling cascade is also an essential regulator of stem cell proliferation and self-renewal, which is supported by the fact that Wnt3a protein, *in vitro* promotes the self-renewal of hematopoietic stem cells (13). With the increasing amount of information obtained in the last few decades, it is clear that aberrant Wnt signal plays a central role not only in various human degenerative diseases but also in tumorigenesis and tumor progression. It is well established that the Wnt signaling is emerging out to be a major pathway in its contribution to the development of human cancer. In this review, we will focus on how aberrant activation of the Wnt cascade affects human breast cancer.

3. TYPES OF WNT PATHWAY

The Wnt pathway primarily consists of canonical and non-canonical pathway (see Figure 1).

3.1. Canonical pathway

The canonical pathway involves beta-catenin as the key component which is conserved from plants to higher animals and asserts its actions by transcriptional activation of target genes in the nucleus. Once Wnt is secreted, it binds to various factors like SFRP (secreted frizzled-related sequence protein) and WIF (Wnt inhibitory factor), however genetic and biochemical evidence show Frizzled (Fz) to be the primary receptors of Wnt proteins. Frizzled are seven-transmembrane receptors with CRD (cysteine rich domain) at the N-terminal for Wnt to directly bind to it (22-24). In canonical Wnt pathway, there is another single-pass transmembrane receptor called LRP6/5 which forms a trimeric complex to transduce the signal (25, 26). Frizzled is required for multiple Wnt pathways (27, 28), but LRP6/LRP5 on the other hand is specifically

required for the Wnt/β-catenin mediated pathway (29, 30). The binding of Wnt to Frizzled configures its heptahelical structure to bind and hyperphosphorylate Dishevelled (Dsh in Drosophila and Dvl in vertebrates) (31) that transduces the signal. Dvl binds to Axin at the C-terminus via its DIX (Dishevelled homologous) domain and N-terminus via its PDZ (acronym from 3 proteins: Post synaptic density protein [PSD95], Drosophila disc large tumor suppressor [DlgA], and Zo-1 protein) domain along with GSK3beta to form a ternary complex which enables Dvl to recruit FRAT1 in the absence of Wnt signal. In the presence of Wnt this complex is disrupted and signal cannot be transduced (32). The cytosolic domain of LRP6 which contains PPP(S)P motif reiterated 5 times, can also activate the Wnt pathway by phosphorylation even at a single PPP(S)P motif (33). It has been shown that LRP6/5 binds to Axin, a scaffolding protein, and localizes it to the plasma membrane followed by its degradation and thus leading to the dispersal of the beta-catenin destruction complex (33, 34). LRP6 is phosphorylated by both GSK3beta and CKI (casein kinase I) (35) as well as by CK1 gamma (36). However, the importance of the intracellular domain of LRP6 has also been shown to constitutively activate the Wnt pathway via Wnt3a-induced LEF1 irrespective of its membrane localization (37). Recent studies report that Wnt-3a triggers the internalization of LRP6 by its interaction with caveolin which facilitates the recruitment of Axin to its phosphorylated PPP(S)P domain, leading to the accumulation of beta-catenin in the cytosol (38). In the cytoplasm, Axin forms a multi-protein complex with APC (adenomatous polyposis coli) (39-41), GSK3beta and CKI (42-45). This complex facilitates beta-catenin phosphorylation by CKI and GSK3beta which enables an F-box protein in the E3 ubiquitin ligase complex, containing beta-transducing repeats, to bind and mark beta-catenin for proteasomal degradation (46-49). GSK3beta is a widely expressed Ser/Thr protein kinase which phosphorylates a variety of substrates at both primed and unprimed sites. Results of an yeast two hybrid analysis showed that GSK3beta interacts with LRP6 at the C-terminal to phosphorylate at the PPP(S)P motif to attenuate GSK3beta activity (50). In response to Wnt signal, titration of Axin from the APC-Axin-GSK3beta complex results in the disruption of the complex as it binds to the phosphorylated PPPSP motif of LRP6 and thus causing beta-catenin to accumulate in the cell cytoplasm.Finally, beta-catenin is translocated to the nucleus and binds to TCF/LEF (51, 52). The TCF family (TCF-1, LEF1, TCF-3,4) contains high mobility group box (HMG) which is responsible for binding to the target DNA (53). The beta-catenin-TCF complex is converted from a transcriptional repressor to transcriptional activator by displacing Groucho and its recruitment of HDAC (histone deacetylase) (54). The displacement of Groucho leads to the recruitment of histone acetylase CBP/p300 (cyclic AMP response element binding protein) which acts as a co-activator (55, 56). Two other protein components, BCL9 and Pygo, are also shown to potentiate the transcriptional activation of beta-catenin-TCF complex (57). The beta-catenin-TCF complex also interacts with various proteins like ICAT (58, 59) which leads to an inhibition of beta-catenin and TCF interaction and also dissociates LEF (lymphocyte enhancer factor) and CBP/p300 from the activating complex (59, 60). Therefore, a controlled mechanism exists inside the nucleus for the tight regulation of Wnt target gene expression.

