



## Review

## Secreted frizzled related proteins: Implications in cancers



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## ABSTRACT

The Wnt (wingless-type) signaling pathway plays an important role in embryonic development, tissue homeostasis, and tumor progression because of its effect on cell proliferation, migration, and differentiation. Secreted frizzled-related proteins (SFRPs) are extracellular inhibitors of Wnt signaling that act by binding directly to Wnt ligands or to Frizzled receptors. In recent years, aberrant expression of SFRPs has been reported to be associated with numerous cancers. As gene expression of SFRP members is often lost through promoter hypermethylation, inhibition of methylation through the use of epigenetic modifying agents could renew the expression of SFRP members and further antagonize deleterious Wnt signaling. Several reports have described epigenetic silencing of these Wnt signaling antagonists in various human cancers, suggesting their possible role as tumor suppressors. SFRP family members thus come across as potential tools in combating Wnt-driven tumorigenesis. However, little is known about SFRP family members and their role in different cancers. This review comprehensively covers all the available information on the role of SFRP molecules in various human cancers.

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## 1. Introduction

Wnt signaling plays an essential role in cell proliferation, patterning, and fate determination during normal developmental processes [1–4]. Wnt signaling pathways are traditionally characterized as  $\beta$ -catenin dependent (canonical) and  $\beta$ -catenin independent (non-canonical) pathways, the latter comprising the non-canonical planar cell polarity and the Wnt/ $\text{Ca}^{2+}$  pathways. However, it has recently been suggested that there is a degree of overlap and interaction amongst these pathways [5]. Major effectors of the Wnt signaling pathway are the Wnt ligands, which are a large family of secreted glycoproteins that are cysteine-rich and highly hydrophobic, and Frizzled receptors (FZD) that bind to Wnt ligands and initiate Wnt driven signaling. There are 19 known Wnt proteins in mammalian systems along with 10 known human frizzled receptors whose expressions are spatially and temporally regulated during development [6].

### 1.1. Canonical Wnt signaling pathway

During the inactive ‘OFF’ state of the canonical pathway,  $\beta$ -catenin is bound by the destruction complex that consists of Axin, adenomatous polyposis coli (APC), and glycogen synthase kinase-3- $\beta$  (GSK3 $\beta$ ), where phosphorylation by GSK3 $\beta$  primes it for  $\beta$ -transducin repeat-containing protein ( $\beta$ -TrCP) mediated ubiquitylation, followed by proteosomal degradation [7]. Prior to phosphorylation by GSK3 $\beta$ , priming phosphorylation of serine 45 on  $\beta$ -catenin by casein kinase 1 (CK1) is required, where CK1 is bound to axin [8]. Concurrently, transcriptional activity of TCF is inhibited by corepressor Groucho [9]. Upon binding of the Wnt ligand to the FZD membrane receptor protein and low-density lipoprotein receptor-related proteins (LRP-5/6), the canonical Wnt signaling pathway is activated (Fig. 1). This interaction causes Axin and the phosphoprotein disheveled (DVL) to bind to phosphorylated LRP5/6, thus inhibiting the function of the destruction complex, which results in an increased level of stabilized  $\beta$ -catenin in the cytoplasm.  $\beta$ -Catenin then translocates to the nucleus and, in concert with the T-cell factor/lymphocyte enhancer factor (TCF/LEF) family of transcription factors, promotes the expression of Wnt-responsive genes such as *c-myc* [10] and *cyclin D* [11]. The downstream targets of Wnt signaling are involved in cell survival, proliferation, and differentiation, and an aberrant activation of Wnt signaling has been frequently associated with tumorigenesis [12–14]. Inhibition of Wnt signaling has been an area of extensive research as a potential target for cancer therapy. One approach of inhibiting Wnt signaling is through Wnt antagonists that keep Wnt signaling in check. Therefore, a better understanding of these antagonists is imperative in modulating Wnt signaling.

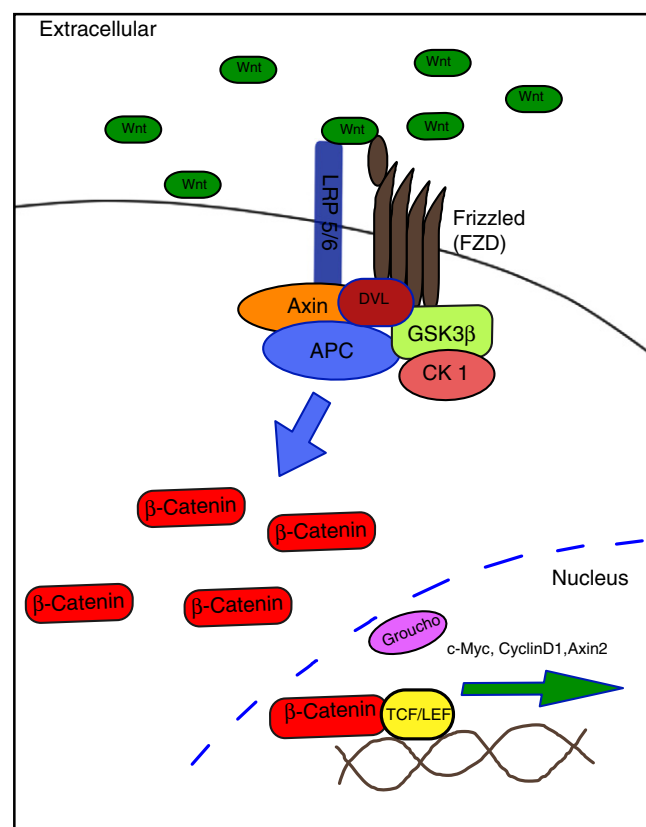
### 1.2. Non-canonical Wnt signaling pathway

In non-canonical Wnt signaling, Wnts are able to initiate downstream signaling and transcription in a  $\beta$ -catenin independent manner. Two of the more well-characterized mechanisms of the non-canonical Wnt signaling pathways are the planar cell polarity (PCP) pathway and the Wnt/ $\text{Ca}^{2+}$  pathway. The PCP signaling pathway is essential for regulating cell polarity during morphogenesis via activating JUN-N-

terminal kinase (JNK)-dependent transcription factors, through a cascade involving small GTPase RAC1 and RHOA, as well as JNK. PCP is activated often by the Wnt5A ligand, and numerous studies have shown the PCP signaling pathway to antagonize the canonical pathway [15]. In the Wnt/ $\text{Ca}^{2+}$  pathway, phospholipase C (PLC) is first activated, resulting in the release of intracellular  $\text{Ca}^{2+}$  stores, which subsequently activates downstream effectors,  $\text{Ca}^{2+}$  and calmodulin-dependent kinase II (CAMKII), calcineurin, and protein kinase C (PKC), and finally activating the transcriptional regulator Nuclear factor of activated T-cells (NFAT). The Wnt/ $\text{Ca}^{2+}$  signaling pathway has been found to be associated with SFRP2 during angiogenesis, another hallmark of cancer in breast cancers, via increased expression of NFAT [115].

