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Article

Understanding and Targeting the Colon Cancer Pathogenesis: A Molecular Perspective

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Colorectal cancer (CRC) one of the leading cause of cancer-related deaths worldwide. With the presently available knowledge on CRC, it is understood that the underlying is a complex process. The complexity of CRC lies in aberrant activation of several cellular signaling pathways that lead to activation and progression of CRC. In this context, recent studies have pointed towards the role of developmental pathways like; hedgehog (HH), wingless-related integration site (WNT/β-catenin) and Notch pathways that play a crucial role in maintenance and homeostasis of colon epithelium. Moreover, the deregulation of these signaling pathways has also been associated with the pathogenesis of CRC. Therefore, in the search for better therapeutic options, these pathways have emerged as potential targets. The present review attempts to highlight the role of HH, WNT/β-catenin and Notch pathways in colon carcinogenesis.

Keywords: Hedgehog. WNT/β-catenin. Notch. Colon cancer. Molecular targets.

INTRODUCTION

Colorectal cancer (CRC) is one of the major causes of morbidity and mortality, representing the second major cause of cancer incidence among females and the third among males (Arnold *et al*., 2017). The occurrence of colon cancer is strongly related to age, with 90% of the cases arising in people who are 50 years or older (Favoriti *et al*., 2016). As per the GLOBOCON 2018 report, the number of cases may raise to 14 million by the year 2035 globally (Ferlay *et al*., 2019). CRC is now the third most common malignant disease in both men and women in Asia (Park, Jee, 2018). The estimated ASR (age-standardized incidence rate) in the year 2010 for rectal cancer in India was 4.3 and 3.5 per 100,000 in males and females respectively (Mohandas, 2011). Colon carcinogenesis is a multistep process and it emanates from a series of molecular and histopathological alterations involving a variety of oncogenes and tumor suppressor genes that transform normal colonic epithelium into an invasive carcinoma (Nguyen, Goel, Chung, 2020). The epithelium of the gastrointestinal tract is continually replaced and to maintain homeostasis of the intestinal epithelium cellular proliferation, differentiation, migration and death must be strictly regulated (Bertrand *et al*., 2012). A few but highly conserved signaling pathways like hedgehog (HH), wingless-related integration site (WNT/β-catenin) and Notch pathways are thought to drive these processes (Bertrand *et al*., 2012; Pandurangan *et al*., 2018). Alterations in the above-mentioned pathways that control developmental processes during embryogenesis and organogenesis have been recognized as hallmarks of cancer. It is not surprising that several of these signaling pathways are altered in oncogenic processes. Research suggests that these pathways do not act in isolation, but are interconnected such that alterations in one lead to alterations in another. Understanding this cross-talking of different pathways is critical to the development of successful targeted therapies. The focus of CRC research is shifting from a clinical perspective towards developing an understanding of the molecular basis via studying the interactions and cross-talk between these pathways that determine the underlying complex pathogenesis of this

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malignancy. Although several research groups have explained the role of different signaling pathways, still the role of developmental pathways in the process of colon carcinogenesis is not completely understood. Thus, the present review attempts to fill this gap in knowledge and shed light on the role of developmental pathways like HH, WNT/β-catenin and Notch signaling in the pathogenesis of colon cancer, since a more thorough understanding of these developmental pathways may contribute to improved strategies for prevention, screening, diagnosis, and therapy for colon cancer.

UNDERSTANDING THE COLON EPITHELIUM

The primary function of the intestinal tract is the digestion and absorption of nutrients. The intestinal lumen is lined with a specialized simple epithelium, which performs the primary functions of digestion and water and nutrient absorption and forms a barrier against luminal pathogens. The gut is anatomically divided into the small intestine and the colon. The small intestine can be divided up into the duodenum, the jejunum, and the ileum. The intestinal epithelium is the most vigorously self-renewing tissue of adult mammals (Heath, 1996). The four differentiated cell types that reside within the epithelium—goblet cells, enteroendocrine cells, Paneth cells, and enterocytes—are visualized through staining with specific markers proliferative cells reside in the crypts of Lieuberkuhn, epithelial invasions into the underlying connective tissue. The crypts harbour stem cells and their progeny, transit-amplifying cells. Transit-amplifying cells spend approximately two days in the crypt, in which they divide 4–5 times before they terminally differentiate into the specific cell types of intestinal epithelia. In the small intestine, the surface area is dramatically distended through epithelial protrusions called villi. Three types of differentiated epithelial cells cover these villi, the absorptive enterocytes, mucous-secreting goblet cells, and hormone-secreting enteroendocrine cells. Three days after their terminal distinction, the cells extend the tip of the villus, undergo impulsive apoptosis, and are removed through the gut lumen (Hall *et al.*, 1994). Paneth cells are unusual in that they settle at the crypt bottoms and represent the only differentiated cells that escape the upward migration. Paneth cells have a function in innate immunity and antibacterial defence, to which ends they secrete bactericidal defencing peptides and lysozymes. The modular organization of the epithelium of the small intestine and colon into crypts is globally comparable. Histologically, two important differences between the two types of epithelia. The colon carries no villi but has a flat surface epithelium. Moreover, Paneth cells are absent in the colon (Sauer, 1998).