3.2. Non-canonical pathway

The non-canonical Wnt pathway can be further divided into two categories: Wnt/PCP (Planar Cell Polarity) and Wnt/Ca++ pathways (61). Both pathways utilize Wnt, Frizzled and Dvl proteins as ligands and receptors, but without the involvement of beta-catenin. In Wnt/PCP pathway, Frizzled activates JNK through Strabismus (Stbm), Dvl, Daam1 and GTPase RhoA and Rho-associated Kinases (62) which modulates the cytoskeletal organization. On the other hand, the Wnt/Ca++ pathway works through the release of calcium via phospholipase C (PLC) and protein kinase C (PKC). The elevated level of cytosolic calcium activates calcineurin phosphatase which in turn dephosphorylates NF-AT and leads to its accumulation in the cytoplasm followed by its translocation to the nucleus to activate the target genes. Given the non-canonical pathway does not require the participation of either LRP6 or beta-catenin, in this review we will focus on the canonical Wnt pathway in relation to breast cancer progression.

4. ROLE OF WNT IN MAMMARY GLAND DEVELOPMENT

Several lines of recent evidence show that the Wnt pathway is critical in the development of normal mammary gland. The most prominent example is the role of Wnt-4 in the lobular development. When mammary epithelial buds from Wnt-4KO (knockout) mice were implanted in the post-natal mammary fat pad, devoid of endogenous epithelium of wild-type mice, it showed a significant reduction of lobular branching (63). Supporting this finding, the overexpression of Wnt-4 in virgin mice induced a pregnancy-like growth pattern in reconstituted mammary gland (64). In addition, Wnt signaling is also required for the development of the bud stage. In this stage, the invagination of epithelial layer takes place to give rise to a bud like structure which serves as the foundation for the mammary gland. Apart from Wnt-4, Wnt10b (previously known as Wnt-12) is also required for mammary bud development as shown by the whole mount *in situ* hybridization (65). The most convincing evidence of beta-catenin involvement in development of mammary gland came from the fact that mammary development is defective in mice with disrupted LEF1 (66). LEF1 is a transcription factor of the TCF family that associates with beta-catenin to stimulate the expression of Wnt target genes. In this context, the secretion of PTHrP by mammary epithelium is essential for the induction of LEF1 expression (67). The growth of the mammary gland requires epithelial-mesenchymal interactions that is critical for its development (68). The mesenchyme surrounding the mammary bud is required for the mammary epithelial cell fate and is mediated by the paracrine signaling of the PTHrP secreted proteins and PTHR1 receptor. Therefore, PTHrP also facilitates the induction of LEF1 for the downstream activation of Wnt signaling mediated by beta-catenin. Hence, these lines of evidence clearly indicate a