## 2. SFRP family of Wnt antagonists

It is suggested that Wnt signaling is regulated by several classes of negative modulators. Wnt antagonists can be divided into 2 classes based on their mechanisms of action [16]. The first class includes the SFRP family, Wnt inhibitory factor (WIF)-1, and Cerberus. Wnt



**Fig. 1.** The canonical Wnt signaling pathway (“ON” State). Binding of secreted Wnt factors to Frizzled receptors on the cell membrane transduces a signal to the Axin complex that inhibits phosphorylation and degradation of  $\beta$ -catenin.  $\beta$ -Catenin then accumulates in the cytoplasm and can translocate to the nucleus, where it interacts with TCF/LEF and other families of transcription factors to regulate expression of target genes.

antagonists belonging to this class bind to Wnt proteins as well as FZD and are able to block all Wnt signaling pathways. The second class consists of members of the Dickkopf (DKK) family that bind to Wnt co-receptors LRP5/6 and inhibit only the canonical  $\beta$ -catenin pathway.

SFRPs, the first Wnt antagonists to be identified, are secreted glycoproteins that modulate the Wnt signal transduction pathway [17]. They are approximately 300 amino acids in length, with a signal sequence and a frizzled-like cysteine-rich domain (CRD) constituting the N-terminal and a hydrophilic heparin-binding region, which makes up the C-terminal. The CRD domain has 10 conserved cysteine residues and exhibits a close sequence homology to the CRD domain on the extracellular part of the frizzled receptors [18,19]. This homology raised the suspicion that SFRP molecules might compete with the FZD receptors to bind Wnt ligands. However, unlike the FZD receptors, the SFRPs lack any transmembrane or cytosolic domain.

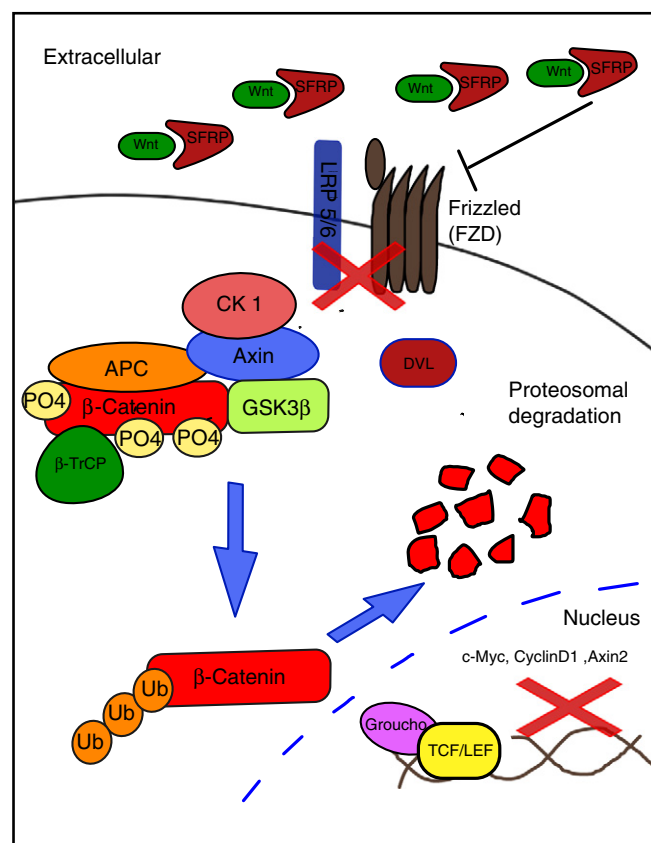
Five mammalian SFRPs (SFRP 1–5) have been identified to date, with additional family members (Crescent, Sizzled, Sizzled-2, and Frzb-2) characterized in *Xenopus* and chick models. Finch et al., purified and cloned the SFRP gene for the first time [20], and they confirmed it to be antagonistic to Wnt signaling. Further evidence to their antagonistic role came from studies where SFRPs had been shown to interact with and bind to Wnt ligands [21–25]. These papers also summarized the antagonizing effects of SFRP molecules on Wnt-induced increases in free  $\beta$ -catenin levels and morphological alterations. Also, SFRPs and SFRP-like molecules affected cell cycle progression and decreased tumor cell proliferation [26]. SFRP proteins have thus emerged as potential Wnt antagonists and a new class of tumor suppressors (Fig. 2).

Numerous studies have reported a downregulation of SFRP members in various cancers [27–29], indicating a loss of function. However, there exist contradictory reports that challenge the antagonistic role of SFRPs [30]. Mii et al. [31], showed that SFRP molecules enhance Wnt diffusion and positively modulate Wnt signaling. Here, in stark contrast to their expected role, SFRP molecules aided the diffusion of Wnt8 and Wnt11 in a *Xenopus* embryo model, leading to the activation of Wnt signaling. In a mouse model, inactivation of SFRP 1 and 2 disrupted Wnt signaling and Wnt-mediated developmental events [32]. The authors showed that depletion of these SFRP molecules impaired Wnt spreading and thus prevented Wnt signaling-dependent specification of the mouse optic cup periphery. However, such a regulation could just have been a tissue-specific effect since many cases and studies have shown SFRP molecules to act as Wnt antagonists. Clinical intervention can therefore utilize the potential of these Wnt inhibitors for the treatment of Wnt-dependent tumors. Further understanding of these secretory molecules would be pivotal in the development and progress of anti-Wnt initiatives.

### 3. Role of SFRPs in cancer

#### 3.1. Prostate cancer

Prostate cancer is the most commonly diagnosed non-cutaneous malignancy and is the third leading cause of death in men of Western descent [33]. This disease is known to be heterogeneous in nature, varying from slow-growing benign tumors to aggressive, metastatic, and malignant tumors. The Wnt signaling pathway is aberrantly activated in prostate cancer and the expression of Wnt ligands is altered. Also, hyperactive Wnt signaling has been linked with androgen-independent growth through crosstalk with the androgen receptor [34], development of bone metastasis [35], and self-renewal of prostate cancer stem cells [36]. The heterogeneity of prostate cancer is also due to a broad spectrum of genetic and epigenetic abnormalities. An epigenetic “catastrophe” occurs during the earliest stages of prostate cancer development and is maintained in a clonal manner through metastatic progression [37]. This catastrophe comprises changes in DNA methylation through *de novo* promoter hypermethylation and silencing of tumor suppressor genes that have important regulatory functions [38].



**Fig. 2.** SFRP mediated inhibition of Wnt. In the presence of SFRPs, canonical Wnt signaling is inhibited as SFRP binds to both Wnts and Frizzled receptors, causing phosphorylation followed by ubiquitylation of  $\beta$ -catenin, priming it for proteosomal degradation.

To understand the crucial role of the Wnt signaling pathway in prostate cancer, Perry et al. [36] examined the role of Wnt antagonists (SFRPs) in prostate cancer cell lines, namely, LNCaP, PC-3, DU145, and 22Rv1, on a set of 20 tumor specimens obtained from men undergoing radical prostatectomy. It was reported that SFRP2 was methylated in 70% of tumor specimens and SFRP5 was methylated in 60% of DU145 and PC-3 cancer cell lines. In 30% of tumor specimens and in all the cancer cell lines tested, the methylation of SFRP3 was detected. However, SFRP1 methylation was only detected in DU145 and PC-3 cancer cell lines and SFRP4 methylation was not noticed in any prostate cancer cell line. Thus, it is plausible that methylation of SFRPs and inactivation of antagonists contribute to the development of prostate cancer through abnormal Wnt signal activation. Several components of the Wnt/ $\beta$ -catenin pathway such as GSK3 $\beta$ , APC, AXIN, and casein kinase were also elevated in tumor specimens and cancer cell lines. Moreover, GSK3 $\beta$  had been shown to suppress androgen receptor (AR)-mediated transactivation and cell growth [39]. AXIN, which is transcriptionally activated through Wnt signaling [40] directs  $\beta$ -catenin for degradation through ubiquitin-dependent proteolysis and was previously reported to be upregulated in prostate cancer cell lines [41].