ABNORMAL GROWTHS IN THE COLON

Maximum colorectal cancers initiate as a polyp – a growth that starts in the inner lining of the rectum or colon and grows to the centre. Most polyps are not cancer. Only certain types of polyps (called adenomas) can become cancer. Taking out a polyp early, when it is small, may keep it from becoming cancer. Over 95% of rectal and colon cancers are adenocarcinomas. These are cancers that start in gland cells, like the cells that line the interior part of rectum and colon. There are some other, more exceptional, types of tumors of the rectum and colon (Alteri *et al*., 2011).

COLON CANCER PATHOGENESIS

The pathogenesis of CRC is very complex and varies according to genetic or epigenetic changes, which are correlated to each other in varying degrees. Such genetic and epigenetic alterations are directly responsible for a specific event within the sequence that leads to CRC, by contributing to the "initiation" of neoplastic transformation of healthy epithelium and/or determining the "progression" towards more malignant stages of the illness.

Variations in genes that control developmental processes during organogenesis and embryogenesis are known as hallmarks of cancer (Bertrand *et al*., 2012). Pathways such as wingless-related integration site (WNT), Hedgehog (HH), and Notch are well-characterized in the developing embryo for establishing cell position, body pattern segmentation, polarity and cell fate decisions (Bienz, Clevers, 2000; Bertrand, *et al*., 2012; Shenoy *et al.,* 2012). It is not astonishing that numerous of these signaling pathways are altered in oncogenic processes. Improved understanding of the cellular basis for colon

cancer and the role that signaling pathways such as WNT, Notch and HH have in the establishment and maintenance of the tumorigenic state will be critical for the development of novel therapeutics. The advent of small-molecule inhibitors for targeting these pathways and their success in other diseases, either as single agents or in combination therapy, provides a rationale for exploiting these pathways as potential targets in the treatment of colon cancer.

The unit of structure in the normal colon is the crypt of Lieberkuhn, which is composed of colon stem cells, transit-amplifying cells and terminally differentiated goblet cells, enterocytes and endocrine cells. The architectural structure of the colon is reflected by a gradient of WNT, HH, and Notch signaling (Takebe, Ivy, 2010; Geissler, Zach, 2012). Beginning at the base of the crypt unit, Notch pathway is maximum in the stem cell compartment and declines as cells move up through the proliferative areas and into the differentiative areas. WNT signaling is similar, with the highest levels of expression being in the earliest stages of the proliferative compartment and tailing off in the differentiative compartment (Burgess *et al.*, 2011). HH expression primarily occurs in the differentiated compartment. Thus, these gradients of developmentally regulated cellular pathways serve to establish the pattern of stem cell/self-renewal, proliferation and differentiation that comprise the colon architecture (Bertrand *et al.*, 2012).

HEDGEHOG SIGNALING

The HH pathway derives its unusual name from the phenotype of hedgehog loss in Drosophila; larvae take on a curled, bristly appearance that may remind some of a hedgehog (Geissler, Zach, 2012). Hedgehog signaling pathway genes are considered as essential components in cell proliferation, differentiation and tissue polarity during embryonic development and also functions in stem cell proliferation, tissue repair, regeneration and oncogenesis (Heretsch, Tzagkaroulaki, Giannis, 2010; Liu, Gu, Xie, 2011). The canonical HH pathway contains several key components, including HH glycoproteins Sonic hedgehog (SHH), Indian hedgehog (IHH), and Desert hedgehog (DHH) (Varjosalo, Taipale, 2008). As shown in Figure 1, upon secretion, SHH glycoproteins bind and inactivate the 12-transmembrane protein Patched1 (PTCH1), which normally inhibits the activity of the 7-transmembrane protein Smoothened (SMO). In the presence of SHH ligand, PTCH1 inhibition of SMO at the primary cilium is abrogated resulting in the nuclear localization of glioma-associated (GLI) transcription factors, which are the terminal effectors of the SHH. PTCH2 receptor shares approximately 54% homology with PTCH1, yet its expression pattern and role in tissue vary significantly from PTCH1. PTCH2 is highly expressed in spermatocytes and helps mediate DHH activity in germ cell development (Carpenter *et al.*, 1998). It has also been revealed that in the absence of SHH ligand binding, PTCH2 has a decreased ability to inhibit SMO (Rahnama, Toftgård, Zaphiropoulos, 2004). In the absence of ligand, suppressor of Fused (SUFU) negatively regulates the pathway by directly binding to GLI transcription factors and anchoring them in the cytoplasm preventing the activation of GLI target genes (Cheng, Bishop, 2002).

