Review Article

Shared signaling pathways in normal and breast cancer stem cells

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Abstract

Recent advances in our understanding of breast cancer biology have led to the identification of a subpopulation of cells within tumors that appear to be responsible for initiating and propagating the cancer: These tumor initiating cells are not only unique in their ability to generate tumors, but also share many similarities with elements of normal adult tissue stem cells, and have therefore been termed cancer stem cells (CSCs). These CSCs often inappropriately use many of the same signaling pathways utilized by their normal stem cell counterparts which may present a challenge to the development of CSC specific therapies. Here, we discuss three major stem cell signaling pathways (Notch, Wnt, and Hedgehog); with a focus on their function in normal mammary gland development and their misuse in breast cancer stem cell fate determination.

Keywords: Cancer stem cell, hedgehog, mammary stem cell, notch, Wnt

INTRODUCTION

Breast cancer is the second most common cancer-related cause of death among women in the United States.[1] While significant advances have been made in our ability to detect and treat certain types of cancer, more targeted therapies are needed to provide improved efficacy with fewer adverse effects. In order to identify targets for new therapies, it is imperative that we continue to expand our understanding of the processes that drive cancer initiation, progression, and maintenance.

Historically, it has been thought that stochastic events causing mutations in any somatic cell will give rise to clonally evolved tumors; and that all cells within the tumor are equally tumorigenic. Recently however, researchers have identified a minor subpopulation of cancer cells within the larger tumor mass that is responsible for tumor initiation, progression and maintenance. Interestingly, these cells display characteristics and markers of normal adult tissue stem cells and have been termed cancer stem cells (CSCs) or tumor initiating cells (TICs). In breast cancer, investigators have shown that as few as 100 CD24low/CD44high/lineage- cells were able to form tumors in mice; in contrast, as many as 10,000 cells that expressed a reciprocal marker profile were unable to form tumors.[2]

The concept of CSCs is consistent with observations made by scientists over many years that there exists a remarkable similarity between normal development and the development of cancer. Mammalian development for example, begins with a single cell that must grow, divide, and differentiate in order to give rise to a complex being. The hallmarks of cancer are all traits that exploit these same processes, but through their
inappropriate utilization. Like normal development, a tumor also arises from a single cell, but grows uncontrollably, fails to differentiate, evades apoptosis, sustains nourishment through angiogenesis, and inappropriately invades surrounding tissues.\(^{[3]}\)

The remarkable parallels between normal development and cancer progression are evident even at the molecular level. In order to accomplish the incredible task of normal human development, complex signaling pathways are in place which allow cells to communicate with each other and their surrounding environment. Not surprisingly, many of the same signaling pathways are (mis)used by tumors and CSCs. Here, we will review three predominant signaling pathways that regulate normal and cancer stem cell function: Notch, Wnt, and Hedgehog.

**NOTCH SIGNALING**

**Overview**

Notch signaling is a crucial regulator of development and cell fate in a variety of tissues.\(^{[4]}\) The Notch transmembrane receptors (Notch1-4) are highly conserved and function as juxtaclirnel mediators of cell–cell interactions with surface-bound ligands (DSL ligands: JAG1, JAG2, Delta-like (Dll)-1, Dll-3, Dll4). The mature Notch receptors are type 1 transmembrane proteins with large extracellular domains, consisting primarily of epidermal growth factor (EGF)-like repeats. Upon binding of an appropriate ligand, the Notch receptor undergoes a two-stage cleavage by the tumor necrosis factor-α-converting enzyme (TACE) and γ-secretase, ultimately resulting in cleavage of the Notch intracellular domain (NICD). The NICD is then free to translocate into the nucleus and interact with the RBP-J (also known as CSL) family of transcription factors (TFs) and the co-activators of the Mastermind (Mam) family\(^{[5,6]}\) (Figure 1).

In the absence of NICD, CSL is associated with ubiquitous corepressors and histone deacetylases (HDACs). Upon binding to CSL, NICD displaces the corepressors and facilitates the recruitment of coactivators, thereby promoting the transcription of Notch target genes such as the Hes and Hey family of transcriptional regulators [Figure 1]. In addition to these, Notch activation has been shown to target genes involved in cell cycle regulation and oncogenesis, such as cyclin D1,\(^{[7]}\) p21/Waf1,\(^{[8]}\) NF-κB,\(^{[9]}\) and c-myc.\(^{[10,11]}\) Thus, Notch signaling is a major regulator of cell proliferation and stem cell fate determination.