Furthermore, in a comprehensive study, SFRP4 was observed to inhibit Wnt signaling and cellular proliferation in androgen-independent prostate cancer cells *in vitro* [42]. SFRP4 overexpression was associated with a decreased rate of proliferation, decreased anchorage-independent growth, and decreased invasiveness in PC-3 cancer cells. Furthermore, in localized human androgen-dependent prostate cancers, increased membranous SFRP4 expression was associated with increased membranous  $\beta$ -catenin expression. These suggest that SFRP4 is an inhibitor of prostate cancer growth and invasion *in vitro* by arresting the Wnt signaling pathway, independent of AR signaling. Consequently, future therapeutic strategies to modulate Wnt signaling by SFRP4 will be

relevant to both localized androgen-dependent prostate cancer and advanced metastatic disease [43].

In addition, in androgen-independent prostate cancer cells, SFRP3 has been shown to suppress prostate tumor growth and cellular invasion. Similarly, evidence points to a role for SFRP4 in the inhibition of cellular proliferation in advanced androgen-independent prostate cancer [44]. Since SFRP4 overexpression in LNCaP cells decreases the growth rate of cells, this suggests that SFRP4 can inhibit prostate cancer growth and metastasis independent of AR signaling, and thus influences the behavior of the benign and malignant form of cancer. Moreover, in a study by O'Hurley et al., immunohistochemical analysis on prostate cancer tissue microarrays with samples from 216 patients was conducted. A strong/moderate SFRP2 expression was observed in epithelial cells of benign prostatic hyperplasia, and negative/weak SFRP2 expression was observed in the majority of tumor epithelia, implying a loss of SFRP2 expression from benign to malignant prostate glands [45]. Based on the aforementioned studies, it can be suggested that SFRP is a negative regulator of androgen receptor activity in prostate cancer.

### 3.2. Breast cancer

Wnt signaling is implicated at several stages of mammary gland growth and differentiation [46], and possibly in the involution of the mammary gland following lactation. Numerous studies establish the Wnt signaling pathway as an accomplice in mammary gland carcinogenesis [47,48]. The Wnt signaling pathway is dysregulated in breast cancer, leading to an upregulation of Wnt players. Wnt ligands are highly expressed in breast tumor tissues and cell lines when compared to their normal counterparts [49]. Wnt ligand activated  $\beta$ -catenin signaling was shown to promote mammary gland hyperplasia [50], and increased  $\beta$ -catenin activation has been observed specifically to be enriched in highly invasive basal-like triple negative breast cancers, leading to poorer prognosis [51,52].

Besides  $\beta$ -catenin activation, breast cancers shown to possess elevated Wnt signaling, have suppressed expression of Wnt antagonists. SFRP1 was shown to be suppressed in highly invasive breast carcinomas [53,54], and its ectopic overexpression in MDA-MB-231 breast cancer cells was found to block the Wnt pathway, downregulate the expression of Cyclin D1, increase the expression of p21, and suppress Wnt mediated xenograft growth and metastasis [55]. Schneider's group in a recent study [56] found that SFRP1 mediated its tumor-suppression role through p53-mediated apoptosis. They showed that mammary epithelial cells from SFRP1 knock-out mice had a decreased expression of p53, caspase-3, along with lesser DNA fragmentation in response to DNA damage, and a recombinant expression of SFRP1 could elevate the levels of pro-apoptotic and p53 mediated gene expression. In another study, loss of SFRP1 expression was thought to be an early event in breast tumorigenesis [57], with its expression inversely correlated with tumor stage ( $p < 0.001$ ) but not tumor grade or lymph node status. This suggests a potential role of SFRP1 as a biomarker for detection of early stage breast cancer. Tumor specific suppression of SFRP1 was seen to be mostly a result of epigenetic modifications [58]. Methylation related studies detected methylation of the SFRP1 promoter in 7 out of 8 breast cancer cell lines, 9 out of 13 grade 2 and 3 ductal carcinomas *in situ* (DCIS), 19 out of 28 invasive ductal carcinomas (IDC), and 6 out of 18 lobular carcinomas, and interestingly, not in any of the 14 normal mammaplasty specimens and mammary epithelial organoids examined. Methylation-based inactivation/suppression of SFRP1 was indicative of poor and unfavorable prognosis among patients [59]. Outside the Wnt signaling pathway, SFRP1 was also seen to block thrombospondin-1 mediated adhesion and migration of the breast cancer cell line MDA-MB-231 [60]. This inhibition was possible because of an interaction between SFRP1 and thrombospondin-1 that involved the netrin domain of the former.

SFRP2 has also been reported to undergo promoter hypermethylation-based silencing in breast cancer [61]. Of the cancerous cell lines studied,

7/8 (88%) lacked SFRP2 mRNA expression due to SFRP2 promoter methylation. Of the primary tumor samples analyzed, expression was strongly reduced in 93 of 125 specimens (74%), with methylation detected in 165/199 primary carcinomas (83%), whereas normal breast tissues remained unaffected by SFRP2 methylation. In the same study, SFRP2 expression was restored with 5-aza-2'-deoxycytidine and trichostatin A treatment, confirming a methylation specific effect. Also, loss of SFRP2 protein expression showed a weak association with unfavorable patient overall survival, and its forced expression in mammary MCF10A cells substantially inhibited proliferation rates.

Apart from SFRP1 and SFRP2, various studies also reported SFRP5 expression to be downregulated and epigenetically inactivated in breast cancer, and this trend dictated a poor overall survival [62]. The expression of the molecule was rescued when cells were treated with DNA-demethylating drugs, confirming the methylation-based silencing of another SFRP tumor suppressor in breast cancer. Suzuki et al. reported epigenetic inactivation of various other Wnt antagonist genes in breast cancer [63]. In their study, frequent and significant methylation of SFRP family genes, including SFRP1, 2, and 5, was found in cancer cell lines and primary breast tumors, and a loss of function of these genes resulted in activation of Wnt signaling in breast carcinogenesis.

A microarray-based study revealed a correlation between high expression of SFRP4 and inhibition of cell growth and proliferation [64]. Studies hint at Wnt-3a being the Wnt ligand that SFRP4 seems to be mediating its effects through. SFRP4 was found to abrogate Wnt-3a-induced  $\beta$ -catenin and Akt/PKB signaling. Using an immunoprecipitation assay, SFRP4 was seen to interact with Wnt-3a and this interaction was assumed to inhibit Wnt-3a induced inhibition of *in vitro* mammary differentiation [65].

Interestingly, there have also been reports that outlined contrasting effects of SFRPs in breast cancers, where SFRP molecules may support tumor progression. One instance is SFRP2, where it was found to be highly overexpressed in canine mammary gland tumors compared to their normal counterparts [66,67]. This increase in SFRP2 expression was shown to block UV-induced apoptosis and induce tumorous transformation in normal mammary epithelial cells. Functionally, SFRP2 associated with the fibronectin-integrin protein complex in the extracellular matrix (ECM) to mediate cell adhesion, and disruption of the same complex abrogated the anti-apoptotic activity of SFRP2 [68]. The same group also characterized the activation of NF $\kappa$ B or the suppression of c-Jun N-terminal kinase (JNK) as being behind the anti-apoptotic role of SFRP2 [69].

In conclusion, SFRPs have been widely shown to be playing an antagonistic role on Wnt signaling. Inhibitors could therefore be designed based on SFRP molecules to target Wnt driven tumors.

