Review

Developmental Signalling in Maintenance and Regulation of Cancer Stem Cells

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Tissue stem cells self-renew throughout the life of an organism thereby maintaining tissue homeostasis and prevent cancer. The major signalling pathways such as Wnt, Notch and Sonic hedgehog control the stem cell regulation and their deregulation leads to cancer. Recent evidences showed that there exists a subset of cells within tumour termed as cancer stem cells (CSCs). These CSCs escape the conventional chemo-radiotherapy and further lead to tumour relapse followed by metastasis. This review focuses on the developmental signalling pathways that are involved in the regulation and maintenance of normal stem cells and CSCs. Understanding the molecular mechanism may be useful to specifically target the CSCs while sparing the normal stem cells to reduce tumorigenecity.

INTRODUCTION

Tumour is composed of a heterogeneous group of cells with different morphologies and behaviour. Research in cancer biology indicates that several cancers are supported by a small subset of cells with stem cell like properties and are termed as cancer stem cells (CSCs) or tumour initiating cells (TIC). Evidences of CSCs involved in resistance to conventional therapies, leading to metastasis and tumour recurrence is abundant (Beck and Blanpain, 2013; Chandler and Lagasse, 2010; Prince and Ailles, 2008).

As early as 1937, Furth and colleagues demonstrated that a single cell was able to produce a haematopoietic malignancy on implantation in mice (Furth et al., 1937). This suggested that certain cells within a tumour may have the ability to give rise to tumour growth (Furth et al., 1937). Later, in 1994, John Dick's group identified human acute myeloid leukaemia-initiating cells using CD34−CD38+ markers and showed that these cells initiated tumour (Lapidot et al., 1994). In 1997, Bonnet and Dick showed for the first time that the CD34−CD38+ population of cells had the self-renewal property. The authors performed limiting dilution assay to show that low numbers of CD34−CD38+ cells were able to form tumours in NOD/SCID mice, identical to donors; whereas considerably higher

Key words: Cancer stem cells (CSCs); EMT, Epithelial to mesenchymal transition; Lineage tracing; β-catenin; NICD, Notch intracellular domain.

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numbers of non-CSCs (CD34+CD38−) were unable to form tumours (Bonnet and Dick, 1997). These cells were coined as cancer stem cells. In 2003, Michael Clarke's group reported the first isolation of CSCs from breast tumour (Al Hajj et al., 2003). Subsequently, the presence of CSCs in other solid tumours like melanomas, hepatocellular carcinoma, glioblastoma, pancreatic cancer, colorectal cancer and head and neck cancer have been identified (Keshet et al., 2008; Li et al., 2007; Ma et al., 2007; Prince et al., 2007; Ricci-Vitiani et al., 2007; Singh et al., 2004). The CSC markers from various cancers are listed in the Table 1. The characterisation of CSCs uses various assays that include: sphere-forming assay, serial transplantation assay in NOD/SCID mice and in vivo lineage tracing. Serial transplantation assay, is considered as 'gold standard' assay, and measures self-renewal as well as the tumorigenic property of CSCs in vivo (Al Hajj et al., 2003; Beck and Blanpain, 2013; Bonnet, 1997; Prince et al., 2007). Recently the strongest evidence for existence of CSCs has come from the lineage tracing experiments in mice model for various cancers such as glioblastoma, skin and colon cancers. The assay showed that the individual fluorescent tagged cells have the capability to give rise to a tumour (Chen et al., 2012; Driessens et al., 2012; Schepers et al., 2012).

