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Review

Targeting Cancer with Paris' Arrow: An Updated Perspective on Targeting Wnt Receptor Frizzled 7

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Abstract:

The Wnt signalling pathway plays a crucial role in tissue homeostasis and cancer biology due to its regulation of cellular processes, including proliferation, migration, and stem cell activity. Frizzled receptor 7 (FZD7) (a member of the F-class G protein-coupled receptors) has emerged as a key Wnt receptor within this pathway, which is elevated in several human malignancies. FZD7 is notably upregulated in gastrointestinal, breast, pancreatic, and hepatocellular carcinomas and transmits oncogenic Wnt signalling through canonical and non-canonical pathways. FZD7 promotes tumour initiation and emerging evidence implicates FZD7 in cancer stem cell maintenance and epithelial-mesenchymal transition (EMT), reinforcing its role in metastasis. Therapeutic strategies targeting FZD7 have shown promise, including FZD7 specific monoclonal antibody-drug conjugates (ADCs), human single-chain fragment variable (scFVs) antibodies, and nanoparticles. Notably, our recent development of FZD7-ADC has demonstrated tumour-selective cytotoxicity with reduced off-target effects, positioning FZD7 as an attractive therapeutic target. Additionally, nanoparticle-based drug delivery systems have enhanced the precision of existing chemotherapies by targeting FZD7-expressing tumour cells. Despite significant advances, clinical translation remains a challenge due to potential on-target toxicity and the complexity of tumour microenvironments. Future research should focus on optimizing delivery systems, refining antibody specificity, and conducting comprehensive preclinical and clinical trials. This review will focus on novel discoveries regarding FZD7 in cancer and provide an update on our original review on this subject in 2016 (Phesse et al. 2016). Additionally, we present new figures generated by our group using the publicly available Pan-Cancer Atlas RNAseq datasets, highlighting FZD7 expression patterns in patient samples. This integrated approach aims to provide updated insights into the function of FZD7 during cancer and its growing status as an attractive target for therapy. In summary, FZD7 stands out as a promising molecular target in cancer therapy due to its selective overexpression in tumours, functional role in Wnt-driven oncogenesis, and potential for innovative therapeutic applications. This review underscores the critical need for continued exploration of FZD7-targeted therapies to improve patient outcomes in cancer treatment.

Keywords: Frizzled Receptor 7; Cancer; Therapy; RNAseq.

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1. Introduction

1.1. Overview of the Wnt Signaling Pathway

Wnt signalling is a highly conserved pathway found in all animals, playing a key role in cell differentiation and body axis polarity [1-3]. Once development has completed, Wnt signalling is required to conserve normal tissue homeostasis through the maintenance of cell renewal and stem cell compartments [1-3]. Deregulation of the Wnt signalling pathway is often associated with disease, specifically cancer [1,2], reflecting overlaps between the hallmarks of cancer and the function of the Wnt signalling pathway [1,2,4]. As the Wnt signalling pathway is often deregulated in cancer, it is an attractive avenue for therapeutic intervention [1-4].

The Wnt ligand family is composed of 19 glycoproteins in vertebrates [3,5]. These ligands are modified in the cytoplasm of the sending cell by O-acyltransferase porcupine (PORCN) which covalently attaches palmitoleic acid to a conserved serine residue [3,5]. Palmitoylation of Wnt proteins by PORCN is required for secretion of all mammalian Wnts and is critical for interaction with Wntless (WLS) which transports the modified Wnt proteins to the plasma membrane [3,5]. There are several mechanisms by which Wnt ligands can be transmitted to receiving cells, including secretion as micelles, diffusion and association with glypicans, attachment to extracellular vesicles or cytonemes [6-8].

The Wnt signalling pathway can be subdivided into two pathways: Canonical and non-canonical (Fig. 1) [1,3,5]. The canonical Wnt signalling pathway is made up of 3 major components: the ligand receptor complex, the destruction complex and β-catenin transcriptional complex [1-4]. The ligand receptor complex is formed when the pathway is "activated" by a Wnt ligand, a family of 19 secreted glycoproteins [1,5]. This activation of canonical Wnt signalling is characterised by the dimerization of FZD Wnt receptors and LDL receptor related protein 5/6 (LRP5/6) co-receptors, facilitated via the binding of a Wnt ligand to FZD's cysteine-rich domain (CRD) (Fig. 1A) [1,5]. A complete ligand/receptor complex leads to the recruitment of Dishevelled (Dvl) to the plasma membrane. Dvl is a phosphoprotein which phosphorylates LRP5/6 when the Wnt ligand/receptor complex is formed and leads to dissociation of AXIN from the destruction complex (Fig. 1A) [1,5]. The lack of destruction complex leads to accumulation of β-catenin within the cytoplasm and its nuclear translocation. Once in the nucleus β-catenin displaces Wnt transcriptional co-repressors such as Transducin-like enhancer of split (TLE) and Groucho, leading to the formation of the β-catenin transcriptional complex (Fig. 1A) [1,5]. The β-catenin transcriptional complex consists of co-transcriptional factors such as T cell factor (TCF) and lymphoid enhancer factor (LEF) [1,5].

In the absence of Wnt ligand, the ligand/receptor complex is unable to form, which subsequently allows the compilation of the destruction complex (Fig. 1B). The destruction complex consists of Axis Inhibition (AXIN), Glycogen Synthase Kinase 3 Beta (GSK3β), Adenomatous Polyposis Coli (APC), Casein Kinase 1 (CK1), Skp1-Cullin-F-box Containing Complex (SCF), Protein Phosphatase 2A (PP2A), and Beta-Transducin Repeat-Containing Protein (β-TrCP) [2,5]. CK1 delivers a phosphorylation modification to β-catenin. This modification at Seri45 allows GSK3β to phosphorylate β-catenin, creating a binding site for β-TrCP that facilitates ubiquitination and subsequent proteasomal degradation of β-catenin (Fig. 1B) [1,5]. This prevents accumulation and nuclear translocation of β-catenin, thus inhibiting formation of the β-catenin transcriptional complex (Fig. 1B) [1,5].