### 3.3. Ovarian cancer

Wnt signaling has been found to be involved in both normal and tumorigenic development of the ovaries [70]; which includes folliculogenesis, luteogenesis, and steroidogenesis, and also tumorigenesis of granulosa cells [71,72]. Wnt ligands are widely expressed in the ovaries [73], are essential for ovarian follicular development and fertility [74], and also contribute to ovarian tumorigenesis through the canonical Wnt signaling pathway [75].  $\beta$ -Catenin, an essential component of the Wnt signaling pathway, contributes to ovarian endometrioid carcinomas [76,77].

SFRP4's pro-apoptotic potential is supported from the observation that it is involved in ovulation related apoptotic processes in the ovaries [78]. Ovulation, which involves the apoptosis mediated degeneration of areas of the follicular and ovarian surface, is guided by the expression of many apoptosis related genes including SFRP4. From the analysis done in the above study, SFRP4 gene expression was found to be upregulated with apoptosis in the ovarian corpus luteum.

The pro-apoptotic role of SFRP4 would only trigger cancer cells to try and avert its action. Loss of SFRP4 was seen to render an aggressive



phenotype of ovarian cancer [79], with poor prognosis for such patients. Immunohistochemical analysis of SFRP4 expression in samples from a cohort of 721 patients, established that SFRP4 was most highly expressed in tubal epithelium and decreased with malignant transformation. Loss of membrane expression was also an independent predictor of poor survival. These results establish a tumor suppressor role for SFRP4 in ovarian cancers. This silencing or downregulation of SFRP4 could be due to promoter methylation, as SFRP1 was also found to be epigenetically silenced in ovarian cancer [80]. SFRP4, with its pro-apoptotic role, is also seen to be downregulated upon establishment of chemoresistance. Its expression is higher in the chemosensitive ovarian cancer cell lines and much lower in the cell lines resistant to Cisplatin [81]. This study also demonstrated that silencing of SFRP4 in the sensitive cell lines rendered them resistant, and re-expression of SFRP4 in the resistant cell lines increased their sensitivity to the drug. Therefore, these findings establish SFRP4 as a novel chemo-response marker in ovarian cancer. Another member of the SFRP family, SFRP5, was seen to be epigenetically silenced in malignant ovarian cancer [82]. Downregulation of SFRP5 promoted the Wnt signaling pathway, tumorigenesis, and chemoresistance to Cisplatin, and restoration of SFRP5 expression decreased Wnt signaling and cancer growth; along with cells' invasive ability and tumorigenicity in mice. SFRP5 also inhibited TWIST mediated epithelial-mesenchymal transition (EMT) and downregulated AKT2 to sensitize the cells to chemotherapy. This reiterates the involvement of SFRP molecules in tumor-suppression and chemo-response.

SFRP4 is generally downregulated in ovarian cancer but contradictory reports exist reporting a high expression of this protein in cancer. High expression of SFRP4 was detected in primary serous ovarian tumors [83], but interestingly, the expression was inversely correlated to  $\beta$ -catenin expression. The mechanism by which decreased  $\beta$ -catenin expression affected Wnt signaling and tumor formation was not studied.

### 3.4. Endometrial cancer

Wnt signaling plays a pivotal role in endometrium development and differentiation, and regulates uterine morphology and function. However, Wnt ligands, the activators of Wnt signaling, show differential expression in the human endometrium [84]. Surprisingly, a downregulation of Wnt members 2, 3, and 5 and an upregulation of 7a, 7b, and 10b, was postulated to be associated with endometrial carcinoma [85].

Physiologically, SFRP4 has been found to be downregulated in the endometrium, when trophoblast induced decidualization and functional changes occurred during early pregnancy [86]. SFRP4 has had a tumor-suppressor role established in endometrial cancer. Its expression was found to be decreased in endometrial stromal sarcomas [87], where  $\beta$ -catenin expression was upregulated. Upon categorisation, SFRP4 was more frequently downregulated in MSI (microsatellite instability) cancers as compared with MSS (microsatellite stable) endometrioid endometrial cancers [88]. Both canonical and non-canonical Wnt signaling pathways have been shown to be activated by Wnt7a, and SFRP4 was shown to antagonize Wnt7a mediated signaling and inhibit proliferation of endometrial cancer cells [89]. Wnt7a is expressed in the luminal epithelium, with SFRP4 localized to the stroma and there was an interaction seen between them by immunoprecipitation, which could possibly explain SFRP4 mediated inhibition in Wnt7a signaling activation.

However, contradictory studies have also shown expression of SFRP4 to be positively correlated with cancer malignancy [90]. Here, SFRP4 mRNA, which also inhibited Wnt8 mediated morphological events, was significantly upregulated in the stroma of the endometrial hyperplasia and carcinoma, and in the stroma of *in situ* and infiltrating breast carcinomas.

As in the case with breast cancer, endometrial cancer studies also observe a dual role of SFRPs in Wnt signaling, with the majority supporting the notion that SFRPs could inhibit Wnt signaling.

### 3.5. Cervical cancer

As with other cancers, SFRPs have been found to be downregulated in cervical cancer and have been postulated as tumor suppressors [91]. Here, an mRNA transcript corresponding to the human SFRP gene (hSFRP) was detected in three normal cervical tissues, but not in three cervical cancer tissues and five human cervical cancer-derived cell lines. Re-expression of hSFRP in HeLa and CUMC-6 cervical cancer cells resulted in a caspase-3 mediated cell death. Apart from apoptosis, studies have also demonstrated the inhibitory capability of SFRP molecules on migration and invasion. SFRP1 and SFRP2 were found to decrease the Wnt mediated invasion abilities of cervical cancer cells [92] and was associated with a decrease in free  $\beta$ -catenin and cell growth.

Tumor specific inactivation of SFRP genes is primarily due to promoter methylation [93]. The rate of occurrences of promoter methylation was 52.2% (12/23), 82.6% (19/23), 65.2% (15/23), and 73.9% (17/23) for SFRP1, SFRP2, SFRP4, and SFRP5 respectively, with the frequencies being significantly higher in cervix adenocarcinomas than in normal matched controls [94]. Here, restoration of SFRP5 inhibited the Wnt signaling pathway and downstream target genes, as well as colony formation and invasive ability of the cancer cells. Another similar study found that the promoter regions of SFRP 1, 2, 4, and 5 genes were significantly hypermethylated in SCC (squamous cell carcinomas) relative to the HSIL (high-grade squamous intraepithelial lesions), LSIL (low-grade squamous intraepithelial lesions), or normal tissues [95].

### 3.6. Colorectal cancer

Colorectal cancer (CRC) is one of the most common malignant neoplasms and leading cause of cancer deaths worldwide [96]. The adenomatous polyposis coli (APC) gene was originally discovered to be involved in a hereditary cancer syndrome termed Familial Adenomatous Polyposis (FAP). FAP patients inherit one defective APC allele causing the development of large numbers of colon polyps early in life. The appearance of adenocarcinomas through clonal evolution is evident from the accumulation of activating mutations in oncogenes or silencing of tumor suppressor genes such as *K-Ras*, *p53*, and *Smad4* [97]. In addition, mutational inactivation of APC leads to the inappropriate stabilization of  $\beta$ -catenin [98,99], which leads to constitutive activation of the canonical Wnt signaling pathway and is regarded as the initiating event in ~90% of CRCs originating from mutations in APC, AXIN (Axis Inhibition Protein), or  $\beta$ -catenin/CTNNB1 [14]. These mutations result in accumulation of free  $\beta$ -catenin in the nucleus, which interacts with T-cell factor/lymphoid enhancer-binding factor, thereby stimulating gene transcription independent of upstream Wnt signals [100]. In addition, numerous upstream Wnt signaling components and Wnt antagonists, such as SFRPs, have been found to be dysregulated in CRC [94].