Table 1. Dysregulation of beta-catenin in clinical samples

IHC staining of Wnt component	% of cases	Type of cancer	Reference
Reduced membrane beta-catenin Reduced cytoplasmic beta-catenin. Nuclear staining beta-catenin	36 72	Breast Carcinoma Phyllodes tumor	73 74 75
Reduced immunostaining Wnt-1	95	Invasive Breast carcinoma	76
Reduced immunostaining Wnt inhibitory factor (WIF)	60	Breast carcinoma	77, 78
Reduced immmunostaining adenomatous polyposis coli (APC)	35	Primary breast cancer	74

Table 2. Animal models of Wnt pathway

Wnt Protein	Mouse model	Mammary phenotype	Status in breast cancer	Reference
Wnt1	MMTV-Wnt1	Adenocarcinoma Breast cancer	Upregulated in grade I tumor Anti-estrogen resistant ER-positive	76, 78, 82, 83 84
Wnt10b	MMTV-Wnt10b	Adenocarcinoma	_	85
Axin	MMTV-axin	Lack of alveoli	Mutation	86, 87
Beta-catenin	MMTV-ΔN89/Δ90 beta-catenin	Precocious alveolar development Adenocarcinoma	Upregulated in cytoplasm of ductal & lobular carcinoma Poor prognosis	69, 75, 88, 89
T-cell factor (TCF)	Tcf1 knockout	Adenocanthomas	_	90, 91
Lymphocyte enhancer factor (LEF)	Lef1 knockout	Arrest of mammary development at E13	_	66
Glycogen synthase kinase3-beta (GSK3-beta)	MMTV-LTR-KImGSK3beta (Kinase inactive)	Mammary tumor with upregulation of beta-catenin and Cyclin-D	Dominant negative	92
Casein kinase2-alpha (CK2-alpha)	MMTV-CK2-alpha	Adenocarcinoma	Overexpression	93, 94

direct involvement of the Wnt signaling pathway in the complex process of normal mammary gland development.

5. ROLE OF WNT PATHWAY IN MAMMARY GLAND CARCINOGENESIS

5.1. Clinical significance of the Wnt pathway in breast cancer

In contrast to the normal breast tissue, there was a remarkable difference in staining pattern of beta-catenin in malignant tissue (69, 70). Alteration in the level of beta-catenin in various stages of breast cancer tissue shows a clear dominance of dysregulation of Wnt pathway. The localization of beta-catenin in the cytoplasm or nucleus is another important criterion to determine the aberrant activation of the Wnt pathway. In addition, immunohistochemical studies have given an apparently contrasting prognostic value of phospho-beta-catenin based on its subcellular location (71). The cytoplasmic localization is associated with prolonged disease free survival whereas nuclear localization has an aggressive and reduced disease-free survival. Thus a more complicated role is played by the stoichiometry, modification and spatial regulation of beta-catenin (see Table 1).

Other than beta-catenin, human breast cancer in relation to canonical Wnt pathway shows deregulation in several stages. Recent study has shown a redundant expression of Wnt ligands for example, Wnt3a, Wnt10b, Wnt6 etc in breast cancer cell lines (72). Furthermore, immunohistochemical studies on primary breast cancer tissues have shown an elevated expression of Cyclin D1 and c-Myc both of which are direct transcriptional targets of canonical Wnt pathway. Immunostaining of Wnt-inhibitory proteins (WIF) can be a reliable parameter to assess the cancer phenotype and is exhibited by reduced immunostaining patterns. The abnormality of APC in the dysregulation of the Wnt pathway is primarily manifested by the truncation mutation though a reduced immunostaining has also been observed in breast cancer specimens. Thus the Wnt signal in the context of mammary gland has dual functions: on one hand, it is essential for the normal development of mammary gland and on the other hand, aberrant Wnt signal in mammary gland is manifested in the form of cancer. Hence, an optimal activation of the Wnt signal with tight control mechanism is necessary for bypassing tumorigenesis and malignant breast carcinoma.