**Notch signaling in normal mammary gland development**

Activation of the Notch signaling pathway has been shown to be involved in normal mammary gland development by influencing cell fate and differentiation decisions. While little is known about the differential downstream signaling of the various Notch receptors (Notch1-4), it is believed that individual receptors may play distinct roles in regulating mammary gland development by cell-type specific distribution of various Notch receptors. For example, Notch-1 and -3 have been shown to be expressed in the luminal cells of the normal mammary gland.\(^{[12,13]}\)

A detailed transcriptome analysis of bipotent and luminal committed progenitors showed that Notch-3 expression was substantially increased in the luminal-restricted colony forming cells (CFCs).\(^{[13]}\) Furthermore, inhibition of Notch-3 by either γ-secretase inhibitors, dominant negative MAML or by shRNA, was sufficient to inhibit in vitro generation of luminal cells from the bipotent CFCs.\(^{[13]}\)

In contrast to Notch-1 and -3, Notch-4 is restricted to the basal and myoepithelial compartments.\(^{[12,14]}\) Mammary stem cells (MaSCs) have also been associated with the basal or suprabasal compartment and it is not surprising then that Notch-4 is reported to be expressed within the MaSC population.\(^{[12,13]}\) Early work suggesting a role for Notch-4 in MaSCs came from Notch-4 (int-3) transgenic mice, a constitutively active form of Notch-4.\(^{[16,17]}\) These studies demonstrated that mammary gland specific expression of Notch-4 (int-3) by insertional mutagenesis of the mouse mammary tumor virus (MMTV) resulted in severely impaired mammary ductal growth and lactation-deficient females.\(^{[16]}\) Furthermore, these mice showed glandular hyperplasia that developed into poorly differentiated mammary adenocarcinomas, which also suggests a potential role for Notch-4 as a proto-oncogene (discussed further below).

Subsequently it was shown that restriction of Notch-4 (int-3) to the secretory mammary epithelium, under the control of the whey acidic protein (WAP) promoter, inhibited the differentiation of secretory lobules during gestation, again suggesting a role for Notch-4 signaling in normal mammary gland development and cell-fate determination.\(^{[18]}\) This work was followed by in vitro studies, which showed that overexpression of the constitutively active form of Notch-4 inhibited normal branching morphogenesis\(^{[19]}\) and disrupted normal alveolar organization / cell polarity.\(^{[20]}\)

Recent studies have shown that activation of the Notch signaling pathway promotes self-renewal of MaSCs, and enhances mammosphere formation (an in vitro assay for stem cell self-renewal) and bipotent CFCs. Conversely, the inhibition of Notch signaling by blocking antibodies or γ-secretase inhibitors completely abolishes secondary mammosphere formation.\(^{[21]}\) Furthermore, in transcriptome
analysis of mammary epithelial cells, Raouf et al. showed that Notch-4, specifically, was highly expressed in bipotent CFCs and that its expression decreased nearly 50-fold during luminal differentiation and to a lesser extent during myoepithelial cell differentiation. Taken together, these studies have clearly demonstrated a critical role of Notch signaling during normal mammary gland development and cell fate determination; in addition, these studies have suggested a potential role of the Notch pathway in aberrant oncogenic signaling.

**Notch signaling in breast cancer and cancer stem cells**

A recurring theme in this field is the utilization of the same signaling pathway in both normal and cancer stem cells. The notch signaling pathway provides a perfect example of the antagonistically pleiotropic effects a signaling pathway can exert. As mentioned earlier, the role of Notch signaling in breast cancer was initially identified as a frequent MMTV integration site. It was not until later that the integration site was recognized as a cause of aberrant expression of the intracellular domain of Notch-4 gene. Further studies have demonstrated that ectopic expression of Notch-4 (int-3) in the non-malignant MCF-10A breast cell line resulted in transformation, aberrant morphogenesis, invasion, and tumor formation, when implanted in immunocompromised
mice. Overexpression of various Notch receptors has now been identified in ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC). More recently, Notch-4 signaling activity was shown to be eight-fold higher in the breast cancer stem cell (CSC) population when compared with the non-stem cell population.