It is known that aberrant Wnt signaling is an early event in colorectal cancer progression [100]. Studies have shown that restoration of Wnt antagonists such as SFRP reduces Wnt signaling in colon cancer cells even in the presence of downstream mutations [101]. For instance, SFRP1 is considered to silence Wnt signaling by binding CRD to Wnt proteins, thus preventing interaction with FZD receptors [102]. Also, HCT116 and SW480 (human colon adenocarcinoma cell line) cancer cells transfected with SFRP genes showed decreased levels of cytoplasmic and nuclear  $\beta$ -catenin following overexpression of SFRP1, SFRP2, and SFRP5, thereby suggesting their role as tumor suppressors.

There is increasing evidence that both genetic and epigenetic variation in Wnt and apoptotic pathways affects susceptibility and progression of colorectal cancer [103]. In one study, SFRP1, 2, 4, and 5 were found to be frequently methylated in colorectal carcinoma and adenoma [104]. The antagonists were more frequently methylated in colorectal tumors as compared to the adjacent normal mucosa. Thus, the expression of SFRP1, 2, 4, and 5 was downregulated in carcinoma and adenoma. However, upon treating the cells with demethylating agents, such

as DAC (5-aza-2'-deoxycytidine)/TSA (Trichostatin A) combination, the silenced SFRP mRNA could be effectively re-expressed in colorectal cancer cell lines. In conclusion, the silencing of SFRP genes tends to increase with the colorectal tumor progression; therefore, the downregulation of SFRP genes may be associated with the progression of colorectal tumors.

In contrast to the above study, Huang et al. found an association of SFRP4 with risk of rectal cancer and early-stage CRC. In 20 CRC patients [105], SFRP4 expression was significantly increased in the cancerous tissues compared to the non-cancerous colorectal mucosa. Moreover, SFRP4 protein was upregulated in 45% of cancer samples compared to match non-cancerous tissues. However, the expression of SFRP1 was downregulated by more than threefold in CRC, and SFRP5 was also found to be downregulated in 80% of CRC samples [101,106]. Although the reason for SFRP4 overexpression in CRC is not clear, it can be proposed that SFRP4 may play a role at the intersection of Wnt and other signaling pathways.

### 3.7. Renal cancer

Wnt antagonist family genes are frequently downregulated in renal cell carcinoma (RCC) as a result of promoter hypermethylation [107]. In one study on renal carcinoma, the methylation frequency of all Wnt antagonists studied was significantly higher in RCC than in the matched normal tissue, with the methylation profile being identical in around 73% of RCC patients. In addition, the methylation status of Wnt antagonist genes in serum DNA was significantly correlated with tumor grade and stage, indicating the potential of the methylation status as a biomarker of RCC.

Other family members have also been studied individually with respect to their promoter methylation and tumor-suppressor activity. SFRP1 was found to be silenced by methylation at the promoter region [108]. Another study also described SFRP1 as a tumor suppressor; whose expression was silenced by methylation [109]. Here, methylation-specific PCR detected hypermethylation in 26/57 (45.6%) conventional RCC cases and a >3-fold decrease of SFRP1 expression in 33/34 (97.1%) conventional RCC cases. Loss of SFRP1 expression corresponded to a tumor phenotype of clear cell RCC (cRCC) [110]. Stable re-expression of sFRP1 in these cells decreased the expression of Wnt target genes, cell growth, and anchorage-independent growth, and also inhibited tumor growth in a xenograft model.

SFRP5 has also been found to act as a tumor suppressor in renal cancer, with its expression epigenetically downregulated in cancer tissues and cell lines [111]. There was increased expression of SFRP5 upon recruitment of histone acetylases to its promoter region when cells were treated with demethylating drugs. On a parallel scale, restoration of SFRP5 inhibited anchorage independent colony formation and cell invasion ability, and apoptosis in RCC cells was also increased.

However, contradictory reports emerged with respect to the role of SFRPs in renal cancer. For instance, SFRP1 was found to be upregulated in metastatic renal cell carcinomas [112], as detected by gene expression profiling in cell lines and immunohistochemistry of renal tissues. Its upregulation is explained by the unmethylated/hypomethylated status of its promoter region. Functionally, knocking down SFRP1 increased apoptosis and decreased the invasive potential of the cells by decreasing matrix metalloproteinase (MMP) 10. Another molecule, SFRP2, was shown to possess oncogenic functions in renal cancer [113]. Stably expressed sFRP2 promoted *in vitro* cell proliferation and *in vivo* tumor growth. This promotion was due to reduced phosphorylation of  $\beta$ -catenin and increased T-cell factor/lymphoid enhancer factor transcriptional activity positively regulating the expression of c-Fos, Bcl2, Bcl-w, cyclin B2, and cyclin E2. Even SFRP3 was seen to be acting as an oncogene in metastatic renal cancer [114]. Tissue microarray analysis showed that the level of SFRP3 protein was low in primary renal cancer tissues but high in metastatic renal cancer tissues. Functional analysis showed increased cell growth, invasion, and tube formation,

and decreased numbers of apoptotic cells in the SFRP3-transfected renal cancer cell line A498. The reverse trend was seen in metastatic cells (ACHN and Hs891.T) when SFRP3 expression was silenced by using a knock-down approach.

### 3.8. Bladder cancer

Exposure to carcinogenic compounds (arsenic, tobacco, etc.) is known to increase the risk of bladder cancer [115,116]. These carcinogens act through an epigenetic mechanism [117], silencing the tumor-suppressor SFRP genes in the process [118]; and frequency of promoter methylation of SFRP genes is associated with tumor stage ( $p < 0.02$ ). In this cancer Urakami et al. determined the predictive potential of methylation of all SFRP genes combined [119]. Fifty four bladder tumor biopsies and their corresponding matched normal controls were analyzed for the methylation and expression levels of six Wnt antagonist genes (SFRP1, SFRP2, SFRP4, SFRP5, WIF-1, and DKK-3). The tumor samples had increased methylation levels and a subsequent lower mRNA expression of the Wnt antagonists. The M (methylation) score of Wnt antagonist genes had a sensitivity of 77% and a specificity of 67% as a diagnostic biomarker. Even the urine-derived DNA from patients had an 80% similarity to the methylation pattern in the tumor samples, providing an opportunity to just use the urine-derived DNA for methylation-based cancer prediction.

This predictive potential of SFRP promoter methylation was increased much further when it was combined with the downregulation of another tumor suppressor, TP53 [120]. Both TP53 alteration and SFRP gene methylation markers were independently associated with invasive bladder cancer, and the combined effect of these alterations produced a >30-fold risk of invasive disease. Thus, classifying tumors based on the presence of these two markers may be a clinically powerful predictor of invasive bladder cancer.