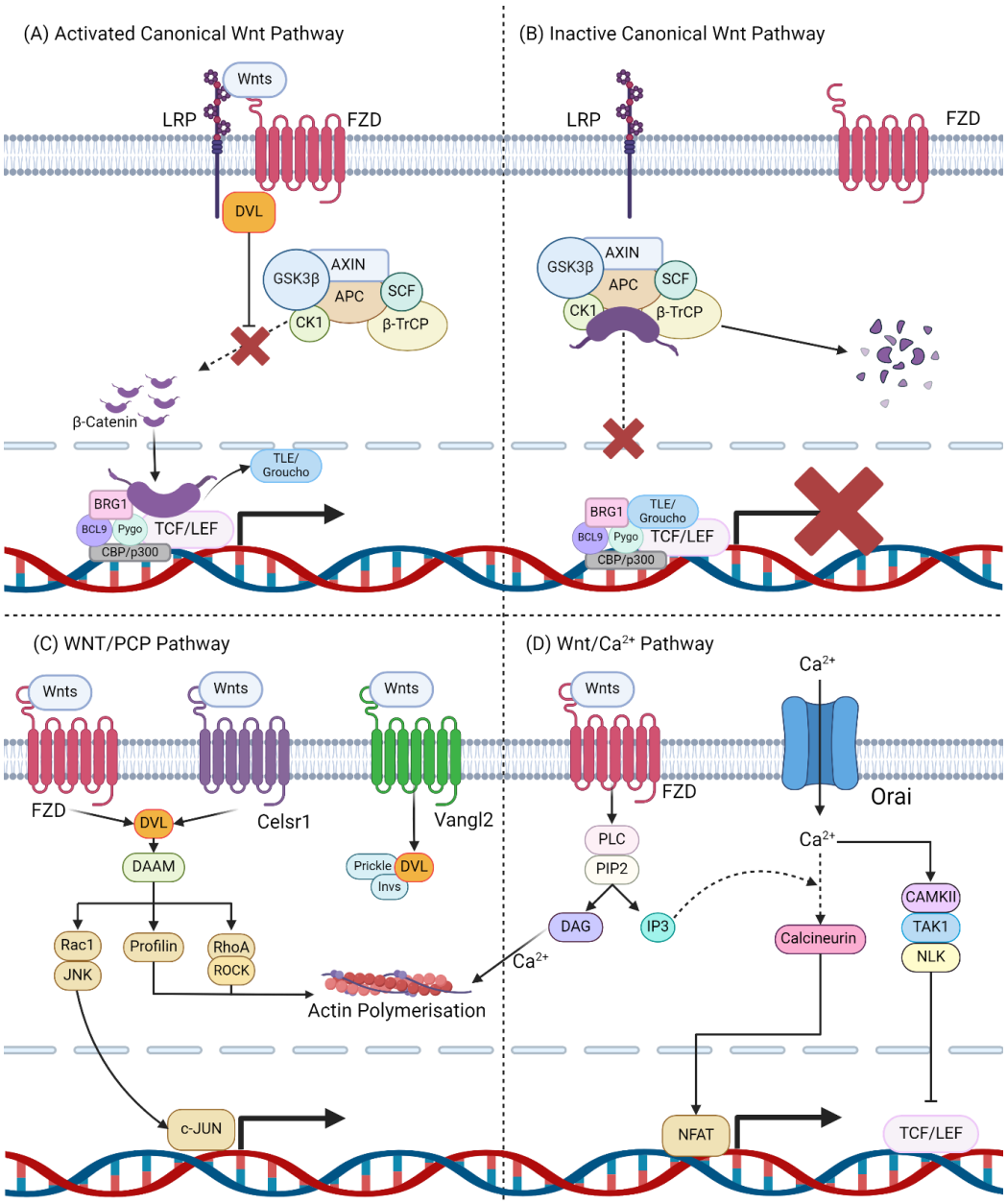

Figure 1: The Wnt Signalling Pathway. Panel (A) shows activated canonical Wnt signalling pathway. Wnt ligands bind to FZD receptors and LRP5/6 co-receptors forming the ligand receptor complex. This leads to recruitment of Dishevelled (Dvl) to the plasma membrane which inhibits the function of the destruction complex. β-catenin accumulates in the cytoplasm and translocases into the nucleus, displacing TLE/Groucho, forming the β-catenin transcriptional complex. Formation of this transcriptional complex leads to activation of canonical Wnt target genes which regulate cellular functions associated with tissue homeostasis. Panel (B) shows inactive canonical Wnt signalling pathway. In the absence of Wnt ligands, the destruction complex, which is composed of AXIN, APC, GSK3β, CK1, SCF and β-TrCP, sequentially phosphorylates cytoplasmic β-catenin. This results in ubiquitination of β-catenin and subsequent proteasomal degradation. Inhibiting β-catenin cytoplasmic accumulation limits its nuclear translocation, ensuring canonical Wnt target gene remain transcriptionally repressed. Panel (C) and (D) show the non-canonical Wnt signalling pathway. Panel (C) shows the Wnt/Planar cell polarity (PCP) pathway. Activation of the Wnt/PCP pathway may occur via multiple receptors, including FZDs, CELSR1 and VANGL2. Binding of specific Wnt ligands to FZD or CESLR1 leads to recruitment of Dvl. This begins a signalling cascade through DAAM which results in either transcriptional activation of Wnt/PCP target genes via c-JUN or activation of actin polymerisation through RhoA, ROCK and Proflin. Panel (D) shows the Wnt/Ca2+pathway. Orai proteins allow calcium to enter the cell. Again, binding of specific Wnt ligands to FZD receptors which results in G protein activation of

PLC which cleaves PIP2 into DAG and IP3. Calcium enters the cell and accumulates within the endoplasmic reticulum, where IP3 binds to its corresponding receptor, inducing a release of calcium into the cytoplasm. DAG and cytoplasmic Ca2+ initiate a signalling cascade to activate actin polymerisation via PKC and CDC42. IP3 mediated cytoplasmic Ca2+ then induce transcriptional repression of β-catenin/TCF/LEF transcriptional complex and activates NFAT via Calcineurin. Created in BioRender, https://BioRender.com/x65q290.

Non-canonical Wnt signalling can be further subdivided into the Wnt/planar cell polarity (PCP) (Fig. 1C) and Wnt/calcium (Ca2+) pathway (Fig. 1D) [1,5]. In the Wnt/PCP pathway FZD receptors work with several co-receptors including Protein Tyrosine Kinase 7 (PTK7), Muscle-Specific Kinase (MUSK), Receptor Tyrosine Kinase-Like Orphan Receptors 1 and 2 (ROR1/ROR2), Receptor like Tyrosine Kinase (RYK), Cadherin EGF LAG Seven-Pass G-Type Receptor 1 (CELSR1), and Van Gogh-Like Protein 2 (VANGL2). Downstream signalling is dependent on the co-receptor however all cascades begin with localisation of Dvl to the plasma membrane (Fig. 1C), reviewed further in Qin et al [5].