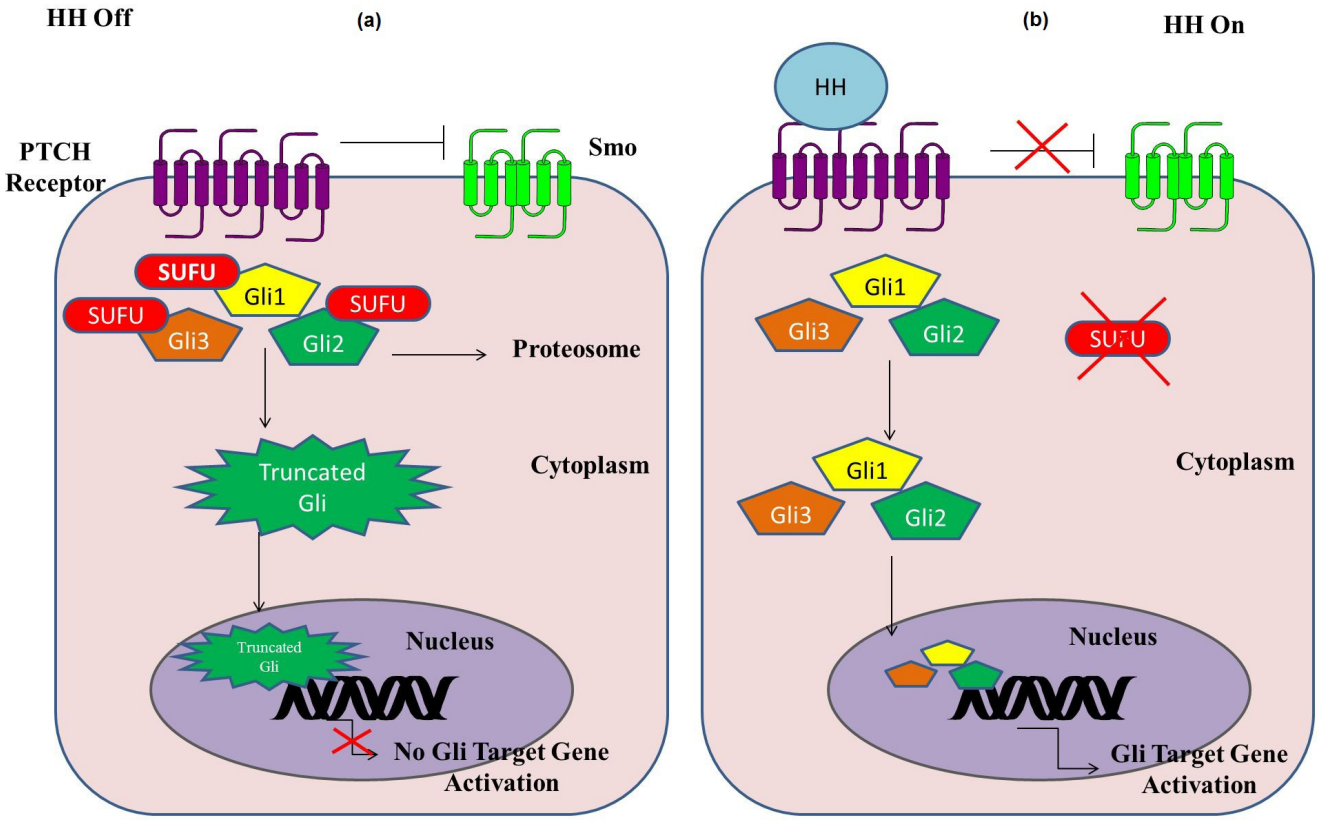

FIGURE 1 -Schematic representation of the Hedgehog (HH) Signaling Pathway (a) In the absence of HH ligand, suppressor of Fused (SUFU) binds to GLI transcription factors in the cytoplasm preventing its translocation to the nucleus. (b) In the presence of HH ligand, the ligand binds to Patched (PTCH) receptors leading to activation of Smoothened (SMO) transmembrane proteins consequently deactivating SUFU and enabling the translocation of GLI transcription factors to the nucleus which ultimately activates downstream target genes.

Cytoplasmic sequestration of GLI transcription factors by SUFU facilitates processing and degradation of GLI proteins, therefore inhibiting SHH pathway (Kogerman *et al.*, 1999). SUFU has also been presented to form a repressor complex leading to communication with DNA-bound GLI1 and suppression of GLI1-induced gene expression (Cheng, Bishop, 2002). Invertebrates, there are three GLI transcription factors (GLI1, GLI12 and GLI3). GLI1 is the only full-length transcriptional activator whereas GLI2 and GLI3 act as either a positive or negative regulators as determined by posttranscriptional and posttranslational processing (Ruiz i Altaba, 1999). In response to SHH ligand binding, GLI2 accumulates in the primary cilium and drives transcriptional activation, overcoming negative regulation by GLI3 (Kim, Hwi, Hung, 2005). Additionally to regulation by SUFU, GLI1 is also controlled by the kinase Dyrk1. Dyrk1 can potentiate GLI1 activity by phosphorylation at multiple serine/threonine sites that have been shown to induce nuclear accumulation and GLI1-mediated transcription (Mao *et al.*, 2002). GLI transcription factors can activate target genes that include targets involved in HH pathway feedback (e.g., GLI1, PTCH1), proliferation (e.g., Cyclin-D1, MYC), apoptosis (e.g., Bcl-2), angiogenesis (e.g., ANG1/2), epithelial-to-mesenchymal transition (e.g., SNAIL), and stem cell self-renewal (Hui, Angers, 2011).