### 3.9. Acute myeloid leukemia

Acute myeloid leukemia (AML) is a heterogeneous group of hematological malignancies and the development of AML requires cooperation between at least two classes of gene mutations; Class I mutations, such as FLT3, RAS, JAK2, PTPN11, and KIT mutations that activate genes in the kinase signaling pathways, thereby conferring proliferation and survival advantage to hemapatoipoietic cells; and Class II mutations, such as RUNX1/RUNX1T1, PML/RAR $\alpha$ , CBF $\beta$ /MYH11, MLL/PTD, AML1/RUNX1, and CEBPA mutations, which affect transcription factors and impair hemapatoipoietic differentiation [121]. Epigenetic modification, such as aberrant methylation in the promoter region of suppressor genes, also affects the development and progression of malignancies [122]. It is known that the Wnt signaling pathway is highly conserved and active in embryogenesis and tissue maintenance [123], and activation of the Wnt/ $\beta$ -catenin signaling pathway has been shown to be essential for the establishment of normal and leukemic stem cells [123,124]. Studies have shown that dysregulation of the Wnt signaling pathway from either overexpression of oncogenes or loss of Wnt inhibitors promotes uncontrolled cell proliferation and survival in AML [99,125,126]. t(8;21)(q22;q22) is the most frequent karyotypic abnormality in AML, which is characterized by the clonal proliferation of hemapatoipoietic stem or progenitor cells [127,128]. In AML, abnormal nuclear localization of non-phosphorylated  $\beta$ -catenin has been demonstrated both *in vitro* and in patient samples [125,129]. Also, studies have shown a correlation between nuclear  $\beta$ -catenin expression and survival in patients with AML [130]. An association between hypermethylation of Wnt inhibitors, SFRPs, and specific chromosomal translocations in AML was reported in a few studies [131,132] where the frequencies of hypermethylation of Wnt inhibitors were found to be 31.6% for SFRP1, 30.1% for DKK-1, 26.0% for WIF-1, 19.3% for SFRP2, 12.6% for SFRP5, and 1.5% for SFRP4 [133].

In another study, a methylation-specific polymerase chain reaction was used to analyze the promoter methylation of SFRP1, SFRP2, SFRP4, SFRP5, DKK-1, and DKK-3. Aberrant methylation of Wnt antagonists was detected in 64% of AML marrow samples. Treatment of the cell lines with DAC induced re-expression of methylated Wnt antagonists and inactivation of the Wnt signaling pathway by downregulating the Wnt pathway genes *Cyclin D1*, *TCF1*, and *LEF1*, as well as reduced nuclear localization of  $\beta$ -catenin. In addition, Western blotting showed a reduction of  $\beta$ -catenin in the nucleus, indicating the inactivation of the Wnt signaling pathway after treatment with DAC [134]. Therefore, the use of hypomethylating agents might be beneficial for AML patients by inhibiting the activation of tumor promoting genes.

Myeloid leukemias can be classified as either chronic or acute, and Wnt signaling has been implicated in regulating the growth of cells. Chronic myelogenous leukemia results from a Bcr-Abl translocation that originates in human stem cells (HSCs) [135] and is dependent on Wnt signaling for growth and renewal [99]. Several studies have examined the presence of DNA methylation of CpG islands in leukemia. Chung et al. [136] reported that methylation of APC was present in more than 50% of patients with T-cell leukemia or lymphoma [137]. Furthermore, after treatment with DAC, APC gene expression was restored in the T-cell leukemia cell lines [138]. Shen et al. reported that with increasing concentrations of DAC, the methylation levels of SFRP1, 2, 4, and 5 decreased in Molt-4 cells and Jurkat ALL cell lines, HL60 cells, and the APL (acute promyelocytic leukemia) cell line. These results suggest that promoter methylation downregulates SFRP gene expression in these cells [139].

### 3.10. Gastric cancer

The incidence of gastric carcinoma (GC) is highest in Eastern Asia, Eastern Europe, and South America while the lowest rates are in Africa and North America [140,141]. During cancer development, aberrant CpG island hypermethylation is found to occur frequently in the stomach [142]. To date, around 87 genes have been characterized to be inactivated by hypermethylation of the promoter CpG islands in GC. Additionally, the number of methylated CpG islands is observed to be higher in *Helicobacter pylori* (HP)-positive GC than in HP-negative GC.

This led Cheng et al. to screen the expression and methylation status of four SFRP members (SFRP1, 2, 4, and 5) in primary gastric cancer samples [143]. Among the four SFRPs examined, only SFRP2 was significantly downregulated in gastric cancer as compared to adjacent non-cancer samples. In addition, promoter hypermethylation of SFRP2 was detected in 73.3% primary gastric cancer tissues. However, the expression of SFRP2 was restored in gastric cancer cell lines upon treatment with a demethylating agent. Moreover, *in vivo* suppression of tumor growth, induction of cell apoptosis, and inhibition of proliferation can be achieved by forced expression of SFRP2. Thus, it can be concluded that epigenetic inactivation of SFRP2 is a common and early event in gastric carcinogenesis and may be a potential biomarker for gastric cancer. To summarize, SFRP2 inhibits tumor cell proliferation and induces cell apoptosis *in vitro*. The frequent silencing of SFRP2 by methylation in gastric cancer as compared to adjacent gastric mucosa suggests a potential tumor suppressor role.

### 3.11. Lung cancer

Lung cancer represents the leading cause of cancer deaths in the world [33,144], and non-small cell lung carcinoma (NSCLC) is the most common variant [145,146]. In a significant number of patients, the disease recurs after therapeutic surgery and adjuvant chemotherapy, ultimately leading to death. The cure rate for lung cancer remains low, with merely 15–20% five-year survival rates [33]. Thus, it is essential to understand the aberrant promoter methylation of cancer-related genes in lung cancer for the identification of high-risk populations, early detection, and prognostic and predictive markers of tumor behavior.

Lung cancer is a molecular disease driven by the multi-step accumulation of genetic, epigenetic, and environmental factors [147,148]. Epigenetic alterations such as DNA methylation, histone modifications, and microRNAs (miRNA) result in silencing of important cancer-related genes. Abnormal methylation of CpG islands, near the promoter region of many critical genes, has been associated with the initiation and progression of lung cancer [149,150].

Genetic as well as epigenetic dysregulation of gene expression during malignant transformation is partly due to the unusual expression of miRNAs [151,152], which are ~21-mer non-coding RNA molecules that regulate gene expression [153]. For instance, cigarette smoke is known to induce the expression of miR-31, which functions as an oncomir during human pulmonary carcinogenesis. Time course experiments revealed that the levels of miR-31 in lung cancers from smokers were higher than those observed in non-smokers. Thus, activation of miR-31 might be a significant phenomenon during human pulmonary carcinogenesis. Furthermore, it was recorded that overexpression of miR-31 diminishes SFRP1, SFRP4, and WIF-1, and increases Wnt-5a expression, suggesting its role as a tumor promoter. It is indicated that overexpression of miR-31 increases the proliferation and tumorigenicity of lung cancer cells while knock-down of miR-31 inhibits growth of these cells [154]. Collectively, these data suggest that miR-31 activates Wnt signaling in cultured lung cancer and normal respiratory epithelial cells [155]. Therefore, efforts should be directed to inhibit the aberrant expression of microRNAs.