5.2. Animal model of breast cancer for Wnt pathway

Wnt signal orchestrates a complex network of developmental programs but its aberrant signaling has been observed in many human cancers. Most of the common human cancers caused by Wnt signaling relates to the mutation of its various components of canonical Wnt pathway for example, beta-catenin, APC and Axin. However, evidence of mutation in Wnt pathway responsible for breast cancer is unexpectedly lacking.

A number of transgenic and knockout animal modelshave been employed to study the intricate role of Wnt pathway in tumorigenesis. Table 2 shows the mouse models with various Wnt component manipulations that lead to the mammary development defects and tumorigenesis. The first transgenic mouse was constructed with Wnt-1 having an MMTV (mouse mammary tumor virus) mediated insertional mutagenesis. This results in the transcriptional activation of Wnt-1 gene that leads to the development of lobuloalveolar hyperplasia and eventually cancer (2). Furthermore, epigenetic changes or mutation at any nodal point of the Wnt pathway also leads to the development of cancer (79-81). It is well established that dysregulation in components of the Wnt pathway are responsible for tumorigenesis, and among them, beta-catenin/TCF component has been widely used in transgenic

mouse model to elucidate and characterize canonical Wnt pathway. Other models with Axin mutation showed defective alveoli formation. Similar dysregulation of mammary development occurred in the LEF-1 knockout mice. A dominant-negative form of GSK3beta also led to the formation of breast tumor with the upregulation of beta-catenin and its downstream target Cyclin-D.

To gather further insights into the role of Wnt pathway in breast carcinoma it is essential to make animal models to delineate the precise role of each of the components which is responsible for the cause and insidiousness of the disease.

5.3. How dysregulation of Wnt pathway leads to tumorigenesis and malignant progression?

The downstream effector of the Wnt signaling, beta-catenin, is the primary component for the Wnt-mediated mammary oncogenesis. Beta-catenin is responsible for the upregulation of cell cycle regulatory molecules such as c-Myc and Cyclin D1 (88, 89). Cyclin D1 is frequently overexpressed in breast cancer and plays a major role in the mammary cell proliferation (95). Cyclin D1 is a target not only of the Wnt signaling pathway but also of other mitogenic signaling pathways (95). It was previously shown that TGF alpha and Wnt cooperatively induce mammary tumorigenesis (96) mediated by the direct interaction between beta-catenin and EGFR/erbb2 heterodimers (97). Recent evidence has shown that the dysregulation of Wnt signaling is coupled with other signaling pathways which lead to breast cancer. It has been found that increased expression of Wnt-1 in HMEC (human mammary epithelial cells) leads to activation of the Notch signaling pathway mediated by DNA damage response. The notch signaling is upregulated by the aberrant expression of notch ligands, Dll1, Dll3, Dll4, that lead to the tumorigenic transformation of the HMEC cells (98). A clear evidence of the crosstalk between erbB and Wnt signaling was shown when Wnt overexpression in HC11 mammary epithelial cells or treatment with conditioned medium from cells expressing Wnt-1 or Wnt-5a increased the expression of Cyclin D1 via the induction of EGFR (99). Cyclin D1 is required for the mitogenic signaling via EGFR in mammary tumor cells (100). The expression of Cyclin D1 was repressed when EGFR kinase activity was inhibited suggesting that Wnt-1 and Wnt-5A activated the MAPK signaling pathway by EGFR and induced mammary tumorigenesis.

Beta-catenin degradation is dependent on GSK3beta activity that is regulated by AKT protein kinase as well as Wnt ligands. Thus, a crosstalk between PI3K pathway and Wnt pathway is relevant for breast cancer progression. It has also been shown that beta-catenin is induced by the PI3K/AKT pathway in the presence of growth factors like insulin, IGFI, FGFI (101, 102). Thus, the degree of complexity of the Wnt pathway increases manifold with the involvement of other pathways and opens up new avenues to develop preventive measures to cure cancer.