Although many different markers for CSCs have been identified in tumours of different tissues, cells isolated by using these markers are not a pure CSC population. Hence, one of the major challenges is the isolation of a pure population of CSCs. Recent study on quantitative proliferation dynamics of hair follicle stem cells showed the isolation of stem cells based on their cell division. This suggests that it may be possible to isolate pure stem cell population (Waghmare and Tumbar, 2013; Waghmare et al., 2008). Another challenge is to understand how these CSC populations are regulated and maintained. Therefore, it is important to study the various signalling pathways that are crucial for survival of CSC population.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Cancer stem cell markers</th>
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<tbody>
<tr>
<td>Leukaemia</td>
<td>CD34+CD38− (Bonnet, 1997)</td>
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<tr>
<td>Breast Cancer</td>
<td>CD44+CD24 (Hajj et al., 2003); ALDH1+ (Ginesteir et al., 2007); CD133+ (Wright et al., 2008)</td>
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<tr>
<td>Head and Neck</td>
<td>CD44+ Lin (Prince et al., 2007); A1DH1+ (Clay et al., 2010; Krishnamurthy et al., 2010); CD133+</td>
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<td>Cancer</td>
<td>Zhang et al., 2010; CD10+ (Fukusumi et al., 2014); CD98 (Matens de Kemp et al., 2013)</td>
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<td>Pancreatic Cancer</td>
<td>CD44+CD24ESA+ (Li et al., 2007); c-Met (Li et al., 2011)</td>
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<tr>
<td>Liver Cancer</td>
<td>CD133+ (Ma et al., 2007); CD90+ (Yang et al., 2008); CD133+ (Haraguchi et al., 2010); OV6+ (Yang et al., 2008)</td>
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<tr>
<td>Glioblastoma</td>
<td>CD133+ (Singh et al., 2004); SSEA1+ (Son et al., 2009); MET (De Bacco et al., 2009)</td>
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<tr>
<td>Melanoma</td>
<td>ABCB5 (Keshet et al., 2008)</td>
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<td>Colorectal Cancer</td>
<td>CD133+ (Ricci-Vitiani et al., 2007); CD166+ (Dalerba et al., 2007; Vermeulen et al., 2008); Lgr5+ (Barker et al., 2007; Vermeulen et al., 2008); CD44+ (Haraguchi et al., 2008); CD44v6+ (Todaro et al., 2014)</td>
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**Embryonic developmental process and cancer stem cells**

Development of an organism is regulated at the molecular level by various signalling pathways, and deregulation in these molecular mechanisms leads to cancer formation. Recent studies have shown various similarities between cancer and development. During the normal developmental process, undifferentiated embryonic stem cells further differentiate and give rise to the differentiated tissues of an organism. Similarly in cancer, undifferentiated CSCs are involved in tumour progression that leads to metastasis (Bellacosa, 2013).

The embryonic stem cells have a core transcriptional network comprising of transcription factors like Oct4, Sox2 and Nanog that contribute to self-renewal and pluripotency (Boyer et al., 2005). Similarly, lung CSCs showed elevated levels of Oct4 and Nanog transcription factors (Chiou et al., 2010). In head and neck cancer, CD44 variant CD44v3 was shown to interact with Oct4-Sox2-Nanog leading to CSC-like properties such as self-renewal and cisplatin resistance (Bourguignon et al., 2012). Recently, it was shown that the lineage ablation of Sox2-expressing cells in both benign and malignant skin squamous cell carcinomas resulted in tumour regression indicating an important role of Sox2 in tumour initiation and CSC functions. Moreover, chromatin immuno-precipitation analysis identified Sox2 target genes involved in controlling tumour stemness (Boumahdi et al., 2014).

Another important phenomena common to both the CSCs as well as the embryonic stem cells is the occurrence of epithelial to mesenchymal transition (EMT). During EMT, the cells lose their polarity and acquire migration capabilities that results in loss of epithelial marker E-cadherin and simultaneous increase in mesenchymal marker N-cadherin. During embryogenesis, EMT is associated with gastrulation required for the formation of the three germ layers. In cancer, EMT leads to invasion, metastasis and cancer stem cell-like phenotype (Kalluri, 2009; Singh, 2010). A recent study showed that Twist1, an EMT promoter protein, is expressed during early stages of tumorigenesis and is required for the initiation of skin tumours (Beck et al., 2015).