ALTERED HEDGEHOG SIGNALING

HH signaling is important during normal embryonic development and its aberrant activation or deregulation is associated with many human cancers like basal cell carcinomas, medulloblastomas and cancers of the oesophagus and bladder (Ruiz i Altaba, 1999). In

gastrointestinal cancers, HH pathway activation occurs not by mutation or amplification of signaling molecules, but via transcriptional upregulation of the HH ligands (Katoh, Katoh, 2009). It has recently been suggested that HH signaling progresses during colon carcinogenesis (Yoshikawa *et al.,* 2009) and in metastatic disease (Varnat, Zacchetti, Ruiz i Altaba, 2010), whereas in normal colonic tissue, it is involved in differentiation (van den Brink *et al.,* 2004; Kasper *et al.,* 2006). A study group shows the significance of HH pathway in cellular survival via activation of Gli1 and Gli2 in human colon carcinoma cells. Activated Gli proteins regulate downstream targets of HH signaling, including Bcl-2, PDGFRa, Fas, and DR5. In the presence of GANT61 (targeting Gli) the functions of Gli activators are inhibited; PDGFRa and Bcl-2 are downregulated, whereas Fas and DR5 are upregulated. GANT61 induces significant cell death, whereas targeting Smo with cyclopamine is less effective at inducing cytotoxicity. These findings underscore the critical role of HH signaling in human colon cancer cells and the possibility of targeting Gli1 and Gli2 activator functions using GANT61 in this disease (Mazumdar *et al.,* 2011). Activation of HH signalling could be seen in various noncutaneous malignancies, including brain cancer, gastrointestinal, prostate, lung and breast malignant tumors (Yang *et al.,* 2010). Besides, evidence support that HH signalling is essential for carcinogenesis and spreading of malignant melanoma, ovarian cancer, leukaemia and B-cell lymphomas (Kasper *et al*., 2006; Ehtesham *et al*., 2007; Lindemann, 2008; Fiaschi *et al*., 2009). However, very little is known regarding the specific role of HH signaling in regulating cell survival and proliferation in colon cancers, and the downstream target genes involved in the determination of cell fate (Mazumdar *et al*., 2011). Additional to the classical (canonical) signaling axis, there are also some non-classical (non-canonical) pathways related to SHH signaling are present. Non-canonical SHH signaling denotes to either: (1) initiation of signaling from PTCH1/SMO but independent of GLI transcription factors; or (2) initiation of GLI transcription factors independent of SHH ligand or PTCH1/SMO. The second is better studied and multiple pathways have been recognised, mostly oncogenic, that can enhance GLI activity. Transcription factors viz. GL1 are completely regulated by K-Ras, TGF, PI3K-AKT, and PKC (Deng *et al*., 2015). K-Ras, in particular, seem like a pathway capable of triggering GLI1 independent of the SHH pathway as knockdown of SUFU does not affect K-Ras-induced GLI1 (Rajurkar *et al*., 2012). Moreover, the GLI proteins are adversely regulated by p53, PKA, and PKC (Sheng *et al*., 2006; Stecca, Ruiz I Altaba, 2009; Makinodan, Marneros, 2012; Yoon *et al*., 2015). GLI1 transcriptional activity has also been revealed to be declined with p53 overexpression and upregulated with p53 knockdown (Stecca, Ruiz I Altaba, 2009). Moreover, p53 has been shown to interrelate with TAF9 leading to suppression of GLI1 activity (Yoon *et al.,* 2015). PKA regulation of GLI1 is very specific as PKA directly phosphorylates Thr374 of GLI1, which promotes cytoplasmic localization and reduced activity of GLI1 (Sheng *et al.,* 2006). Interestingly, our laboratory findings have shown that targeting the HH signaling colon cancer cells enhances the cytotoxicity. We showed that plant-derived compound, andrographolide inhibited the proliferation of colon cancer HCT-116 cells via downregulating the expression of GLI1 and Smo in-vitro **[Unpublished].** Thus, indicating the importance of HH signaling pathway in targeted inhibition of colon cancer. Furthermore, the role of HH is also explained in the resistance mechanisms in colon cancer (Das, Islam, Lam, 2020). It is associated with the drug resistance in patient-derived organoid cultures (Palle *et al*., 2015). The nuclear mediator GLI1, is thought to be the key molecule it is shown to enhance the resistance of LoVo colon cancer cells against 5-Fluorouracil (5-FU) (Usui *et al*., 2018). Also, HH mediated the drug resistance mechanism through GLI1 in patient-derived air-liquid interface (ALI) organoids from colon cancer patients against Irinotecan, 5-FU and Oxaliplatin. GLI1 gene, when knockdown results in the decrease of drug resistance in these cells (Usui *et al*., 2018).

WNT SIGNALING

This pathway is highly conserved throughout the animal kingdom (Clevers, 2006). The central player in the canonical WNT pathway is β-catenin. As explained in Figure 2, during the lack of a WNT

signal, β-catenin is targeted for proteasomal degradation through sequential phosphorylations occurring at its N terminus. A degradation complex, consisting of the tumor suppressors axin and adenomatous polyposis coli (APC) and the constitutively active kinases glycogen synthase kinase 3β and casein kinase I, regulates β-catenin phosphorylation status in a cell. When, WNT ligands signal through their Frizzled and low-density lipoprotein receptor-related protein (LRP) receptors, the destruction complex is inactivated. As a result, β-catenin is no longer phosphorylated and accumulates in the cell. The coincident translocation of β-catenin into the nucleus results in the binding of β-catenin to transcription factors of the T cell factor/lymphocyte enhancer factor (TCF/LEF) family. TCF/LEF-β-catenin forms an active transcriptional complex that activates target genes. In the absence of a WNT signal, transcriptional repressors like Groucho bind TCF/LEF transcription factors (Cavallo *et al.*, 1998).