### 3.12. Parathyroid adenoma

Primary hyperparathyroidism (pHPT), a condition common in postmenopausal females, is a result of excessive secretion of parathyroid hormone, resulting in hypercalcemia. This condition is commonly caused by parathyroid adenoma, a benign type of tumor in 85% of the cases; parathyroid carcinoma is rare [156]. Apart from surgical therapy, there is no effective treatment for pHPT, and parathyroid carcinoma is associated with death. Aberrations in the Wnt/ $\beta$ -catenin signaling pathway have been identified in parathyroid tumors, and an aberrantly spliced, truncated variant of LRP5 causes accumulation of  $\beta$ -catenin in the nucleus. It has been noted that normal parathyroid tissue displays low levels of hypermethylation, parathyroid adenomas display intermediate levels of hypermethylation, and parathyroid carcinomas display hypermethylation of all CpG islands. Genes that show significant and frequent hypermethylation are mainly involved in cell cycle regulation and transcription (*CDKN2B*, *CDKN2A*, *RB1*, *WT1*, *RASSF1A*, and *RIZ1/PRDM2*), and members of the Wnt/ $\beta$ -catenin signaling pathway (*APC*, *SFRP1*, *SFRP2*, and *SFRP4*). Upon treatment with the demethylating agent DAC in cell cultures, the gene expression of the hypermethylated genes was restored, suggesting that the epigenetic alterations are reversible and can be studied to inhibit the abnormal activation of the Wnt signaling pathway.

### 3.13. Oral cancer (esophageal carcinoma)

The incidence of esophageal adenocarcinoma is increasing at a startling rate in Western countries [157]. It is known to arise from Barrett's esophagus, a metaplastic condition in which the normal squamous epithelium is replaced by an intestinal-type epithelium [158]. Inactivation of tumor suppressor genes by promoter hypermethylation has been considered as an important mechanism involved in the development of Barrett's esophagus [159]. Several tumor suppressor genes, especially p16, have been shown to undergo epigenetic changes in Barrett's esophagus [160,161].

To explore the role of Wnt signaling and SFRP methylation in the cancer progression of Barrett's esophagus, the extent of methylation of SFRP genes was determined in esophageal adenocarcinomas [162], Barrett's esophagus, and normal epithelia. Hypermethylation of SFRP1, 2, 4, and 5 was detected in the majority of cancers. In contrast, protein



expression of SFRP1, 2, and 4 was downregulated in 87%, 67%, and 90% of cancers respectively, and the expression correlated inversely with grade and stage of cancers as well as grade of dysplasia. Demethylation treatment effectively re-expressed SFRP mRNA in cancer cell lines; thus, hypermethylation of SFRP genes is an early event in the evolution of esophageal adenocarcinoma, and methylation of SFRP1, 4, and 5 might serve as biomarkers for Barrett's neoplasia.

Oral squamous cell carcinoma (OSCC) is one of the most commonly occurring cancers of the head and neck region [163,164]. Recent studies have demonstrated that DNA methylation causes epigenetic silencing of cancer-related genes and plays an important role in OSCC carcinogenesis. To study inactivation of SFRP1, SFRP2, SFRP4, SFRP5, WIF-1, and DKK-3 in paraffin embedded oral cancer, methylation-specific PCR (MSP) was utilized. In particular, SFRP2, SFRP4, SFRP5, WIF-1, and DKK-3 showed methylation of their promoter in OSCC. This suggests that an epigenetic fingerprint may not only improve current diagnostic tools, but also contribute to therapeutics of oral neoplastic and pre-neoplastic lesions.

### 3.14. Hepatocellular cancer

Activation of  $\beta$ -catenin is observed in 50%–83% of hepatocellular carcinomas (HCCs) [165], and upregulated TCF/LEF transcription is frequently detected in liver cancer cell lines [166], although mutation of AXIN is less frequent [167,168] and mutation of APC is also not seen in HCC. This suggests that mechanisms other than these mutations may contribute to the aberrant activation of Wnt signaling in liver carcinogenesis, and it indicates that SFRPs may play a key role in liver carcinogenesis. In a study performed by Carruba et al., the authors tested Li-7, HuH-1, HepG2, HLF, Hep3B, HuH-7, JHH-4, CHC4, and CHC32 cell lines and observed that SFRP1 was methylated in 75% of cell lines tested, SFRP2 was methylated in 58% of cell lines, SFRP5 methylation was recorded in 58% of cell lines, and SFRP4 methylation occurred in 25% of cell lines. On the contrary, in normal liver tissue, no SFRP methylation was detected. In liver cancer cell lines, SFRP2 was silenced in HepG2 and Hep3B cells and, upon treatment with DAC, the expression of SFRP1, 2, and 5 was restored in these cells [169]. Thus, this suggests that SFRP methylation plays a key role in liver cancer progression.

### 3.15. Pancreatic cancer

Pancreatic cancer is one of the most malignant types of tumors, usually having extremely poor prognosis, in addition to a currently unclear cause of pathogenesis [170]. However, it has been discovered that loss of expression of tumor suppressor genes such as p16 [171] and RASSF1A [172] occur frequently in pancreatic cancer. The frequencies of methylation of SFRPs were also examined, where methylation of SFRP-4 was observed to be the highest (76.7%) in pancreatic cancer samples as compared to SFRP1, 2, and 5. Loss of expression of SFRP1, 2, 4, and 5 was found in the majority of the 60 pancreatic cancer samples studied. It was observed that the frequencies of hypermethylation and expression loss of SFRPs in pancreatic cancer samples were significantly higher than those in the adjacent normal tissue samples, suggesting that hypermethylation and subsequent expression loss of SFRPs occur early and play an important role in the pathogenesis of pancreatic cancer.

### 3.16. Malignant mesothelioma

Malignant mesothelioma (MM) is an aggressive cancer associated with past asbestos exposure, and is characterized by rapid progression, late metastases, and poor prognosis. Investigation of the role that Wnt signaling plays in the pathogenesis, progression, and resistance to apoptosis of MM has found evidence for elevated  $\beta$ -catenin protein levels in mesothelioma tumors and models, although activating mutations of CTNNB1 ( $\beta$ -catenin) have not been found in MM [173–175]. Several studies have examined specific molecules and have reported overexpression of Wnt1, Wnt2, and DVL in mesothelioma cells, with biochemical

consequences for cell proliferation and apoptosis [174,176,177]. Aberrant downregulation of SFRPs through promoter methylation of SFRP1, 4, and 5 has been reported in MM tumors and cell lines [178,179] as well as SFRP downregulation in mouse MM models [180]. The SFRP4 promoter has been reported to be hypermethylated in MM cell lines, and overexpression of SFRP4 resulted in growth suppression and apoptosis in mesothelioma cells [176]. However, these mechanistic studies employed  $\beta$ -catenin deficient MM cell lines. Current evidence indicates that MM tissue and cells are overwhelmingly  $\beta$ -catenin positive [173,174,181], suggesting that studies in  $\beta$ -catenin deficient cells may not be particularly relevant to the disease.

## 4. Role of SFRPs in cancer stem cells

Cancer requires the generation of an actively dividing cell. This cell may be an actively dividing stem cell, a stem cell stimulated to divide by tissue damage or inflammation, or a differentiated (mature) cell acquiring the property of self-renewal. Mutations inactivating tumor suppressor genes or activating oncogenes convert the activated stem cell into a cancer stem cell (CSC). CSCs are hypothesized to be the pathological counterpart of normal somatic tissue stem cells [182].