A number of recent studies indicate that the Wnt pathway is also responsible for EMT (epithelial mesenchymal transition). EMT is required for gastrulation, neural crest formation, organ morphogenesis and wound healing. It is found that the EMT is also a key regulator in the process of acquisition of invasive property of cancer cells via which it traverses the ECM (extracellular matrix) during dissemination leading to metastasis. During EMT, the epithelial cells are transformed to the mesenchymal, fibroblast-like property characterized by the loss of cell adhesion and increased cell motility. It has been well documented that beta-catenin which interacts with adherens junction molecule, E-cadherin, is responsible for maintenance of tight cell-cell interaction. Due to aberrant Wnt signaling, the titration of membrane associated beta-catenin is reduced, resulting in the increased cytosolic beta-catenin which subsequently enters the nucleus to upregulate the Wnt target genes. Results of a recent immunocytochemical analysis of breast carcinoma specimens showed that there was a significant reduction of E-cadherin with concomitant increase in the expression of Snail and Slug, both of which are zinc-finger transcription factors that bind to the E-boxes in the E-cadherin promoter to repress its expression (103). This was also accompanied by the aberrant expression of MMP9 (matrix metalloprotease) that is responsible for the degradation of basement membrane of ECM (104). In another study, elevated expression of frpHE (human stromal protein of the secreted frizzled gene family) mRNA has also been observed in the stroma of *in situ* and infiltrating breast carcinoma (105).

Among other components, Axin acts as a negative regulator of the Wnt cascade. LOH (loss of heterozygosity) at a region of human chromosome 17q23-q24, where the Axin gene is located, has been observed in breast as well as other forms of cancer (106). On the contrary, Axin2 homologue has been shown to play a positive role in breast cancer by stabilizing the transcription factor Snail-1, a key regulator of EMT, in a Wnt-Axin2-GSK3beta cascade (107). Thus, it is clearly evident that dysregulation of multiple nodal points in the Wnt cascade leads to tumorigenesis and pathogenesis of cancer.

5.4. Wnt pathway, stem cells and breast cancer

Stem cells are defined as those cells which are endowed with the property of self renewal that are able to generate daughter cells, capable of giving rise to a repertoire of cells found in mature tissue. There are two different types of stem cells: a) those responsible for tissue renewal and b) those requiring appropriate stimulus for repairing of damaged tissue. The stem cells usually have a slow cycling with innumerable replicative potential and thus are favorable for the development of cancer. Several lines of evidence that show the existence of stem cell niche in mammary gland (108-112) and thus it is postulated that there is a direct relationship between mammary stem cells and breast cancer. A recent concept of cancer stem cell is redefining the origin and development of cancer. Cancer stem cell is defined as a cell within a tumor with the ability to self-renew and to cause the heterogeneous lineages of cancer. Cancer stem cells can only be defined experimentally by their ability to recapitulate the generation of a continuously growing tumor. Cancer stem

cell can also be called as "tumor initiating cell'' and ''tumorigenic cell.''

Wnt has been a subject of great attention in recent years for its multifunctional property in cell fate decision during development as well as in self renewal of cells. It has long been established that Wnt signaling plays an important role in the process of self renewal in hematopoietic stem cells (HSC) where elevation of beta-catenin takes place with the activation of TCF/LEF-1 promoter activity (113). Studies with purified Wnt-3a proteins have shown to induce self renewal properties in HSC (13) and TCF-4 which has also been shown to be essential for the maintenance of crypt stem cells in the small intestine (114). It has been recently shown that a single mammary cancer stem cell which has the power of self-renewal and multipotency, is capable of developing a complete mammary gland in premalignant tissue in MMTV-Wnt-1 mice (115). Other studies have also shown a direct role of Wnt signaling in self renewal in epidermal (116) and gut cells (117). Hence, these recent advancements in the development of cancer stem cell theory is interesting as numerous approaches can be developed to target specific cancer stem cells to curtail the development of cancer.