The Wnt/Ca2+ pathway predominantly signals through Wnt5a and FZD2 (Fig. 1D) [5]. Binding of the Wnt ligand to FZD2 results in PLC activation and cleavage of PIP2 [5]. This results in cytoplasmic influx of Ca2+, resulting in cellular polarization and activation of the downstream components of the Wnt/Ca2+ (Fig. 1D) [5].

There are 10 Frizzled Wnt receptors in vertebrates, which recognise and bind the fatty acyl groups on Wnt ligands, which mediates association with co-receptors to form the ligand/receptor complex to transmit Wnt signalling into the receiving cells [9]. Frizzled (Fzd) receptors have seven transmembrane domains, an extracellular N-terminal with a conserved cysteine rich domain (CRD), which is responsible for binding to Wnt ligands, and an intracellular C-terminal with a putative PDZ binding domain [1]. The interaction of specific Wnt ligands with specific Wnt receptors is poorly understood and is dependent on the availability or receptors, co-receptors and other pathway components [10]. FZD7 has been reported to transmit both β-catenin/canonical and non-canonical Wnt signalling, although this is not unique to FZD7 [1].

1.2. Frizzled Receptor 7 (FZD7) in the Wnt Pathway

The frizzled (FZD) receptor family consists of 10 members (FZD1-FZD10) which belong to the F class of G coupled receptors [11]. FZD7 is highly expressed throughout embryonic development, playing a key role in PCP pathway mediated prechordal plate progenitor cell protrusion formation and migration/mesoderm differentiation during gastrulation [12,13]. FZD7 has been extensively studied in the gastrointestinal tract with genetic deletion demonstrating its key, non-redundant role for the function of intestinal and gastric stem cells [14-17]. In the mouse intestine, Lgr5+ stem cells require FZD7 to maintain normal homeostasis and regeneration [14]. Alternatively in the stomach, FZD7 is not required to regulate the activity of stem cells in the gastric epithelium, Fzd7 deficient Lgr5+ were able to lineage trace as per normal Fzd7 proficient Lgr5+ cells [1,14-17]. FZD7 differential expression has been shown in many cancer types when comparing tumours to patient matched healthy tissue samples, where it transmits oncogenic Wnt signalling to promote tumorigenesis and malignant progression [1,18,19]. Although this review focusses on the growing body of work identifying FZD7 as an attractive target for cancer therapy, several other FZD Wnt receptors are also implicated in cancer [20,21], suggesting they may also be targets for therapy.

Inhibition of FZD7 has been used to demonstrate its requirement in several cellular functions that are important to cancer, including proliferation, stemness, differentiation,

migration, invasion and regulating the location of differentiated cells in normal tissue [15,22-24]. These studies, and many others described in this review, have helped identify FZD7 as a regulator of tumorigenesis and malignant progression, with increased FZD7 often associated with poor clinical outcome [25-27]. Tables 1 highlight the experiments investigating how FZD7 expression alters cancer function *in vitro* and *in vivo*. Table 2 displays the range of approaches taken to target FZD7 since 2016 identified in this review. Table 3 shows all cancer relevant miRNA interactions with FZD7 identified since 2016. (Table 1, 2, 3.)

Table 1. FZD7 Mechanism in Cancer

Cancer Types	Investigating FZD7 Role in Cancer Function		Reference
	In Vitro	In Vivo	
Gastric	FZD7 knockdown reduces colony forming ability, c-MYC and cyclinD1 expression in MKN28 & MKN24.	FZD7 knock out reduces tumour burden, c-MYC, CD44 and cyclinD1 expression in gastric GEMM ($gp130^{F/F}$).	[17]
Colorectal	circCSPP1/miRNA-944 mediated knockdown of FZD7 in CRC cells has been shown to reduce tumorigenic traits and reduces chemoresistance	shRNA targeting cricCSPP1 (a positive regulator of FZD7) reduced tumour size in subcutaneous CRC model	[28]
Breast	FZD7 knockdown via shRNA in MDA-MB-231 and BT-20 TNBC BCa cells resulted in reduced migration, invasion, proliferation and colony formation.	FZD7 shRNA knockdown in MDA-MB-231 reduced xenograft tumour size in subcutaneous model. Additionally, Axin2, Cyclin D1 and MYC expression were all downregulated.	[29-31]
Hepatocellular Carcinoma	shRNA knockdown of FZD7 in HCC cell lines results in NF-κB induced apoptosis through downregulation of Bcl-2 and Bcl-XL. siRNA-mediated FZD7 knockdown was able to sensitise HCC cells to several chemo-therapy agents (including 5-FU, MMC and ADR) significantly reducing their IC50 dosages. Increased FZD7 expression promotes colony formation in HCC cells.		[23,32-35]
Lung	Downregulation of FZD7 mRNA via miR-27b-3p promotes apoptosis and suppress cancer cell viability.		[36]
Ovarian	shRNA downregulation of FZD7 results in epithelial-like phenotype and reduction of EMT associated gene expression.	FZD7 knockout in MA-148 cells was unable to prevent tumour formation in a subcutaneous xenograft model of OC. However direct comparisons of FZD7 proficient and deficient cells were not made.	[37-39]
Leukaemia	Changes in FZD7 expression in CML cells mirror those in BMSCs. Manipulation of FZD7 expression (knockdown or overexpression) in		[40]

	BMSCs influences FZD7 expression in surrounding CML cells. Direct knockdown of FZD7 in CML cells reduced the expression of Wnt target genes MDR1 and CD44, suggesting that FZD7 acts through the canonical Wnt/β-catenin pathway.	
Prostate	Overexpression of FZD7 led to increased invasion and proliferation in PCa cells, indicating FZD7 plays an oncogenic role in PCa.	[41]
Pancreatic	Knockdown of FZD7 resulted in significantly lower β-catenin levels and ABCG2 in pancreatic cell lines in the presence of Wnt5a, suggesting FZD7 as the bridge between Wnt5a and ABCG2. Additionally, knocking down FZD7 led to increased apoptosis of capan-2 cells when treated with gemcitabine, highlighting the role of FZD7 in gemcitabine resistance in pancreatic cancer.	[24,42]
Melanoma	FZD7 knockdown via miR-486-5p expression resulted in a reversible loss of invasion and proliferation capacity in melanoma cell line. Furthermore, FZD7 is required for melanoma melanosphere formation and invasion via the non-canonical Wnt signalling pathway.	[43,44]
Renal	Inducing overexpression of miR-613 (negative regulator of FZD7) significantly reduced proliferation and invasion of FZD7 dependant RCC cells	[22]

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Table 2. List of Approaches Targeting FZD7 since 2016 167