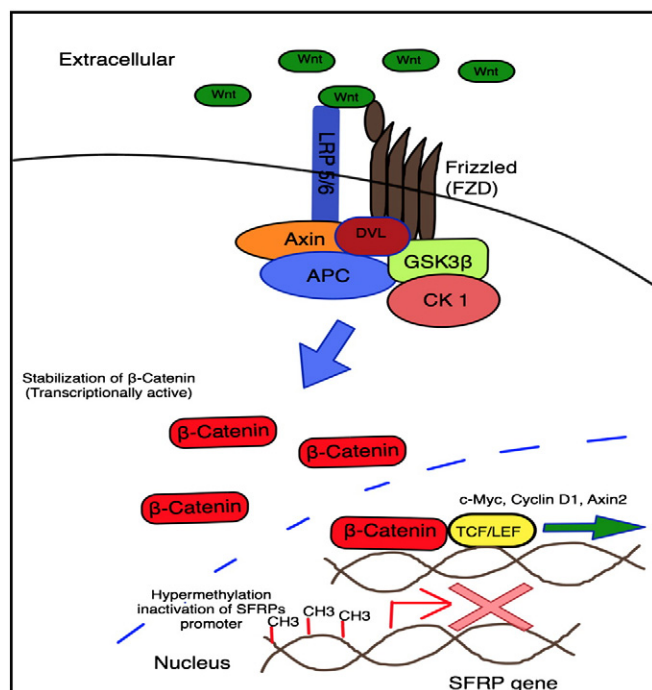
The evidence for the importance of the Wnt pathway in CSC biology and its role in the maintenance of CSCs was seen in myeloid leukemia. Zhao et al. [183] demonstrated the requirement of  $\beta$ -catenin for self-renewal of normal hematopoietic stem cells and CSCs in chronic myeloid leukemia in a mouse model. Moreover expression of the Wnt antagonist and tumor suppressor, Wnt inhibitory factor (WIF), was downregulated in astrocytomas by aberrant promoter hypermethylation [184]. Additionally, hypermethylation of the promoters of SFRP1 and SFRP2 is a significant event in primary glioblastoma multiforme (GBM), whereas hypermethylation of the antagonist Dickkopf (Dkk) was associated with secondary GBM [185]. Furthermore, SFRP4 was seen to substantially decrease the glioma stem cell (GSC) population and reduce the expression of stemness markers in GSCs derived from the glioma line U138MG (Warrier et al. in press). This inhibition was reversed by Wnt3a, indicating that the Wnt signaling pathway plays an important role in the acquisition and modulation of chemoresistance in glioma tumors and glioma stem cells.

## 5. Re-activating the SFRP genes

Aberrant Wnt pathway activation is a bona-fide driver of a wide range of tumorigenesis. Numerous academic pursuits and industrial investments have been dedicated at finding a cure for perturbations in Wnt signaling, with efforts being made to find inhibitors that could suppress or, at least, control the associated signaling events. As reviewed in [9], drugs such as NSAIDs (Non-Steroidal Anti-Inflammatory Drugs), Vitamins, and Imatinib mesylate, have been speculated as potential drug inhibitors of Wnt signaling. Although therapies targeting Wnt signaling sound attractive and plausible in theory, in the clinical setting they have fallen short of being an effective therapeutic regimen against Wnt related tumors. Garber's review [186] clearly outlines the problems faced in finding drugs against the Wnt pathway. Failure to do so has been attributed to the ubiquitous presence of Wnt signaling in a wide range of cellular processes, thereby affecting the efficacy and specificity of any promising therapeutics. Nevertheless, new technologies and an ever-increasing interest in Wnt signaling are helping the scientific community understand more about it and possibly develop better drugs.

As clearly evident from the current review, one possible area of intervention against Wnt signaling could be drug modulators involving SFRP molecules. SFRP proteins or SFRP-like molecules would compete with the FZD receptors for Wnt ligands, thus suppressing canonical signaling. On one hand, efforts could be made to design SFRP-like molecules or small-sized drug inhibitors that would reach the site of the tumor and inhibit Wnt mediated signaling. On the other hand, as SFRPs genes are often silenced via hypermethylation (Fig. 3), drugs





**Fig. 3.** Hypermethylation of SFRP. SFRP promoters, when hypermethylated, are silenced, resulting in reduced SFRP expression and an increased Wnt pathway activation.

that affect the methylation status of gene promoters could be used to alter the methylation status of SFRP gene promoters and result in their concomitant re-activation. Epigenetic gene silencing has been known to suppress many tumor-suppressor genes [187,188]. Interventions that affect epigenetic modifications have been considered as an approach to re-activate epigenetically silenced genes, including SFRPs. Karpf et al., [189] have explored the various avenues of re-activating epigenetically silenced genes.

Epigenetic modifications are mainly mediated by methyl transferases (DNA Methyl Transferases or DNMTs) and histone deacetylases (HDACs) that are recruited to the CpG islands to remodel the chromatin to a compact state (formation of heterochromatin regions). Inhibitors of DNMTs and HDACs are being tested as effective drugs against epigenetic silencing of tumor suppressors. Pharmacological inhibition of DNMTs using 5-aza-2'-deoxycytidine and decitabine, and genetic manipulation using antisense and siRNA technology, has been the most extensively studied approaches. Both the approaches showed a marked re-activation of tumor suppressor genes [190,191]. Due to the short half-life and toxicity of DNA-incorporating nucleoside-based 5-azacytidine drugs, other non-nucleoside based drug research has gained importance. Compounds such as arsenic trioxide [192] and quinoline-based DNA hypomethylating agents [193] have been found to be effective in their potential to inhibit and degrade DNMTs. Another group tested a class of acridine compounds such as quinacrine [194]. These DNA-intercalating agents desilenced SFRP genes, among many other hypermethylated genes including p16 and E-cadherin, and also inhibited the activity of DNMT enzymes *in vitro*. Histone deacetylases, important players involved in hypermethylation, were targeted using sodium butyrate, an HDAC inhibitor [195]. Here, the drug induced demethylation and histone modification at the promoter region of SFRP-1 and -2 genes in human gastric cancer cells, thus restoring their expression.

Thus, more studies are needed to better understand the processes and different players involved in the silencing of SFRP genes. Existing and novel drugs have to be tested to induce demethylation and re-activation of epigenetically silenced genes such as SFRPs. Methylation inhibitors, hypomethylating agents, and HDAC inhibitors are a few drug classes that have to be studied further and optimized for their efficacy in demethylating the silenced genes. A key aspect to look at is the

effect on Wnt signaling events and Wnt mediated tumorigenic effects upon usage of these drugs. Drugs that desilence SFRPs and also inhibit Wnt mediated effects are to be prioritized for further research.

## 6. Conclusion

In summary, the Wnt signaling pathway plays a key role in stem cell maintenance and differentiation of hematopoietic progenitors. Oncogenic activation of the Wnt/ $\beta$ -catenin signaling pathway is common in various cancers. In this review, we have provided a comprehensive summary of the roles of Wnt antagonist SFRPs in diverse cancer types, and their involvement in causing aberrant canonical and non-canonical Wnt signaling. The SFRP function as negative regulators of Wnt signaling and have important implications in carcinogenesis, where they are downregulated by promoter hypermethylation in many tumors. Restoration of SFRP function attenuates Wnt signaling and induces apoptosis in a variety of cancer types. Wnt signaling inhibits apoptosis through activation of  $\beta$ -catenin/TCF-mediated transcription. Results from various studies described in this review provide evidence for a potential role of SFRPs and associated Wnt signaling in cancer, and opens the possibility for treatment of cancer by developing novel therapeutic approaches that target this pathway.

In addition, the involvement of SFRPs has also been implicated in cancer stem cells. Cancer stem cells, unlike the bulk of the cells within a tumor, are elusive to drug treatment and remain untouched upon chemotherapy and radiotherapy, being the central cause for tumor initiation and recurrence. This additional role of SFRPs further complicates the roles of this class of Wnt antagonists in cancer. Further elucidation of the functions of SFRPs in Wnt mediated pathways and cancer would be pivotal in finding possible novel therapeutics for cancer through targeting these SFRPs.

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