In addition to Notch-4 signaling in breast cancer, Notch-1 and -3 have also been identified as proto-oncogenes. Notch-1 has been particularly well studied since its role in carcinogenesis was first identified in MMTV/myc transgenic mice. This study reported that a high proportion of c-myc-induced tumors harbored activating mutations in Notch-1. The role of Notch-1-ICD in oncogenesis is not, however, restricted to myc-induced tumor models. Notch-1 has also been identified as a target for MMTV insertional activation in MMTV/neu transgenic mice. To validate these findings, the truncated 3' Notch-1 ICD was shown to be sufficient for transformation of HC11 mouse mammary epithelial cells in vitro. Notch-1 was also shown to be a downstream mediator of oncogenic Ras signaling that was necessary to maintain the neoplastic phenotype in Ras-transformed human cells. Finally a proto-oncogenic role for Notch-1-ICD was demonstrated by generating Notch-1-ICD transgenic mice. In addition, this study also generated Notch-3-ICD transgenic mice and found that both Notch-1- and Notch-3-ICD strains developed mammary gland tumors and exhibited very similar phenotypes.

Thus, it is evident that Notch signaling is crucial to mammary gland development, CSC renewal, and cell fate determination.

**WNT SIGNALING**

**Overview**

Like most important developmental signaling pathways, Wnt signaling is an ancient and highly conserved system that participates in the coordinated development of various organisms. Wnt was initially identified as a porto-oncogene (int-1), similar to many of the Notch genes discussed earlier. It was later found to be the mammalian homolog of the Drosophila gene wingless (wg), and thus the name Wnt was created as a fusion of the two terms.

The Wnt family of proteins consists of 19 secreted glycoproteins that function as ligands for receptor-mediated signaling pathways. These signaling pathways participate in a variety of developmental processes throughout embryogenesis and in adults. The receptors for the Wnt ligands belong to the Frizzled family of proteins, which are seven-pass transmembrane domain G protein-coupled receptors (GPCRs). In addition to the Frizzled (Fz) family of receptors, Wnt ligands have recently been shown to activate receptor tyrosine kinases Ror2 and RYK, which may indicate a degree of promiscuity for Wnt ligands.

Activation of the Fz receptors by Wnt ligands can result in two distinct signaling pathways: canonical and non-canonical. Non-canonical Wnt signaling (which functions independent of β-catenin) will not be discussed in detail here, instead we will focus on canonical Wnt signaling, which is most suitable in the context of this review. The canonical Wnt signaling is initiated by the binding of the Wnt ligand to the Fz receptor and its co-receptors, low-density lipoprotein receptor-related protein 5 or 6 (LRP5 or LRP6). In the absence of this interaction, cytoplasmic β-catenin is continually degraded by the Axin complex, which is made up of glycogen synthase kinase 3 (GSK3), casein kinase 1α (CK1α), Axin, and the tumor suppressor adenomatous polyposis coli (APC). This complex promotes the phosphorylation of the amino terminal region of β-catenin, which targets the protein for ubiquitin-dependent proteasomal degradation. Lack of nuclear β-catenin allows the T-cell factor / lymphoid enhancer factor (TCF / LEF) family of transcriptional activators to be inhibited by Groucho. However, in the presence of Wnt signaling, the Fz-LRP5/6 complex activates the Dishevelled (Dvl) scaffolding protein, which phosphorylates LRP5/6 and promotes the recruitment of the Axin complex to the receptors and away from cytoplasmic β-catenin. This allows for stabilization and nuclear accumulation of β-catenin, which displaces Groucho on the TCF / LEF proteins and recruits other co-activators (e.g. CBP, BCL9, and Pygo), thereby allowing transcription of target genes [Figure 1].

**Wnt signaling in normal mammary gland development**

Early evidence of Wnt signaling in mammary gland development came from MMTV-driven expression of Wnt1, which, in addition to causing adenocarcinomas, also resulted in an increase in the number of terminal end buds (TEB). Further evidence for the role of Wnt signaling in mammary gland development came from mice lacking the Lef-1 transcription factor or the Lrp6 co-receptor, and from mice overexpressing the Wnt inhibitor Dickkopf, all of which failed to develop an appropriate mammary bud. More recently, experiments using reporter mice of Wnt activity (Axin2-LacZ) showed positive staining in nearly all branches of the mammary ductal system; more importantly, Wnt activity was detected in cells located in the basal layer of the mammary ducts which has been suggested to be the MaSC niche.