Compound Name	Mechanism of Action	Cancer Types	Reference
OMP18R5	Noncompetitive inhibitor of FZD1, 2, 5, 7 & 8.	Gastric	[17,45]
FZD7 specific Single-chain fragment variable antibodies	Binds and inhibits FZD7 mediated signalling. (binding region not mentioned)	CRC	[46]
Soluble recombinant FZD7 decoy receptor	Binds to ligands associated with FZD7, limiting the formation of the Wnt ligand/receptor complex.	Gastric & CRC	[47]
FZD7 targeting nanoparticles	Deliver doxorubicin or beta-catenin siRNA directly to FZD7 expressing cells.	Breast	[35,48]

SHH002-hu1	Humanised antibody which competitively inhibit FZD7 mediated signalling.	Lung	[49]
FZD7-ADC	Humanised antibody which noncompetitively binds to FZD7 and delivers a microtubule inhibitor.	Ovarian	[50]
Fz7-21	Small peptide inhibitor of FZD7 mediated signalling.	N/A	[51]

Table 3. List of miRNA interactions with FZD7

miRNA	Mechanism	Cancer Types	Reference
miR-27b-3p	Directly downregulate FZD7 expression in lung cancer.	Lung	[36]
miR-485-5p	Bind to the 3′-untranslated region of FZD7, increased miR-485-5p expression led to reduced FZD7 expression and when FZD7 was restored, the invasive and proliferative capacity of the melanoma cells was restored.	Melanoma	[43]
miR-504	binds directly to the 3′ untranslated region of FZD7 mRNA, reducing its expression. Downregulated in HCC.	HCC	[33]
miR-542-3p	downregulation in HCC is associated with an increase in clonogenicity and increased FZD7 expression.	HCC	[32]
miR-613	Bind within the 3'-UTR of FZD7, reduces FZD7 expression. Overexpression of miR-613 significantly reduced proliferation and invasion in ACHN and 786-O RCC cells, whereas artificially inducing miR-613 overexpression appears to counteract FZD7-dependant tumour cells.	Prostate & RCC	[22,41]
miR-944	FZD7 is upregulated via circCSPP1 mediated down regulation of miR-944 in doxorubicin resistant patient samples.	CRC	[28]

1.3. Wnt Signaling Dysregulation in Cancer

The Wnt signalling pathway is deregulated in multiple cancer types, due to mutations or deregulation of Wnt signalling components [2-4]. As previously mentioned, the Wnt signalling pathway plays a key role in adult tissue homeostasis through regulating cellular function including proliferation, migration and invasion [2-4]. Additionally, Wnt signalling is also involved in the regulation of stem cell compartments. Deregulation of both

cellular function and stem cell compartments can result in aggressive disease with poor prognosis [2-4].

2. Gastrointestinal Cancers

Gastrointestinal (GI) cancers are reported to attribute to approximately 26% of the global cancer burden and are responsible for up to 35% of all cancer-related mortalities worldwide [45,47,52].

Gastric tumours show an up regulation of FZD7 expression when compared to surrounding healthy tissue [45,47,53]. FZD7 is accumulated on the surface of cells containing mutations affecting genes involved in FZD receptor cell surface turnover such as RNF43 in ~54% of patients [17]. FZD7 expression has also been correlated with poor patient prognosis [52]. Genetic deletion of FZD7 *in vivo* inhibits tumour initiation and growth in Wnt driven (mutant *APC*) and cytokine driven (mutant *GP130*) mouse models of gastric cancer [17]. Similarly, over-expression of the transcription factor B-cell lymphoma 6 (BCL6) results in transcriptional repression of FZD7, and an associated decrease of proliferation, migration, and invasion in AGS and SGC-7901 gastric cancer cells [54].

Pharmacologically targeting FZD7 using OMP18R5 (Vantictumab), which binds FZD1/2/5/7/8, showed a reduction in the formation of both SGC-7901 and MKN-45 gastric cancer spheroids [45]. This was underpinned by a reduction in gastric cancer stemness markers CD44 and ALDH1, which re-sensitised SGC-7901 chemo-resistant cells to cisplatin [45]. Further *in vivo* studies have shown OMP18R5 drastically reduces weight and number of tumours in $gp130^{F/F}$ mice which develop spontaneous STAT3-dependant tumours [17]. Additionally, OMP18R5 reduced the expression of multiple Wnt target genes including Myc and CD44 as well as Fzd6 and Fzd7 [17]. An upregulation of Fzd2 has also been identified in gastric tumours upon OMP18R5 treatment, however Fzd2 elevated expression is unable to rescue Fzd7 loss [14,17]. This non-redundant role is consistent with Fzd7's role during intestinal regeneration [14] and mesoderm differentiation [55]. However, $Fzd7^{-/-}$ mice are viable whilst $Fzd7^{-/-}$; $Fzd2^{-/-}$ mice are embryonic lethal indicating redundancy during development [56].

Colorectal cancer (CRC) attributes to ~9.2% of cancer-related mortality in both sexes and 10.2% of total diagnosed cancer cases globally [57]. The Wnt signalling pathway in spontaneous CRC is often mutationally deregulated with ~60% of patients presenting with APC mutations [57-59]. Other Wnt regulators including RNF43, CTNNB1 and AXIN2 are also mutated however the frequency is reported to be much lower at ~5% [58]. FZD7 is upregulated in ~37% of CRC tumours and positively correlates with poor patient survival [19,57]. To supplement the literature, we analysed publicly available RNAseq data, which shows the correlation between FZD7 mRNA expression and CRC stage (Fig. 2). Notably, knockdown of FZD7 in CRC cells has recently been shown to reduce tumorigenic traits, including cell proliferation and migration [28]. Furthermore, FZD7 is upregulated via circCSPP1 mediated down regulation of miR-944 in doxorubicin resistant patient samples [28]. This implies FZD7 plays a role during chemotherapy resistance and presents an opportunity to re-sensitise tumours to chemotherapy agents by targeting FZD7 [28].