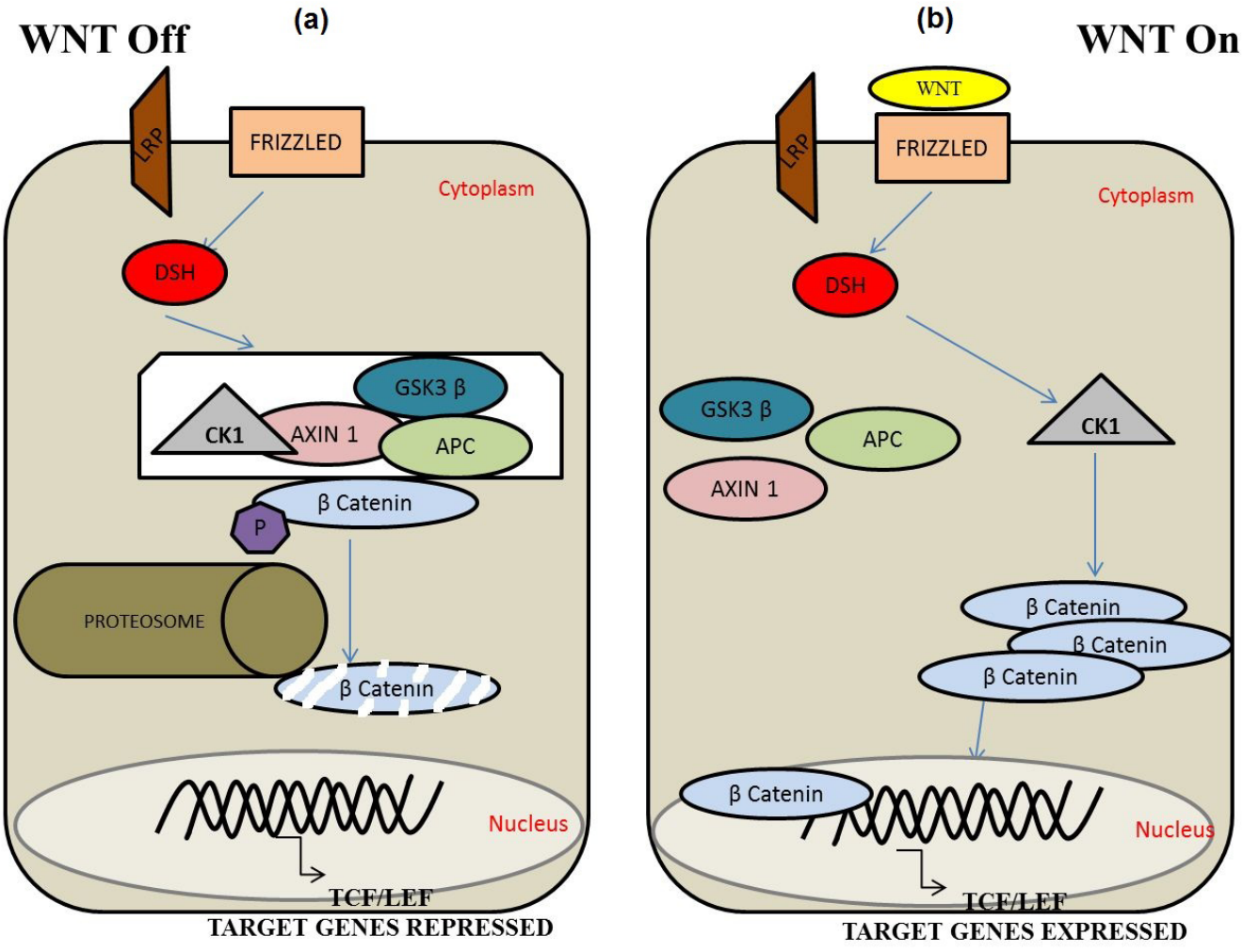

FIGURE 2 - Schematic WNT signaling (a) In the absence of ligand, A degradation complex, consisting tumor suppressors axin-1 (AXIN 1), adenomatous polyposis coli (APC), the constitutively active kinases glycogen synthase kinase 3β (GSK3β) and casein kinase I (CK 1) phosphorylates β-catenin leading to proteasomal degradation. (b) In the presence of ligand, the ligand binds to Frizzled and low-density lipoprotein receptor-related protein (LRP) receptors, leading to the inactivation of destruction complex. β-catenin accumulates in the cytoplasm and is translocated into the nucleus binding to the transcription factors of the T cell factor/lymphocyte enhancer factor (TCF/LEF) family.