6. TARGETING WNT PATHWAY FOR POTENTIAL THERAPY

It is conceivable that targeting various components of the Wnt pathway, which is dysregulated in the process of cancer progression, would provide a rationale for pharmacological intervention. In the past few decades, a number of attempts have been made to curb aberrant Wnt signaling that is responsible for a wide spectrum of human cancers. The Wnt pathway has been targeted at various levels: a) the extracellular Wnt ligands b) intracellular protein level of various Wnt components, c) aberrant expression of the critical mediator, beta-catenin level and d) downstream targets of the Wnt pathway. There are also various natural inhibitors of Wnt signaling pathway and a rational approach to potentially downregulate the activated Wnt pathway. In this section, we look into the various approaches key regulators of Wnt signal for cancer therapy in general.

6.1. Beta-catenin

A multiple number of cancers arise due to beta-catenin abnormality and thus it is one of the most promising targets of the Wnt pathway. A number of strategies including RNAi, antisense and protein knock-down have been developed. Antisense approach has been used in colon cancer which resulted in the reduction of beta-catenin both at the mRNA as well as protein level that subsequently affects its downstream targets TCF and Cyclin D1 by reducing their expression (118, 119). Similar results were obtained by RNAi method not only in colon cancer (118, 120) but also in esophageal cancer (121), leukemia and lymphoma cell lines (122). NSAIDS (non-steroidal anti-inflammatory drugs) like celecoxib approved by FDA (food and drug administration) as well as EMEA (European Medicines Agency) (123, 124) are effective in decreasing the nuclear levels of beta-catenin and subsequently reduce the formation of multiple polyps in FAP patients (125). In a cell-based small molecule screening process hexachlorophene has been found to degrade beta-catenin expression by a Siah-1 (126) mediated pathway in colon cancer cells (127).

The beta-catenin–TCF complex in the nucleus is responsible for the modulation of Wnt target genes. Hence, targeting this complex would be the most appropriate approach to develop cancer therapeutics. Several studies show the presence of a constant level of beta-catenin-TCF complex in human cancer. Rational design combined with high-throughput screening can lead to the development of drugs which can disrupt the beta-catenin-TCF complex (128). The elucidation of the crystal structure of beta-catenin-TCF has probed into the molecular mechanism by which it interacts to form a stable transcription factor complex (129-131). Thus drug development utilizing the disruption of beta-catenin-TCF complex holds great promise. There has been a great deal of speculation regarding the use of NSAIDS to treat cancer as it inevitably causes serious side-effects including alimentary canal and kidney damage. Therefore, there is a considerable amount of skepticism regarding the use of NSAIDS. However, new generation NSAIDS like NO-releasing aspirin (NO-ASA) has been shown to arrest growth in colon cancer cells by inhibiting beta-catenin-TCF interaction (135,136), with a thousand-fold more efficacy than traditional aspirin administration (137-139). However, it should be noted that beta-catenin also interacts with overlapping domains with E-cadherin (132) and APC (133) and these interactions are negative contributors to the Wnt signaling. Therefore, it is a real challenge to develop small molecules which can effectively and selectively disrupt beta-catenin-TCF complex without affecting its interaction with E-cadherin or APC.