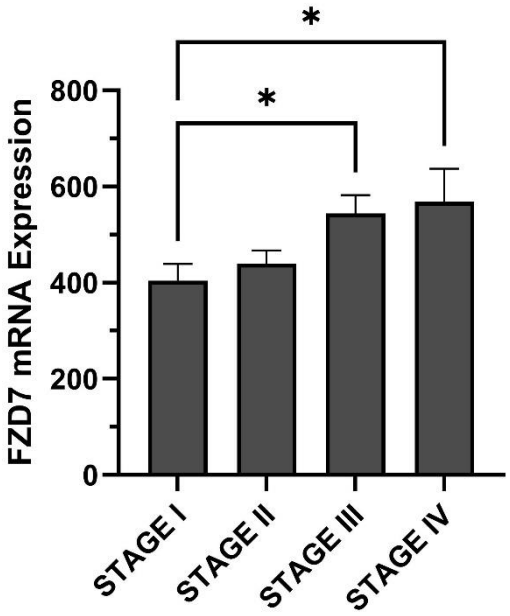

Figure 2: FZD7 mRNA expression is increased in late-stage colorectal adenocarcinoma. FZD7 mRNA expression levels (RSEM, batch normalized from Illumina HiSeq_RNASeqV2) across American Joint Committee on Cancer (AJCC) neoplasm disease stages (Stages I, II, III, and IV). Bars represent mean expression ± standard error of the mean (S.E.M). Statistically significant differences were observed between Stage I and Stages III and IV ($p < 0.05$, denoted by *). Statistical analysis was performed using one-way ANOVA. Data included: Stage 1 (n = 91), Stage 2 (n = 188), Stage 3 (n = 151) and Stage 4 (n = 68). Data on patient samples were obtained from the Colorectal Adenocarcinoma dataset (TCGA, PanCancer Atlas) and accessed Dec 2024 via https://www.cbioportal.org/.

Targeting FZD7 in CRC cells using human single-chain fragment variable antibodies (scFVs) showed an 83% inhibition of growth via an MTT assay in SW480 cells when compared to FZD7-negative SKBR-3 cells [46]. However, FACS analysis revealed ~8% non-specific binding to the FZD7-negative cells [46].

Alternatively, to targeting the FZD7 receptor directly, recombinant soluble FZD7 decoy receptors can be used to inhibit ligand receptor complex formation [47]. A soluble version of the Wnt ligand binding CRD domain of FZD7 is reported to significantly increase apoptosis and decrease expression of β-catenin and cyclin D1 in both AGS (gastric cancer) and SW480 (CRC) cells [47]. This approach leads to a reduction in the formation of the Wnt ligand/receptor complex and a subsequent reduction in canonical Wnt signalling downstream markers. However, this may prove a difficult therapeutic approach as previously *in vivo* studies have used FZD8 decoy receptors which were able to inhibit tumour growth, however this was reversable when treatment was discontinued [60].

3. Breast Cancer

Breast cancer (BCa) is the most commonly diagnosed cancer, and the main cause of cancer associated death in women with 2.3 million cases [61]. In 2020, there were 685,000 deaths associated with BCa worldwide [61]. In the UK, the incidence of BCa in 2020 was 53,889 and there were >11,800 BCa associated deaths per year [62]. The current treatments for BCa cancer include surgery, endocrine therapy, bone-modifying therapies, chemotherapy, and immunotherapy [63]. Unfortunately, these treatments have several adverse effects and have been shown to reduce in efficacy due to therapeutic resistance, thus novel therapeutic approaches to tackling BCa are required [63].

FZD7 mRNA expression is increased in human triple negative breast cancer (TNBC) when compared to non-TNBC [29,35,48], suggesting FZD7 is an attractive target for therapy [29,30]. Using publicly available datasets, we have presented FZD7 mRNA levels in a range of different subtypes of BCa, with highest expression observed in TNBC (Fig. 3). This presents a link between FZD7 and TNBC which is associated with poor prognosis, although FZD7 levels were not significantly different between different stages of disease progression in each subtype.

FZD7 knockdown via shRNA in MDA-MB-231 and BT-20 TNBC BCa cells resulted in reduced migration, invasion, proliferation, colony formation and stemness *in vitro*, and blocked the growth of TNBC xenografts [29]. Analysis of the FZD7 knockdown in TNBC xenografts identified that β-catenin dependent canonical Wnt signalling was reduced as several transcriptional Wnt target genes were decreased including AXIN2, CyclinD1 and MYC [29,30]. Interestingly, Yin et al., suggested that Wnt5a/5b (previously known to be associated with non-canonical Wnt signalling) facilitated BCa progression in a model of TNBC, confirmed by means of co-immunoprecipitation of Wnt5a/5b and FZD7 [31]. Knockdown of FZD7 reduced the expression of Wnt5a/b, which was rescued when FZD7 was upregulated. Further bioinformatic analysis associated Col6a1 with FZD7 and Wnt5b expression, and is considered to drive BCa stemness and metastasis [31]. The expression of Col6a1 was reduced in FZD7/Wnt5b knockdown tissues while Col6a1 inhibition reduced the expression of Wnt5a/b in BCa cells [31]. In addition, knockdown of Col6a1 impaired BCa migration, invasion and mammosphere formation, suggesting that Col6a1 mediates FZD7/Wnt5b to regulate BCa cancer stem cells and progression [31].

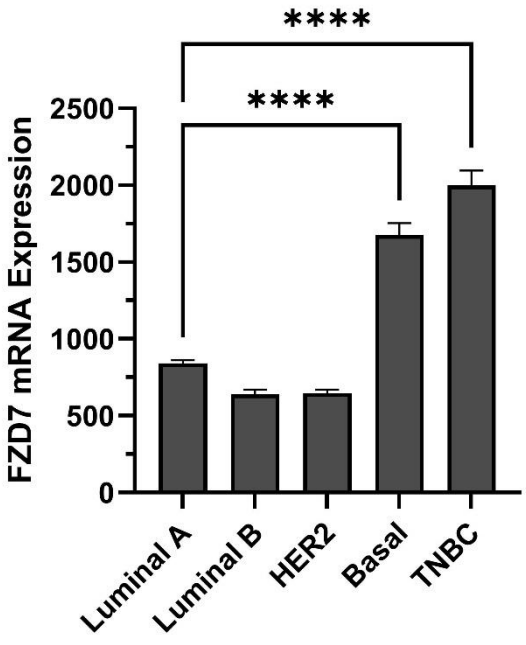

Figure 3: FZD7 mRNA expression across breast cancer subtypes. FZD7 mRNA expression levels (RSEM, batch normalized from Illumina HiSeq_RNASeqV2) in different breast cancer subtypes: Luminal A (n = 379), Luminal B (n = 244), HER2-enriched (HER2) (n = 230), Basal (n = 363) and triple-negative breast cancer (TNBC) (n = 251). Bars represent mean expression ± standard error of the mean (S.E.M). Statistically significant differences were observed between Luminal A and Basal or TNBC ($p < 0.0001$, denoted by ****) Statistical analysis was performed using one-way ANOVA. Non-specific luminal disease group removed. Data was obtained from the following GEO series (GSE), 12276, 12763, 12777, 13787, 16446, 17907, 20685, 20713, 21653, 31448, 45827, 48391, 65216, 76275.