All these indicate that regulation of embryonic stem cells and CSCs share similar mechanisms. Therefore, it suggests that deregulation of various developmental pathways are involved in cancer formation and CSC regulation and maintenance. Hence, studying the developmental signalling pathways will shed light on the regulation of CSCs.

**Developmental signalling pathways and CSCs**

The various pathways which are deregulated in cancer include Wnt, Notch, Hedgehog, EGFR, PI3K, NFkB, etc. Among these, three
well-known pathways such as Wnt, Notch and Hedgehog play an important role in the development and normal homeostasis. Conversely, deregulation of these pathways is shown in CSC regulation and maintenance (Ailles, 2012; Purow, 2012).

**Wnt Pathway**

Wnt pathway is evolutionarily conserved and is involved in various organisms. It was first discovered in *Drosophila*, when a mutation in wingless (*wg*) gene led to a distinct phenotype including absence of wings and halters. Later, Nusse's group showed that the insertion of Mouse Mammary Tumour Virus (MMTV) in mice led to mammary tumour by proviral activation of the *int* oncogene. The *int* oncogene was later demonstrated as the mouse homologue of the Drosophila *wg* gene. From these two studies, a new nomenclature *Wnt* (combination of *wg* and *int*) was obtained (Nusse et al., 1984; Rijswijk et al., 1987; Sharma, 2013).

There are 19 highly conserved Wnt ligands discovered till date. These ligands are secreted hydrophobic glycoproteins found to be associated with cell membranes and extracellular matrix. In Wnt producing cells, the endoplasmic reticulum produces Wnt ligands, lipid modified by porcupine (Mikels, 2006; Willert et al., 2003). Wnt ligands can act through two general categories of pathways: canonical and non-canonical. The canonical pathway is β-catenin dependent, while the non-canonical pathways include Wnt/Ca²⁺ and Wnt/JNK pathways. In the canonical pathway shown in Fig. 1, Wnt ligands bind to the conserved cysteine rich domain (CRD) of the frizzled receptors (Fz) which in turn forms co-receptors complexes with low-density lipoprotein like receptors (Lrp5/6). Further, this interaction recruits the Dishevelled (Dsh)

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**Figure 1: Wnt Pathway.** A) In the absence of the Wnt ligand, β-catenin is phosphorylated by destruction complex (APC, CK1α, GSK3 and Axin) and is subjected to proteasomal degradation resulting in no transcription of the Wnt target genes. B) In the presence of the Wnt ligand, the destruction complex is disrupted and thereby β-catenin enters the nucleus and brings about the transcription of Wnt target genes. APC: Adenomatous Polyposis Coli; CK1α: Casein kinase 1α; GSK3: Glycogen synthase kinase 3; TCF: T cell factor; LEF: Lymphoid enhancing factor; Dsh: Dishevelled; LRP: Low-density lipoprotein like receptors.
protein to the cytoplasmic tail of Fz receptor and brings about inhibition of destruction complex surrounding β-catenin. The components of the destruction complex comprise of scaffold protein Axin, Glycogen synthase kinase 3β (GSK3β), Casein kinase 1α (CK1α) and adenomatous polyposis coli (APC). In the absence of the Wnt ligands, the destruction complex hyper-phosphorylates β-catenin and targets it for proteasomal degradation by ubiquitination. The binding of Wnt ligand to Lrp5/6 causes phosphorylation of the cytoplasmic tail of Lrp6, which in turn recruits Axin to the receptor complex that disrupts the destruction complex and stabilises β-catenin. The stable β-catenin translocates to the nucleus and binds to the lymphoid enhancing factor/T-cell factor (LEF/TCF) thereby transcriptionally activating the different target genes involved in cell fate determination during embryonic development and tissue homeostasis (Mikels, 2006; Willert et al., 2003).