There are three natural compounds PKF115-584, PKF-222-815 and CPG049090, which are obtained from high-throughput screening (HTP) of natural compounds, which has shown to inhibit Wnt signaling in colon cancer cells (134) and also in Xenopus embryo (51). It is well established that recruitment of various co-activators are necessary for efficient activation of beta-catenin-TCF target Wnt genes. Thus if co-activators like CBP (CREB-binding proteins) and BCL9 (B-cell lymphoma)/pygopus are effectively inhibited to interact with beta-catenin-TCF complex, it would lead to the downregulation of Wnt pathway. Indeed a small molecule inhibitor, ICG-001, which selectively binds to the CBP resulted in the titration of CBP from beta-catenin-TCF complex followed by reduction of Wnt signaling effectively in colon cancer cell. Furthermore, the inhibition of Wnt signaling was accompanied by the reduced expression of anti-apoptotic gene, survivin (135).

In an adenoviral based approach, the FADD (Fas-associated via death domain) gene under the tight control of promoters containing TCF-responsive elements was introduced in colon cancer cells which effectively killed the cells, substantiating the validity of the approach.

Other viral based approaches include the generation of oncolytic viruses. The tumor cells that exhibit higher beta-catenin-TCF activity have augmented therapeutic effect of the viruses by replication in the target tumor (136). This was validated by engineering replicating viruses which express the viral E1B and E2 genes from promoters containing TCF-response elements. This was highly effective in colon cancer cells but showed a 50-100 fold decrease in lung cancer cells and normal fibroblast lacking an active beta-catenin-TCF signaling (136).

6.2. Extracellular components of Wnt pathway

6.2.1. sFRP

The sFRP (secreted Frizzled–related proteins) comprises a family of five glycoproteins that binds to the Frizzled receptor and antagonizes the Wnt signal. Reduction in sFRP's has led to the development of various types of cancer including breast and it has been shown that restoration of sFRP inhibited the growth and promoted apoptosis (76, 78, 137-143). Hence, sFRPs are potential targets to curb adverse effects of Wnt pathway by inducing apoptosis and restricting cell growth.

6.2.2. Wnt

Various methods have been employed to knock down Wnt-1 expression by antisense RNA (144). Apart from that, a monoclonal antibody (145, 146) which can neutralize the effect of Wnt-1 has also been developed. This antibody proved to be effective in a number of human cancers like breast and non-small cell lung cancer by inducing apoptosis accompanied by reduction in tumor growth in animal models (146). Similar results were observed when Wnt-2 monoclonal antibody was used to treat melanoma (147) and non-small cell lung carcinoma (148) that effectively induced apoptosis resulting in the inhibition of malignant progression.

6.3.3. Dkk

Dkk is another antagonist of Wnt pathway which prevents binding of Wnt to LRP5/6 (30) and thus has a considerable potential to serve as a therapeutic target. Dkk-1 is the most important member of the Dkk family of proteins that includes Dkk2, Dkk3, and Dkk4 (149, 150). It has been shown that expression of exogenous Dkk3 leads to cell growth inhibition in non-small cell lung carcinoma (151) as well as reduced invasion and motility in osteosarcoma cells (152).

Therefore, multiple strategies are applied to treat aberrant Wnt signaling and subsequently curb specific human cancer. However, most of the drugs are still at an infant stage. Due to the highly complex nature of Wnt signal, it is imperative to develop drugs with high specificity and efficacy.

7. CONCLUSION AND FUTURE DIRECTION

The complexity of the Wnt pathway is increasing with the identification of more key regulators and its cross-talk with other major pathways. In the context of breast cancer, evidence regarding the mutational status of various components of the Wnt pathway is sparse. Yet, dysregulation of Wnt pathway is emerging as a major cause in the development of breast cancer. The crucial questions which need to be addressed include what factors mediate the hyperactivation of Wnt pathway and what mediators stabilize beta-catenin, thereby activating the downstream effectors and Wnt target genes as well. More fundamental questions need to be elucidated, as how the negative and positive regulators co-ordinate and integrate in the cellular milieu to constitutively activate the Wnt pathway. Identification of the crucial nodal points which are responsible for the etiology of breast cancer can provide therapeutic targets to develop drugs with high efficacy opening new avenues in the treatment of breast cancer.

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