In recent years, nanoparticles have been utilised to improve Fzd7-directed therapies in breast cancer [35]. The innovative technique of delivering doxorubicin (a chemotherapy agent used to treat BCa) in poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) covered with antibodies targeting FZD7, proved to be an efficient treatment [64]. When doxorubicin was delivered by anti-FZD7 nanoparticles in MDA-MB-231 cells, it was able to localise into the nucleus of FZD7-positive BCa cells, enhancing the treatment efficacy compared to freely delivered Doxorubicin [64]. Furthermore, an elegant technique combined anti-FZD7 antibody nanoparticles with β-catenin small interfering RNAs (siRNAs), which resulted in a significant decrease in proliferation, migration and spheroid formation of TNBC cells [35]. Tumour growth and metastasis were inhibited when both the FZD7 receptor and β-catenin were simultaneously targeted using nanoparticle technology *in vivo* [35].

4. Hepatocellular carcinoma

Hepatocellular Carcinoma (HCC) is the sixth and fourth highest cancer in both incidence and cancer-related mortality respectively [65]. FZD7 is upregulated in HCC when compared to healthy liver tissue suggesting it is a good target for therapy [23]. Indeed, shRNA knockdown of FZD7 in HCC cell lines results in NF-κB induced apoptosis through downregulation of BCL-2 and BCL-XL [23]. FZD7 expression in hepatitis B virus infection associated HCC is no different to expression in spontaneous HCC [34]. This data suggests that FZD7 is a suitable target for both hepatitis B virus infected and non-virus induced HCC [34]. RNAseq data from patient samples further supports a positive correlation between FZD7's mRNA expression and disease progression in HCC (Fig. 4).

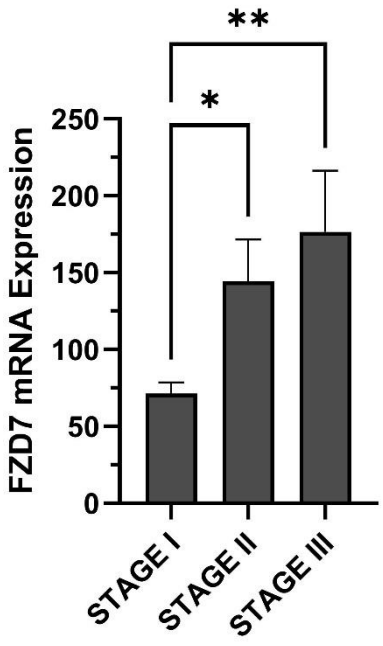

Figure 4: FZD7 mRNA expression status in liver hepatocellular carcinoma. FZD7 mRNA expression (RSEM, batch normalized from Illumina HiSeq_RNASeqV2) across neoplasm disease stages (Stages I, II, and III) based on the American Joint Committee on Cancer (AJCC) classification. Bars represent mean expression ± standard error of the mean (SEM). Significant differences were observed between stages ($p < 0.05$, denoted by *; $p < 0.01$, denoted by **). Statistical analysis was performed using one-way ANOVA. Data included: Stage I (n = 165), Stage II (n = 81) and Stage III (n = 78). Patient's data was obtained from the Liver Hepatocellular Carcinoma dataset (TCGA, PanCancer Atlas) accessed Dec 2024 via https://www.cbioportal.org/.

Since 2016, few advances have been made on specifically targeting FZD7 using small molecule agents in HCC. However, FZD7 has since been identified to play a role in multi-drug resistance (MDR) in HCC [66]. siRNA-mediated FZD7 knockdown was able to sensitise HCC cells to several chemotherapy agents (including 5-FU, MMC and ADR) significantly reducing their IC50 dosages [66], indicating that targeting FZD7 may sensitise HCC patients to chemotherapy.

Additionally, regulators of FZD7 gene transcription have been identified in HCC specifically miRNA-542-3p [32]. miR-542-3p downregulation in HCC is associated with an increase in clonogenicity and increased FZD7 expression, suggesting miR-543-3p may negatively regulate FZD7 expression via an antagonistic mechanism of action [32]. miR-504 is also downregulated in HCC and is able to bind directly to the 3′ untranslated region of FZD7 mRNA, reducing its expression [33]. Indeed, reduced miR-504 expression leads to increased FZD7 expression and promotes colony formation in HCC cells [33]. These transcriptional inhibitors of FZD7 could potentially be utilised therapeutically to reverse cellular changes induced through an overexpression of FZD7 in HCC.

5. Lung cancer

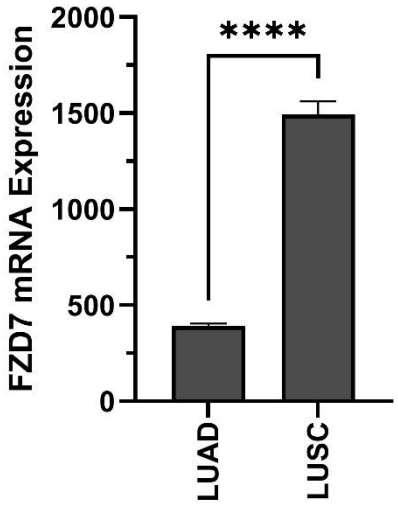

Figure 5: FZD7 mRNA expression in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). FZD7 mRNA expression levels (RSEM, batch normalized from Illumina HiSeq_RNASeqV2) in lung adenocarcinoma (LUAD) (n = 510) and lung squamous cell carcinoma (LUSC) (n = 484). Bars represent mean expression ± standard error of the mean (S.E.M). LUSC exhibited significantly higher FZD7 mRNA expression compared to LUAD ($p < 0.0001$, denoted by ****). Statistical analysis was performed using an unpaired t-test. Data were obtained from 994 patient samples in the Lung Adenocarcinoma (TCGA, PanCancer Atlas) and Lung Squamous Cell Carcinoma (TCGA, PanCancer Atlas) datasets and accessed Dec 2024 via https://www.cbioportal.org/.

Lung cancer is the leading cause of cancer-related mortality worldwide [49]. It has been previously shown that Wnt signalling, specifically FZD7, plays a major role in both lung tumorigenesis and tumour progression [49]. Using publicly available RNAseq datasets we have identified upregulation of FZD7 mRNA in Lung Squamous Cell carcinoma (LUSC) when compared to Lung Adenocarcinoma (LUAD) (Fig. 5), with no significant difference between NSCLC and SCLC observed. This data suggests a potential link between FZD7's mRNA expression and poor lung cancer prognosis.