**Wnt signalling in normal development and cancer**

Wnt pathway is involved in different biological processes such as embryonic development, self-renewal, proliferation, morphogenesis, etc. Wnt3a and Wnt1 knock out in mice led to deficiencies in neural crest derivatives and neural tube formation during the development (McMahon et al., 1990; Yoshikawa et al., 1997). Wnt3 knock out in mice led to early gastrulation defect and perturbations in the establishment of apical ectodermal ridge during development (Liu et al., 1999). Further, absence of Wnt4 ligand led to defects in female development, while Wnt7a deletion led to female infertility in mice (Jeays-Ward et al., 2004; Parr et al., 1998). Axin1 knockout in mice led to neuro-ectodermal and cardiac abnormalities (Zeng et al., 1997). Wnt signalling was shown to be crucial in hair follicle development as targeted deletion of β-catenin in the epidermis led to failure in placode morphogenesis (Huelsken et al., 2000). Absence of Lef1 led to defects in the pro-B-cell proliferation and abnormalities in several organs like teeth, mammary glands, whiskers and hair (Reya et al., 2000; VanGenderen et al., 1994); while the knockout of Tcf1 led to thymocyte proliferation and differentiation defects (Schilham et al., 1998). Using the Wnt reporter, Axin2-LacZ, Wnt responsive cells were localised to the sub ventricular zone (SVZ) of the developing brain and basal layer of the mammary ducts, which are the stem cells niches. Furthermore, these Wnt responsive cells showed high sphere forming ability and were able to differentiate. Hence, the Wnt pathway plays an important role in normal development and tissue homeostasis (Logan, 2004; VanAmerongen et al., 2009).

There are strong evidences showing involvement of Wnt pathway in regulation of various cancers. Frequent somatic mutations
in β-catenin were observed in both mice and human hepatocarcinomas (Coste et al., 1998), prostate cancers and colon cancers (Voeller et al., 1998). During intestinal adenoma initiation, the first step was APC inactivation followed by β-catenin stabilization, while progression from adenoma to carcinoma required the synergistic action of k-ras activation and β-catenin nuclear localization (Phelps et al., 2009). β-catenin was shown to be essential for retaining tumorigenicity of MDA-MB-231 breast cancer cell lines both in vivo and in vitro. Further, β-catenin knockdown cells implanted into mice showed decrease in the tumour size. In addition, an in vitro study in breast cancer cell lines showed reduction in aldehyde dehydrogenase 1 (ALDH1) positive cells (Xu et al., 2015). Wnt3a expression was associated with EMT and promoted colon cancer progression (Qi et al., 2014). Moreover, deletion of Axin1 was reported in sporadic medulloblastomas and hepatocellular carcinomas (Dahmen et al., 2001). Increased expression of Dsh protein in non-small cell lung carcinoma and mesothelioma have been reported (Uematsu et al., 2003).