Although Wnt-1 and Wnt-3 have clearly been implicated in
driving MMTV-associated tumors.\cite{30, 41, 42} neither is expressed in the adult mammary gland. However, other members of the Wnt family, such as Wnt-2, 4, 5a, 5b, 6, and 7, are expressed at various stages of mammary development.\cite{43, 44} Genome-wide transcriptome analysis has shown that Wnt-2, 5a, and 7b are enriched in the TEB, whereas Wnt-4, 5b, and 6 are enriched in both the TEB and the mature duct.\cite{46} Levels of the various Wnt proteins also change according to the stages of mammary gland development. In the virgin mouse mammary gland, Wnt-2, 5a, and 7b are strongly expressed, but these are downregulated during pregnancy; in contrast, pregnancy strongly induces the expression of Wnt-4, 5b, and 6.\cite{44}

Functional studies have confirmed the essential role of Wnt proteins in mammary gland development. For example, overexpression of Wnt-4 was shown to result in an increase in ductal branching,\cite{47} suggesting its role in pregnancy-induced side branching, which is consistent with its temporal expression.\cite{44} This was further substantiated by observations that the mammary tissue of Wnt-4-/- mice exhibited significantly reduced ductal branching compared to their wild-type counterparts.\cite{48}

**Wnt signaling in breast cancer and cancer stem cells**

Wnt1 was initially identified, by Nusse and Varmus as an insertion site for MMTV-associated oncogenesis and was therefore classified as a proto-oncogene.\cite{30} Since then, we have learned a great deal about the Wnt signaling pathway and not surprisingly, many Wnt family members and downstream effectors have been implicated in oncogenesis. Soon after the identification of Wnt-1, Wnt-3 was identified by Nusse and colleagues as yet another common insertion site for MMTV-induced oncogenesis.\cite{41, 42} More importantly, mammary gland–specific expression of stabilized β-catenin, the effector molecule of canonical Wnt signaling, has been shown to result in aggressive adenocarcinomas, consisting predominantly of glandular and undifferentiated cells.\cite{49}

Interestingly, mutations in Wnt signaling are not common in breast cancer although one study has shown that approximately 50% of clinical cases exhibit stabilized β-catenin.\cite{30} In line with these findings, Bafico et al. found that some human breast and ovarian cancer cell lines exhibited high levels of stabilized β-catenin without mutations in downstream components. The authors further demonstrated that extracellular Wnt inhibitors could attenuate β-catenin levels, suggesting aberrant autocrine signaling as a possible mechanism for increased levels of uncomplexed, transcriptionally-active β-catenin in these cancer cell lines.\cite{51}

In addition to alterations in β-catenin, there are many reports documenting the inactivation of negative regulators of the Wnt signaling pathway or overexpression of positive regulators. For example Dvl, a positive regulator of Wnt signaling, is amplified in 50% of breast cancers.\cite{52} In contrast, Frizzled-related protein 1 (FRP1), a secreted Wnt inhibitor, was reportedly lost in 78% of malignant breast cancers making it one of the most frequent alterations in breast cancer if these findings are confirmed.\cite{53} Finally, expression of APC, a negative regulator of Wnt signaling, is lost in approximately 36 – 50% of breast cancers either by mutation, loss of heterozygosity, or hypermethylation.\cite{54, 55} Transgenic mouse studies confirm the role of APC in breast cancer. Using K14-Cre or WAP-Cre, Kuraguchi et al. deleted a single APC allele in either the mammary stem / progenitor population or luminal cells of lactating mice, respectively. Interestingly, mammary tumors only developed when APC was deleted in mammary progenitor cells, suggesting Wnt-induced tumorigenesis targets the stem / progenitor population.\cite{56}

Further support for the role of Wnt-1 in targeting MaSCs for oncogenesis comes from Shackleton et al., who found a 6.4-fold expansion of MaSCs in premalignant MMTV-Wnt1 transgenic mammary glands.\cite{40} Finally, Varmus and colleagues have shown that the pre-neoplastic lesions and tumors of Wnt-1 mice have expanded stem / progenitor cell populations, as identified by keratin 6 and Sca-1 markers.\cite{57} The tumors are also found to contain a mixture of luminal and myoepithelial cells with identical mutations, suggesting a common cell of origin. Notably, similar results have been obtained from transgenic mice expressing β-catenin or c-myc, two downstream components of Wnt signaling. However, mammary tumors in transgenic mice expressing Neu, H-Ras or plvoma middle T antigen do not show a similar enrichment for MaSCs.\cite{57}

Taken together, these studies provide compelling evidence that Wnt signaling is not only an important oncogenic pathway, but that MMTV-Wnt1 preferentially targets mammary stem / progenitor cells for transformation.