The role of FZD7 within lung cancer has been extensively studied, miR-27b-3p has been identified as a potential tool to directly downregulate the expression of FZD7 mRNA to

promote apoptosis and suppress cancer cell viability [36]. Alternatively, a humanised FZD7 antibody, SHH002-hu1 has also been shown to be effective in both *in vitro* and *in vivo* studies [49]. SHH002-hu1 treatment can inhibit migration and invasion assays, in non-small cell lung cancer cell lines, to similar levels compared to FZD7 siRNA-mediated knockdown [49]. Additionally, SHH002-hu1 was more efficacious than XAV-939 (A Tankyrase inhibitor) in various lung cancer xenograft models [49]. This preclinical data suggests that targeting FZD7 with SHH002-hu1 could be a potent therapeutic approach for non-small cell lung cancer patients in the future. Although further investigation into FZD7 expression levels across subtypes of lung cancer would aid in the translation of this compound.

6. Ovarian Cancer

In 2020, ovarian cancer (OC) was the third most common cancer globally with an incidence of 313,959 cases annually and a mortality rate of 207,252 cases per year [67]. Due to its high prevalence and mortality rate, considerable research efforts have gone into finding novel therapeutic targets [67]. FZD7 has been shown to have a role in driving ovarian cancer progression. Indeed, a number of studies have found that FZD7 is upregulated in ovarian cancer and targeting this receptor results in reduced ovarian cancer progression, presenting FZD7 as an actionable target in ovarian cancer [39,68].

New findings suggest that FZD7 also plays a role in regulating the activity of ovarian cancer stem cells (OCSCs) and extracellular matrix (ECM) remodelling [37]. Tissue transglutaminase (TG2) is a protein that links epithelial cells into the ECM via fibronectins and integrins [38]. Recently, Condello et al., found an association between TG2, fibronectin and integrin β1 as ECM contributors of ovarian cancer stemness via FZD7 [37]. FZD7 can also act as the receptor for TG2 to activate ovarian cancer stem cells in OC cells [37]. Integrin-linked kinase (ILK) is another ECM molecule that is reported to interact with FZD7 to support OCSCs [69]. In a study carried out by Atwani et al, ILK was associated with lower survival rate in patients with OC and increased expression of ILK was also found in metastatic samples from patients, suggesting its progression in OC [69]. OCSCs spheroids treated with the ILK inhibitor compound 22 (cpd-22), showed decreased expression of FZD7 and Myc. The direct link between ILK and FZD7 was later confirmed by OC spheroids obtained from patients and treated with Wnt3a, showing increased ILK and FZD7 expression by IF staining, co-IP and a proximity ligation assay, which detects proteins localised within 40nm distance in tissues [69]. Moreover, when the ILK-FZD7 pathway was disrupted by cpd-22, it led to reduced OCSCs spheroid formation and lower intraperitoneal metastasis *in vivo*, especially when combined with carboplatin [69].

FZD7 has also been implicated as a regulator of OC cancer stem cell activity via epigenetic regulation of TWIST via H3K4me3 and H3K27ac at the TWIST1 proximal promoter. Similarly, FZD7 has been implicated in OC resistance to cell death via TWIST regulation of BCL2 [39]. This data strongly suggests that FZD7 promotes tumour initiation/growth/progression in OC, but there are no papers demonstrating that direct inhibition of FZD7 can inhibit OC disease progression. However, recently there has been a novel antibody drug conjugate (ADC) using a FZD7 specific antibody (septuximab vedotin) which has been shown to be able to specifically kill FZD7-positive OC cells in vitro, and dramatically regress OC xenograft tumours [50]. The use of a humanised mouse has also demonstrated that FZD7-ADC treatment did not cause any noticeable toxicity [50]. This is a substantial innovation in targeting FZD7 expressing cancer cells as its high specificity reduces toxicity concerns which were raised previously due to OMP18R5's translational issues [50,70].

7. Leukemia

Chronic myeloid leukaemia (CML) is a myeloproliferative cancer originated from hematopoietic stem cells (HSCs) characterised by a mutation in the BCR-ABL oncogene [71]. CML can be treated by tyrosine kinase inhibitors (TKIs) such as Imatinib Mesylate (IM), however, most patients eventually develop resistance and therefore new therapies are required [72].

FZD7 is upregulated in CML when in contact with bone mesenchymal stem cells (BMSCs) [40]. Co-culture studies showed that silencing FZD7 in BMSCs cells maintain a lower expression of FZD7 in CML cells, and conversely, increased FZD7 expression in CML cells when FZD7 was overexpressed in BMSCs [40]. Moreover, knockdown of FZD7 in CML cells reduced the expression of Wnt target genes *MDR1* and *CD44*, suggesting that FZD7 acted through the canonical Wnt/β-catenin pathway [40]. Silencing FZD7 in BMSCs sensitised co-cultured CML cells to IM treatment, opening a possible combination of IM and FZD7 inhibition for CML treatment [40]. This is one of the first papers to describe how FZD7 in a non-cancer cell can regulate the function of neighbouring cancer cells, suggesting FZD7 is an attractive target for this type of pro-tumour mechanism in CML and potentially other cancers.

The upregulation of FZD7 in human HSCs during the chronic phase of CML has also been detected by microarray, identifying FZD7 as a possible regulator of CML at different stages [73]. In acute lymphoblastic leukaemia (ALL), the role of long noncoding RNA (lncRNA) and FZD7 has been investigated by Wang et al, revealing a competing endogenous RNA (ceRNA) network that included differentially expressed mRNAs from the bone marrow of patients with ALL, and identified FZD7 as one of the main hub genes [74]. Additionally, FZD7 mRNA had a high correlation with the LncRNA Wilms tumour 1 homolog antisense RNA (WT1-AS). It has been suggested to act as tumour suppressor in cervical cancer, gastric cancer, papillary thyroid carcinoma, non-small cell lung cancer and hepatocellular carcinoma [75-79]. However, in patients with breast cancer and colorectal cancer, upregulation of WT1-AS was associated with poor prognosis, suggesting a dichotomic role for WT1-AS depending on its expression status in individual cancer types [80,81]. Based on this evidence, further studies are needed to clarify the role of lncRNA WT1-AS and its association with FZD7 in ALL [74].

8. Prostate Cancer

Recently, a number of studies have highlighted the importance of FZD7 in prostate cancer (PCa) [41,82]. Ren et al., established that miR-613 has a binding site within the 3'-UTR of FZD7 [41]. Overexpression of miR-613 in PCa cells resulted in a significant decrease in FZD7 mRNA and protein levels. Conversely, inhibition of miR-613 increased FZD7 expression, further validating FZD7 as a target for miR-613 [41]. Overexpression of FZD7 also led to increased invasion and proliferation in PCa cells, indicating FZD7 plays an oncogenic role during PCa [41].