Wnt signalling in normal and cancer stem cell regulation and maintenance

Wnt signalling is important in adult stem regulation and has been shown to be involved in stem cell proliferation, self-renewal and maintenance. In hemato-poietic stem cells (HSC), overexpression of β-catenin increases the stem cell pool size suggesting that Wnt pathway is critical to maintain the hematopoietic stem cell homeostasis (Reya et al., 2003). In mice hair follicle stem cells, live cell imaging showed that β-catenin activation in hair follicle stem cells was involved in hair follicle tissue growth (Deschene et al., 2014). Further, Wnt target gene Lgr5, a G-protein coupled receptor was identified as an intestinal stem cell marker indicating an important role of Wnt pathway in the regulation of intestinal stem cells (Ailles, 2012, Valkenburg, 2011). The deletion of Tcf4, a Wnt downstream gene showed loss of stem cell activity and reduced proliferation of the intestinal epithelium (Korinek et al., 1998). In addition, Lgr5 was identified as a marker of hair follicle stem cells (Jaks et al., 2008) with multipotent properties. Moreover, Wnt inhibitor SFRP1 was shown to play an important role in hematopoietic stem cell maintenance through extrinsic regulation (Renstrom et al., 2009). Over-expression of Sfrp1 led to enhanced mesenchymal stem cell function in angiogenesis (Dufourcq et al., 2008). Besides, Sfrp1 was over-expressed in hair follicle stem cells as compared to the non-stem cells (Tumbar et al., 2004; Zhang et al., 2009). Recently, it was shown that Sfrp1 gene is critical for maintaining proper mammary gland development wherein loss of Sfrp1 promotes mammosphere formation; however the role in mammary stem cells needs further investigation (Gauger et al., 2012).
In cancer, various reports have shown that deregulation of Wnt pathway is crucial for the CSC regulation. Human head and neck CSCs treated with Wnt antagonist, secreted frizzled-related protein 4 (sFRP4), the CSCs showed reduction in the sphere-forming capacity and decrease in the stemness markers like CD44 and ALDH1 (Warrier et al., 2014). In another report, β-catenin was shown to be required for maintenance of cutaneous CSCs since deletion of β-catenin led to reduction in the CSCs and tumour regression (Malanchi et al., 2008). CSCs isolated from mammary tumours of radiation treated p53-null mice showed altered DNA repair in response to radiation as well as β-catenin activation (Zhang et al., 2010). In prostate cancer, Wnt signalling induced tumour initiation, EMT and metastasis. Additionally, in prostate cancer cell lines and primary cultures, Wnt3a treatment increased the self-renewal capacity of putative prostate CSCs, emphasizing that Wnt signalling plays an important role in prostate cancer (Barker, 2006; Valkenburg, 2011; Verras et al., 2004). Moreover, the inactivation of APC in Lgr5-positive stem cells at the intestinal crypts led to transformation within days; while inactivation of APC in progenitors or differentiated cells did not lead to tumour formation even after 30 weeks (Barker et al., 2009). In addition, the deletion of CD44, a CSC marker and a Wnt target gene in mice having heterogeneous APC mutation (APCM<sup>Min+</sup>), attenuates intestinal tumorigenesis (Zeilstra et al., 2008).

**Notch Pathway**

*Notch* gene was first discovered in *Drosophila* by Morgan and Bridges where they showed that a mutation led to wings notching and hence the name “Notch” was coined (Morgan and Bridges, 1916; Mohr, 1919; Poulson, 1940). There are four *Notch* genes, three *Delta-like* and two *Jagged* genes in mammals, that are translated into different Notch ligands, Delta and Jagged. Recently, it was shown that for cell fate determination during development, complex of Notch receptor-Delta-Jagged acts in concert (Fiuza, 2004; Boaretoa et al., 2015).

Since the Notch ligands such as Delta and Jagged proteins, as well as Notch receptors are transmembrane proteins, cell-cell contact is important for the signalling cascade. The Notch receptors contain an extracellular subunit, having multiple EGF-like repeats, and a transmembrane subunit (Wharton et al., 1985). When the Notch ligand binds to its receptor, the extracellular domain of the Notch receptor is dissociated from the transmembrane domain and the S2 cleavage site is exposed (Fig. 2). This site is cleaved by ADAM (a disintegrin and metalloprotease) generating an intermediate that is further cleaved by γ-secretase to generate Notch Intracellular Domain (NICD). NICD then translocates to the nucleus where it binds to ubiquitous transcription factor CSL (CBF-1, Suppressor of Hairless, Lag-1). This complex displaces a co-repressor complex containing...
Further, it recruits a co-activator complex containing a MAML (Mastermind-like protein), p300 and other chromatin modifying enzymes, thereby bringing about transcription of different Notch target genes (Ailles, 2012; Andersson, 2011; Fiuza, 2004).

**Notch signalling in normal development and cancer**

Notch pathway is also evolutionarily conserved and is important in cell to cell communication that regulates cell fate determination during development, cell proliferation, differentiation and apoptosis. The loss of Notch function in vertebrates is associated with disruption of neurogenesis, somite formation, angiogenesis, and lymphoid development. In Drosophila, Notch is shown to control the fate of various cell types in the eye. In vertebrates, Notch is involved in the establishment of the central and peripheral nervous systems, spermatogenesis, oogenesis, myogenesis and imaginal disc development (Artavanis-Tsakonas et al., 1999). In normal mammary development, Notch pathway activation is required for regulation of cell fate, proliferation and stem cell self-renewal.