**HEDGEHOG SIGNALING**

**Overview**

Hedgehog (Hh) was initially identified in *Drosophila* larvae as a segment polarity gene.\cite{58} The signaling pathway is now recognized as an essential signaling pathway that controls tissue polarity, patterning, and stem cells maintenance in a variety of tissues.\cite{59} In vertebrates, there are three Hh homologs known as: Sonic hedgehog (Shh), Desert hedgehog (Dhh), and Indian Hedgehog (Ihh). The Hh proteins are post-translationally modified before they are secreted by the transmembrane receptor, Dispatched.\cite{56} Receptors for
Hh ligands are the Patched (Ptc1 or Ptc2) 12-pass transmembrane proteins. In the absence of Hh, the Ptc receptor inhibits the coreceptor Smoothened (Smo), by preventing its localization to the primary cilium. Inhibition of Smo results in the phosphorylation and cleavage of two transcription factors Gli2 and Gli3. The cleaved forms of these transcription factors act as repressors of target gene transcription. Upon binding of Hh to its receptor, Ptc no longer represses Smo and consequently Gli2 and Gli3 are not cleaved and instead function as activators of Hh target genes[61] [Figure 1].

**Hedgehog signaling in normal mammary gland development**

The three vertebrate Hh ligands (Shh, Dhh, and Ihh) are expressed at various stages of mammary gland development; however, no clear temporal pattern of expression has emerged. For example, Shh and Ihh are expressed early in embryonic epithelial bud formation (E12.5)[62] whereas Ihh and Dhh are expressed in the pubertal mammary gland, but only Ihh levels are upregulated during pregnancy and lactation.[63] Despite the expression of these Hh ligands, current evidence from knockout models suggests that the individual Hh ligands are not indispensable for mammary gland formation.

Transplantation studies of mammary gland tissues from Shh knockout mice into either the cleared fat pad or renal capsule of wild-type mice showed no differences in response to pregnancy when compared to wild-type transplants.[64] The same results were observed in the transplanted Ihh knockout tissue.[64] Notably, Ptc1 levels were unchanged between the Shh knockout tissue and wild type, suggesting that Shh and Ihh may function in redundant pathways. Furthermore, Dhh knockout female mice showed no obvious phenotypes and were able to nurse their pups, suggesting that the mammary gland function was largely unchanged.[65] Again, the lack of a phenotype may be explained by compensatory mechanisms from the remaining Hh homologs.

In order to better gauge the role of Hh signaling in mammary gland development, targeting a common downstream component of the signaling pathway, such as Ptc1 or Gli family members, may yield more relevant findings. Indeed, Ptc1 haplo-insufficiency has been shown to cause ductal hyperplasia, dysplasia, and failure of post-pubertal ductal elongation[63, 66]. Furthermore, Ptc1−/− mice were found to have an expansion of mammary progenitor cells defined as Lin−/CD24−/CD29low[67]. Furthermore, while the embryonic lethality of Ptc1-null mice prior to rudimentary mammary gland development makes direct functional analysis difficult,[68] haplo-insufficient Ptc1 mutants exhibit a complete failure of mammary gland development or defects in ductal elongation and growth.[69]

In contrast to Ptc1, functional analyses of Gli1 have provided conflicting results. Gli1-LacZ reporter was found to be absent throughout embryonic and postnatal mammary development.[69] Furthermore, targeted deletion of Gli1 showed no apparent phenotype.[70] In contrast, both Gli2 and Gli3 are expressed in the mammary epithelium at various stages of development, in addition to being present in the mammary stromal compartment.[69, 71] Further evidence of the role of Gli2 in the mammary gland came from transplantation of the Gli2-null mammary tissue, which resulted in abnormally branched and distended ducts.[71] These results suggest that abnormal constitutive Hh signaling results in an expansion of an undifferentiated progenitor cell population.