ALTERED WNT SIGNALING

Mutations in the WNT pathway cause colon cancer through constitutive activation of the nuclear β-catenin/TCF transcription factor complex (Bienz, Clevers, 2000; Burgess *et al.*, 2011). The most well-documented WNT pathway mutation in colon cancer is the loss of the APC tumor suppressor gene, resulting in constitutive stabilization of â-catenin and activation of WNT pathway genes, namely TCF, which are required for colon crypt maintenance, This results in inappropriate proliferation that presents as colon polyps (Medema, Vermeulen, 2011; Clevers, Nusse, 2012). Interestingly, point mutations in β-catenin have been identified in the approximately 15% of sporadic colon cancers (Morin *et al.*, 1997). These mutations in β-catenin render it insensitive to de-stabilization by the Axin/GSK-3β/APC complex and result in constitutive WNT signaling (Morin *et al.*, 1997; Bienz, Clevers, 2000). In the lack of a WNT signal, transcriptional repressors like Groucho bind TCF/LEF transcription factors (Roose *et al.*, 1998). The WNT pathway regulates its transcriptional target genes through TCF target sites located in promoters and/or enhancers. These reporters consist of concatamers of the binding motif cloned upstream of a minimal promoter. A large variety of WNT/TCF target genes have been described since the discovery that this pathway represents the dominant force behind the proliferative activity of the healthy intestinal epithelium as well as behind colorectal cancer (CRC). Active WNT signaling is essential for the maintenance of crypt progenitor compartments in the intestine. This is evidenced by mice lacking the Tcf4 transcription factor by the conditional depletion of β-catenin from the intestinal epithelium and by transgenic inhibition of extracellular WNT pathway through the secreted Dickkopf-1 WNT inhibitor (Kuhnert *et al.*, 2004). In all cases, a dramatic reduction in proliferative activity was observed. In the converse experiment, activating the WNT pathway through transgenic expression of the WNT agonist R-Spondin-1 resulted in a massive hyperproliferation of intestinal crypts (Kim, Hwi, Hung, 2005). WNT signals in the crypt not only control the proliferation of transit-amplifying progenitors but also are utilized by post-mitotic Paneth cells for their terminal maturation (van Es *et al.*, 2005). Thereby, targeted inhibition of the WNT pathway holds great potential for the prevention and treatment of colon cancer.

NOTCH SIGNALING

Notch signaling plays a vital role in the maintenance of the normal intestinal epithelia (Shenoy *et al.*, 2012). It is essential for regulating the differentiation of colonic goblet cells and stem cells/progenitor cells (Malanchi *et al.*, 2008). Thus, essential in maintaining intestinal development and homeostasis. It is well known that colonic crypts are the principal niche for colonic stem cells. As illustrated in Figure 3, Notch signaling component include, four receptors and several downstream target genes (Hes-1, 5, 6, 7 and Math1) are expressed in normal mouse intestinal crypts of various stages of differentiation and development (Yeung *et al.*, 2010). Notch receptors Notch1, Notch2 and Notch3 were highly expressed at the basal crypt of the human colon, and CSL and Notch ligand Jagged1 were highly expressed at the top of the crypts (Ricci-Vitiani *et al.*, 2008). Such an expression pattern has some functional implications. Notch pathway is essential for regulating the proliferation of crypt progenitor cells and the differentiation of colonic epithelial cells. Suppression of Notch is signaling by depletion of Hes-1, the most abundant and direct downstream target gene of Moreover, it was associated with a significant increase in the secretory lineage of intestinal epithelial cells (Qiao, Wong, 2009). On the other hand, activation of Notch pathway not only promotes the proliferation of stem cells in the crypt but also redirect the gut progenitor cells to differentiate toward absorptive but not secretory lineage cells. Hes-1 regulates the expression of Math1 that is another important gene controlling intestinal differentiation. Mice deficient of RBP-Jj or Hes-1 or those treated with c-secretase inhibitor exhibited increased numbers of secretory epithelial cells (Schröder, Gossler, 2002). The role of Notch signaling in the control of gut crypt differentiation and proliferation was recently confirmed by a study in inducible gut-specific Notch-mutant mice, which showed that it is involved in the regulation of cell cycle progression of crypt progenitor

cells (Riccio *et al.*, 2008). In addition, Notch appears to be necessary for the functional maintenance of WNT signaling in the gut. Other studies have revealed that inhibition of Notch and/or WNT pathways were able to increase the expressions of some colonic differentiation markers such as villin2, muc20 or TFF1 (Fodde, Brabletz, 2007). These results indicate that under the physiological condition, activation of Notch signaling is probably involved in the maintenance of proliferative potential of intestinal epithelial cells.