The Notch pathway is also shown to be important for tip-cell formation during mammalian astrocyte differentiation and angiogenesis. In vertebrates, the Notch pathway leads to patterning during inner ear hair cell formation and insulin-secreting pancreatic β cell production (Ailles, 2012; Fortini, 2009).

Notch signalling has been shown to be involved in various cancers. For instance, Notch1 regulates breast cancer cells by inducing Slug expression (Shao et al., 2015). Notch4 promotes growth of gastric cancer cells through activation of Wnt1, β-catenin.
Notch signalling plays an important role in a number of hematopoietic and solid tumours, but the strongest evidences for its role in CSC regulation has been shown in breast cancer, embryonal brain tumours and gliomas (Fan et al., 2006; Pannuti et al., 2010). In various human breast cancer cell lines and primary patient tissues, a significant decrease in mammosphere formation after Notch inhibition has been demonstrated (Abel et al., 2014). Further, studies on the human mammary mammospheres have shown a feedback loop between Her2/Neu and Notch, as well as promotion of a hypoxia resistant phenotype (Pannuti et al., 2010). In brain tumours, blockade of Notch led to a 5-fold reduction in the CD133+ cell fraction and total depletion of the side population cells. Additionally, differentiated cell growth was observed after Notch inhibition, but lacked formation of tumour xenografts efficiently, indicating that the CSCs required for tumour propagation were absent (Fan et al., 2006). A recent study on primary human pancreatic xenografts showed upregulation of the notch pathway components in pancreatic CSCs. Additionally, inhibition of notch pathway reduced CSC percentage and tumour-sphere formation significantly (Abel et al., 2014).
Sonic-hedgehog Pathway

Hedgehog pathway is delineated in *Drosophila* and determines the anterior-posterior orientation of developing structures (Nusslein-Volhard and Wieschaus, 1980). Similar to Wnt and Notch, the key components of the Hedgehog pathway are evolutionarily conserved, although differences are observed in the mammalian and *Drosophila* Hedgehog signalling. While *Drosophila* has only one hedgehog gene, three homologues have been identified in vertebrates namely, Sonic (Shh), Desert (Dhh), and Indian hedgehog (Ihh). The Sonic Hedgehog pathway is extensively investigated in the vertebrate system (Chen et al., 2005; Varjosalo et al., 2006). The other components of the Hedgehog pathway include patched (Ptc) and smoothened (Smo), constituting a 12-pass transmembrane glycoprotein and a 7-pass transmembrane protein, respectively (Varjosalo, 2008; Wicking et al., 1999).

In the Sonic-hedgehog pathway elaborated in Fig. 3, absence of the hedgehog ligand, Smoothened (Smo) is inhibited by being bound to Patched (Ptc). When the hedgehog ligand binds to Ptc, inhibition of Smo is released which acts on protein complex comprising of fused (Fu), suppressor of fused (Sufu) and cos-2-costa-2 (Wicking et al., 1999; Merchant, 2010). These proteins are generally bound with Gli thereby inhibiting its action. Once the complex is disrupted, Gli translocates to the nucleus and brings about transcription of different downstream targets (Sasaki et al., 1999; Ruiz, 2007; Stecca, 2010).