**Hedgehog signaling in breast cancer and cancer stem cells**

The first evidence of a role for Hh signaling in cancer came from the identification of human Ptc1 mutations in patients with Gorlin’s syndrome, characterized by the early onset of multiple basal cell carcinomas (skin cancer).[72] However, these patients do not have an increased susceptibility to breast cancer; in fact, mutations within the Hh signaling pathway have only been identified at low frequencies in breast cancer, while others have found no differences.[73-75]

In addition, studies of the expression levels of Ptc1 have yielded conflicting results. Recent comparative genomic hybridization analyses have found a loss of the chromosomal region that includes Ptc1 in 19% of primary breast cancer specimens and 33% of breast cancer cell lines.[76] In line with these findings, others have shown that Ptc1 levels are reduced in 50% of DCIS and IDC.[78, 79] In contrast, other studies have found a positive correlation between the expression of Ptc1 in IDC and the proliferative index marker Ki-67.[77]

Analyses of the remaining components of Hh signaling have provided slightly more consistent findings. The co-receptor Smo, for example, was found to be increased in DCIS and IDC in several independent studies.[77, 79, 80] Furthermore, expression of a constitutively-active form of Smo (SmoM2) in transgenic mice resulted in increased proliferation, altered differentiation, and ductal dysplasia;[79] however, long-term studies with these mice have not yet shown an increase in tumor formation.[81] In breast cancer cell lines showing high levels of Hh signaling proteins, inhibition of Smo by cyclopamine has been shown to induce apoptosis.[80]

Finally, the Hh target genes Gli1 / 2 were also increased...
in DCIS and IDC.\textsuperscript{173} The Gli1-overexpressing transgenic mouse was the first Hh pathway mouse model to produce tumors. The tumors that arise in this model exhibit a basal epithelial phenotype, with a basal keratin expression profile.\textsuperscript{192} Notably, it has been reported that the basal subtype of breast cancer is highly enriched for breast cancer stem cells. Consistent with these findings, Liu et al. found that Gli1 and Gli2 were overexpressed in both normal and breast cancer stem cells and that inhibition of Hh signaling resulted in a reduction of mammosphere formation.\textsuperscript{185}

Thus, there is emerging evidence that hedgehog signaling is important for both mammary gland development and cancer stem cell maintenance.

**STEM CELL SIGNALING CROSSTALK**

While the stem cell regulators described here have distinct signaling pathways and functions, it has also become clear that these pathways are coordinated during development through direct and indirect crosstalk. Often, reciprocal activation of various components within two distinct signaling pathways has been observed. For example, there is a correlation between elevated levels of mTOR and Notch signaling in poorly differentiated breast cancers.\textsuperscript{184} Furthermore, Notch receptor activation has been shown to induce the expression of Shh in neural stem cells via cytoplasmic signals, such as, Akt, STAT3, and mTOR.\textsuperscript{185} Conversely, Hh signaling through Gli1 upregulates the expression of the Notch target gene \textit{fag2}.\textsuperscript{186} Other reports suggest that Shh signaling helps reinforce the cell fate decisions executed by Notch.\textsuperscript{187}

Interactions between Wnt and Hh have also been identified. In this context, Hh signaling inhibits Wnt signaling via Gli1 / 2-dependent upregulation of the secreted frizzled-related protein 1 (sFRP1). In mouse embryonic fibroblasts, ectopic Gli1 expression is sufficient to prevent cytoplasmic accumulation of β-catenin; notably, this phenotype can be rescued by inhibiting sFRP1 expression.\textsuperscript{188} Identification of Wnt and Notch as MMTV-associated oncogenes also suggests a possible relationship between these pathways. Early studies established a role for Notch signaling in Wnt-1 induced transformation; Wnt-1 transformed primary human mammary epithelial cells (hMECs) upregulate Notch signaling through the expression of the Notch ligands Dll1 / 3 / 4.\textsuperscript{189} More importantly, inhibition of Notch ligands prevents Wnt-1-induced transformation.\textsuperscript{189}

Together, these interactions help us better understand the dynamics of stem cell maintenance. The interactions among the Notch, Wnt, and Hh pathways will become important as we begin to understand complex developmental programs, maintenance of tissue homeostasis, and tumorigenesis. As we focus on targeting cancer stem cells for the next generation of anti-cancer therapies, a more comprehensive understanding of the crosstalk between these pathways will be crucial for developing effective and selective combination therapies.

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