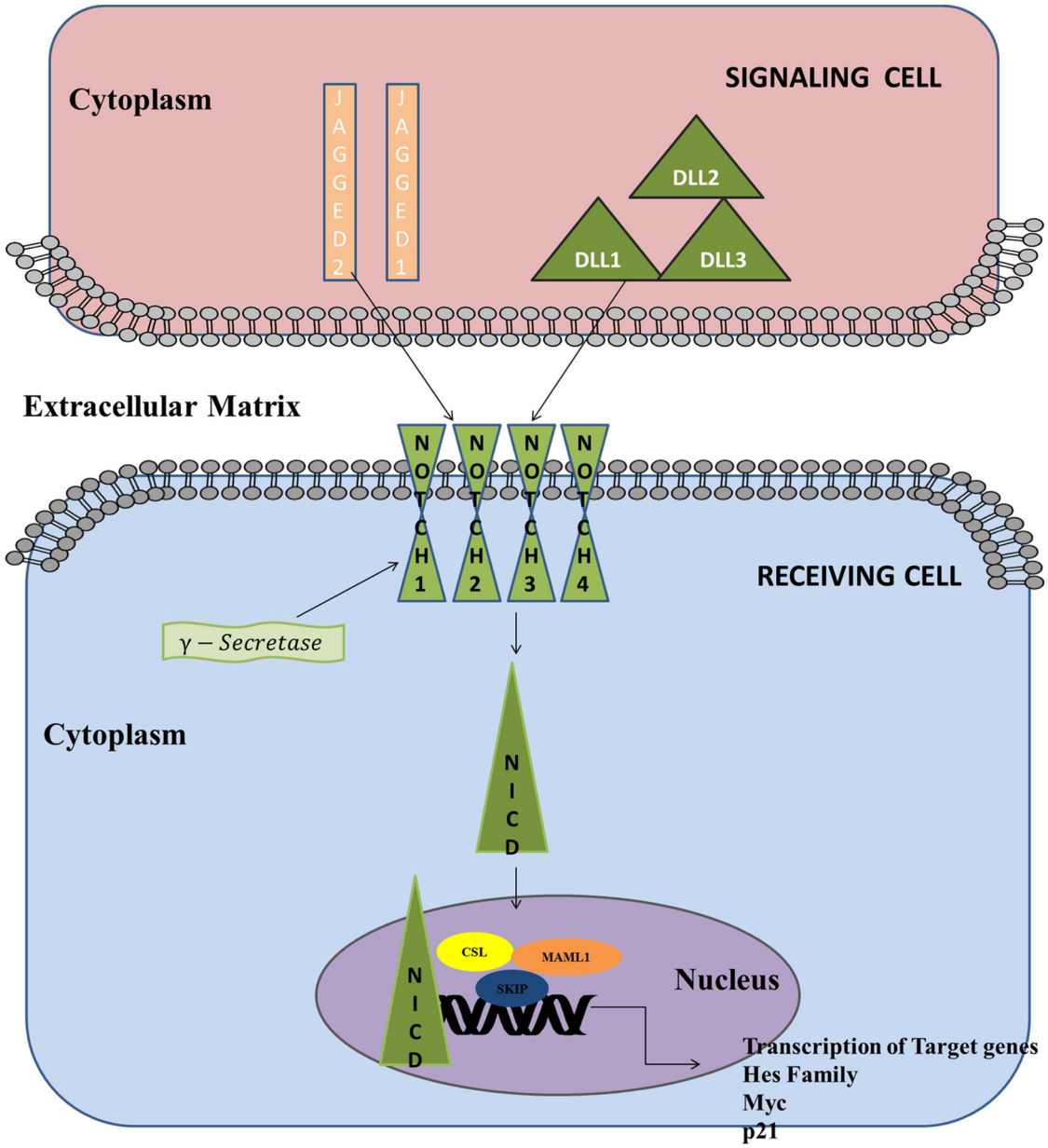

FIGURE 3 - Notch Signaling Pathway: It is active between two cells in close proximity, one acts as a signal transducer and the other as the signal receiver. The signalling cell releases the ligands; Jagged1, Jagged2, Delta-like-1 (Dll-1), Delta-like-3 (Dll-3) and Delta-like-4 (Dll-4), which binds to the receptors; Notch-1, -2, -3 and -4 present on the receiving cell. This activates γ-secretase which in turn cleaves Notch intracellular domain (NICD) of the receptor. NICD translocates to the nucleus and binds with CSL protein, Ski-interacting protein (SKIP) and mastermind-like proteins (MAML) and ultimately affect the expression of target genes.

ALTERED NOTCH SIGNALING

Notch signaling plays an important role in the maintenance of the colon crypt compartment. More recently, inappropriate activation of Notch signaling has been associated with the pathogenesis of colon cancers (Miyamoto, Rosenberg, 2011). The Notch ligand Jagged1 is expressed at a significantly higher level in CRC tissues than in their matched normal colonic mucosa (Zagouras *et al*., 1995). Besides, we observed that higher level of Jagged1, Jagged2, DLL1, DLL3, DLL4, Notch receptors 1–4 and some downstream targets of Notch signaling (Hes-1, Deltex and NICD) are present in 75% of CRC tissues compared with normal colonic tissues (Qiao, Wong, 2009). Consistent with these findings, Notch pathway genes are not only highly expressed in CRC tissues but are also functionally active (Reedijk *et al*., 2008). Its activation is found to be associated with the development of primary CRC rather than metastatic colon cancers (Qiao, Wong, 2009) indicating that activation of Notch may be an early event in CRC development. Activation of Notch signaling may also contribute to the treatment resistance of CRC. For example, the resistance of CRC cells to Oxaliplatin, a platinum-derived chemotherapeutic drug, was closely correlated with a dose-dependent increase in Notch1 expression and NICD production, indicating that cancer cells may adaptively develop mechanisms to overcome therapy-induced cell killing via upregulating Notch pathway (Meng *et al*., 2009). The role of Notch-signaling activation in chemoresistance was further supported by the finding that Numb, a negative regulator of Notch pathway, is downregulated in advanced CRC (Meng *et al*., 2009). The mechanisms of constitutive activation of Notch signaling in CRC are not well understood, but like in any other cancers, genetic mutations at the Notch receptor loci may play some roles (Vieira *et al*., 2015). However, significant mutations of Notch signaling components in CRC have not been reported. In CRC, however, the vast majority of published references indicate that it plays an oncogenic role. Moreover, our preliminary laboratory investigation has also shown that the proliferation of colon cancer SW480 cells was inhibited by targeting the aberrantly activated Notch pathway. As per our findings, the treatment andrographolide downregulated the expression of NOTCH1 and JAGGED1 dose-dependently (Khan *et al*., 2020)**.** Thus, inhibition of Notch signaling may be of therapeutic benefit against colon cancer. Carcinogenesis is a very complex process involving multiple cellular pathways. Thus, anticancer agents selected and employed in modern medicine can inhibit cancer cell proliferation via inducing apoptosis and cell cycle progression. It is also understood that anticancer compounds that can inhibit multiple pro-cancer processes are more likely to inhibit a wider range of cancers and that are of greater importance (Pezzuto, 2002). On the other hand, the suppressing agents can facilitate their effects in different ways including signaling cascade, blocking cell cycle, altered gene expression, induction of cell senescence and inducing cell differentiation or apoptosis (Joo, Visintin, Mor, 2013). In recent years cancer stem cells (CSCs) have been of great interest to researchers, in this regards the role of developmental pathways in CSCs and drug resistance has also been described (Das, Islam, Lam, 2020). Ex-vivo cultures of colonospheres from colon cancer patients and chemoresistant colon cancer HCT-116 cells have shown upregulated Notch signaling compared with normal control cells (Huang *et al*., 2015). The study showed that 5-FU and Oxaliplatin demonstrated effective cytotoxic effect in normal parental cells, on the contrary, chemoresistant cells and colonospheres showed significant resistance towards these drugs. Further, the gene expression revealed that Notch1 expression was also found to be upregulated in chemoresistant cells and colonospheres. The results suggested that Notch signalling is associated with the drug resistance in these cells. Furthermore, they inhibited the Notch signalling with DAPT (Notch inhibitor), which resulted in the increased efficacy of 5-FU and Oxaliplatin in chemoresistant cells and colonospheres (Huang *et al*., 2015).