**Sonic-hedgehog signalling in normal development and cancer**

In vertebrates, the Sonic Hedgehog (Shh), is expressed widely throughout the developing central nervous system (CNS), limb, gut, teeth and hair-follicle. Dhh is involved in development of the germline, while the Ihh is

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**Figure 3: Hedgehog Pathway.** A) In the absence of Hedgehog ligands (Indian, Sonic and Dessert), the Patched receptor (ptc) exerts inhibitory action on the Smoothened receptor (smo). The Gli complex (Gli1 and Gli2) remains in the cytoplasm followed by no transcription of Hedgehog target genes. B) In the presence of Hedgehog ligands, the inhibitory action of Patched (ptc) on Smoothened (smo) is released, and hence Gli complex translocates to the nucleus and brings about transcription of Hedgehog target genes. Fu: Fused; SuFu: Suppressor of Fused; Cos 2: cos-2-costa-2.
involved in development of the skeletal system (Bitgood et al., 1996; Wicking et al., 1999). Shh also plays a role in neural stem cells, determining the neuronal cell fate (Merkle et al., 2007). It was demonstrated that during repair of acute airway injury, the Hedgehog pathway gets activated in the airway epithelium (Watkins et al., 2003). Hedgehog signalling components Ptc, Gli1, and Gli2 were over-expressed when mammary progenitor cells grow as mammospheres (Liu et al., 2006). These reports indicate that the Hedgehog pathway plays a role in normal stem cell regulation (Ailles, 2012).

Hedgehog signalling is involved in various cancers. For example, Ptc1 mutation was observed in patients with medulloblastoma and rhabdomyosarcoma (Hahn et al., 1996; Johnson et al., 1996; Pietsch et al., 1997). Sufu as well as Smo mutations were observed in medulloblastoma (Xie et al., 1998; Taylor et al., 2002); Gli1 and Gli3 mutations were seen in pancreatic adenocarcinoma; and Gli1 gene amplification was seen in glioblastoma (Clement et al., 2007; Jones et al., 2008). Further, Kern et al showed that Gli and PI3K/AKT/mTOR signalling act synergistically to initiate and maintain chronic lymphocytic leukemia (Kern et al., 2015). Another report showed that Sonic hedgehog ligand over-expression led to increased number and size of intestinal adenomas in APC (HET) mice, while loss of Indian Hedgehog almost completely blocks intestinal adenoma development (Buller et al., 2015).

**Sonic-hedgehog signalling in normal and cancer stem cell regulation and maintenance**

Hedgehog signalling is involved in stem cell regulation of various tissues. Shh regulates self-renewal of neural stem cells (Palma et al., 2005). The components of Hedgehog pathway such as Ptc, Gli1 and Gli2 are expressed in the mammary stem cells and down regulated during differentiation (Liu et al., 2006). Hedgehog is involved in controlling neural stem cells through the p53-independent regulation of Nanog (Po et al., 2005).

In colon carcinoma, Hedgehog signalling is activated in CSCs with higher expression of Gli1 and Gli2. In non-small cell lung cancer, the malignant phenotype of the tumours is maintained by ligand-dependent Hedgehog pathway activation (Watkins et al., 2003). Furthermore, Bmi1, which is a downstream target of the Hedgehog pathway was activated in breast CSCs and is also shown to regulate normal and leukemic stem cells (Liu et al., 2006; Takebe, 2011).

**CONCLUSION**

Tumour maintenance and progression is regulated by a subset of cells that are known as cancer stem cells (CSCs). Recently, due to increase in evidences on the existence of
CSCs, they have gained more attention but how these CSCs escape the chemo-radiotherapy is still unknown. Moreover, how the CSCs are maintained in the tumour microenvironment remains elusive. Several reports showed signalling pathways such as Wnt, Notch and Sonic-hedgehog are deregulated in cancer, and also involved in the CSC regulation and maintenance. In addition, evidence of cross-talk between the signalling pathways exists. Therefore, understanding these signalling pathways at the molecular level will be of utmost importance. The study will enable counteracting the issue of signalling cross-talk, and perhaps, multi-targeted drugs approach can be fruitful. Hence, further detailed research on deregulation of the developmental pathways in CSCs needs to be investigated. Eventually, elucidation of the signalling mechanisms will enable to specifically target CSCs without affecting the normal cells.

ACKNOWLEDGEMENTS
The authors acknowledge Mr. Rahul Sarate and Mr. Gopal Chovatiya for their suggestions.

CONFLICT OF INTEREST
The authors claim no conflict of interest.

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