Molecular crosstalk between WNT, Notch and HH signaling

There is ample evidence suggesting the crosstalk between WNT, Notch and HH signaling at several molecular nodes of intersection (Bertrand *et al*., 2012; Geissler, Zach, 2012). The earliest report by Axelrod et al. explained the connection between WNT and Notch

signaling was in fruit flies (Axelrod *et al*., 1996). Also, WNT pathway can influence the expression of HH signaling through Gli3 proteins and vice versa (Watt, 2004; Alvarez-Medina *et al*., 2008). Also, WNT and HH signalings are shown to control Notch ligands; Jagged1 and Jagged 2 respectively and thereby directly effecting the Notch signaling cascade (Estrach *et al*., 2006, Chen *et al*., 2010). Cooperation between the Notch and WNT signaling is required for the proliferation of intestinal precursor cells but not for the subsequent differentiation of the intestinal epithelial cells (Fre *et al*., 2009). Moreover, the downstream target of Notch signaling, Hes-1 can be stimulated by HH signaling as well (Wall *et al*., 2009; Wall, Wallace, 2009; Sang, Roberts, Coller, 2010). Kwon et al. also explained the interaction between β-catenin and the cytoplasmic domain of Notch receptors which consequently stimulates the degradation of β-catenin thereby directly modulating the WNT pathway (Kwon *et al*., 2011). GSK-3β has a central role in WNT pathways and stands as a key component and its interaction with Notch pathway and its influence on the oncogenic process has been explained very recently (Bertrand, 2020). GSK-3β takes part in the process of Notch receptor stability, activation and cleavage (Espinosa *et al*., 2003; Singh *et al*., 2018). Zheng and Connor showed that expression of GSK-3β and Notch-1 was inversely related and inhibition of GSK-3β resulted in upregulation of Notch-1 and NICD (Zheng, Conner, 2018). Interestingly, GSK-3β is also shown to phosphorylate the Notch-1, -2 and -3 leading to the ubiquitin-dependent proteolytic degradation (Jin *et al*., 2009). These GSK-3β mediated phosphorylation ultimately leads to suppression of Notch-mediated gene transcriptions in HEK-293T and NIH-3T3 (Espinosa *et al*., 2003). These finding suggest the mechanistic interconnection between WNT and Notch pathways. It is explained that, during the differentiation and proliferation of thymocyte, HH and Notch signaling share exact Spatio-temporal window in a non-redundant manner, thus maintaining an intracellular balance (Pelullo *et al*., 2019). The present knowledge and understanding of these signaling pathways and their corsstalk is not complete, however it provides a window showing that the interplay and connection could lead to identification of potent targets for designing targeted treatment strategies.

CONCLUSION AND FUTURE PROSPECTS

Colon carcinogenesis is a complex multistep process and it emanates from a series of molecular and histopathological alterations involving a variety of oncogenes and tumor suppressor genes that transform normal colonic epithelium into invasive carcinoma. The epithelium of the gastrointestinal tract is continually replaced and to maintain homeostasis of the intestinal epithelium cellular proliferation, differentiation, migration and death must be strictly regulated. A few but highly conserved signaling pathways like HH, WNT/β-catenin and Notch pathways are thought to drive these processes. Alterations in the above-mentioned pathways that control developmental processes during embryogenesis and organogenesis have been recognized as hallmarks of cancer. It is not surprising that many of these signaling pathways are altered in oncogenic processes. In the light of recent knowledge of developmental pathways in colon cancer, and therefore, targeting these deregulated embryonic pathways holds promise in clinical therapeutic development for colon cancer. Therefore, the present review overlays a platform to better understand and enable the development of better therapeutic approaches for the prevention and management of colon cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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