Recent work has also identified a novel link between FZD7 and GIPC2 during PCa metastasis [82]. GIPC2 is part of the GAIP-interacting protein family containing a C-terminus domain (GIPC). GIPC2 is comprised of GIPC homology 1 (GH1), PDZ, and GH2 domains and is associated with familial hearing loss and cancer [82]. GIPC2 levels are increased in metastatic PCa clinical samples compared to primary PCa [82]. Overexpression of GIPC2 increased the migration, invasion and cell adhesion hallmarks in metastatic PCa cells, while the metastatic potential of PCa cells *in vivo* was reduced when GIPC2 was inhibited [82]. GIPC2 overexpression reduced GSK3β levels in PCa cells and activated β-catenin signalling, and conversely inhibition of GIPC2 reduced β-catenin

signalling [82]. Co-immunoprecipitation assays revealed that GIPC2 directly interacts with FZD7, specifically through the PDZ domain in GIPC2 [82]. Overexpression of GIPC2 resulted in increased PCa metastasis which was reduced when FZD7 was knockdown in PCa cells [82]. These studies identify and validate FZD7 as an attractive target for PCa.

8. Pancreatic Cancer

Pancreatic cancer is recognised as one of the most aggressive cancers with a 5-year survival rate which is lower than 10% [83]. In 2020, pancreatic cancer accounted for an incidence of 49,5773 new cases and 46,6003 deaths worldwide [83]. The low survival rate of patients with pancreatic cancer is reflective of late diagnosis and ineffective therapeutic approaches. Analysis of publicly available RNAseq data suggests FZD7's expression levels are associated with increased histological grade which in tone is correlated with poor disease prognosis (Fig. 6).

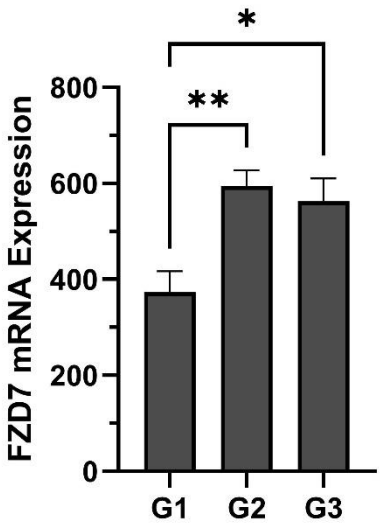

Figure 6: FZD7 mRNA expression status in pancreatic adenocarcinoma. Bar graph showing FZD7 mRNA expression (RSEM, batch normalized from Illumina HiSeq_RNASeqV2) across neoplasm histologic grades (G1 (n = 31), G2 (n = 94), and G3 (n = 48)). Bars represent mean expression ± standard error of the mean (SEM). Statistically significant differences were observed between grades ($p < 0.05$, denoted by *; $p < 0.01$, denoted by **). Statistical analysis was performed using one-way ANOVA. Data include 173 patient samples and were obtained from the Pancreatic Adenocarcinoma dataset (TCGA, PanCancer Atlas) accessed Dec 2024 via https://www.cbioportal.org/.

Recently, FZD7 has been implicated in chemoresistance in pancreatic cancer [42]. ATP binding cassette subfamily G member 2 (ABCG2) is one of the ABC transporter superfamily related to multidrug resistance in pancreatic cancer by transporting anti-cancer drugs outside the cancer cells [42]. Tissue samples from pancreatic cancer patients showed increased expression for ABCG2 and Wnt5a compared to adjacent healthy tissue via immunohistochemistry (IHC) [42]. Survival rate was shown to be significantly lower in patients with high ABCG2 expression. Wnt5a is reported to regulate ABCG2 in capan-2 pancreatic cells in a dose-dependent manner [42]. Knockdown of FZD7 resulted in significantly lower β-catenin levels and ABCG2 in pancreatic cell lines in the presence of Wnt5a, suggesting FZD7 as the bridge between Wnt5a and ABCG2. Additionally, knocking down FZD7 led to increased apoptosis of capan-2 cells when treated with gemcitabine, highlighting the role of FZD7 in gemcitabine resistance in pancreatic cancer [42].

Analysis of publicly available databases as well as tissue from patients with pancreatic cancer, revealed FZD7 and WNT7b levels are increased in pancreatic tumours and positively correlate with poor clinical outcome [24]. Knockdown of FZD7 and WNT7b inhibited the proliferation of pancreatic cancer stem cells, reduced the levels of ABCG2 and made the cells more susceptible to gemcitabine treatment [24]. Conversely, when FZD7 and WNT7b were overexpressed, the opposite effects were observed [24]. The studies of Zhang et al., highlight how different Wnt ligands act through FZD7 to induce pancreatic cancer progression and chemoresistance [24]. Moreover, the high expression of FZD7 in pancreatic cancer has been associated with hepatic metastases in patients, and in vitro assays suggest that FZD7 promotes EMT as FZD7 silencing reduced invasion and migration linked to reduced Vimentin, Zeb1 and Slug [84]. Most recently, bioinformatics analysis from diverse databases suggest that FZD7 is upregulated in late stages of pancreatic cancer, a characteristic that could be associated with hepatic metastasis, suggesting FZD7 as a potential therapeutic target for pancreatic cancer and hepatic metastasis [85,86].

9. Melanoma

FZD7 is highly expressed in melanoma tissue when compared to healthy tissue [43]. Consistent with other cancer types, FZD7 has recently been shown to be regulated by miRNAs. miR-485-5p expression is decrease in human melanoma tissue compared to normal tissue [43]. Enforced miR-485-5p expression decreased melanoma cell proliferation and invasion, key features of cancer growth and progression that were restored when miR-485-5p was inhibited [43]. Bioinformatic analysis revealed that miR-485-5p can bind to the 3′-UTR region of FZD7, which was confirmed by a dual-luciferase assay [43]. Further studies confirmed that increased miR-485-5p expression led to reduced FZD7 expression and when FZD7 was restored, the invasive and proliferative capacity of the melanoma cells also reverted [43]. This data identifies FZD7 as a regulator of melanoma growth and progression, indicating that FZD7 presents a novel potential target for therapy against melanoma.

Most recently, it has been suggested that FZD7 supports melanoma melanosphere formation and amoeboid invasion via non-canonical Wnt/β-catenin pathway [44]. The Wnt11 ligand binds to FZD7 and its downstream effector DAAM1 to activate the non-canonical Rho-ROCK1/2–Myosin II signalling pathway in melanoma melanosphere [44]. When FZD7 was inhibited, there was a significant reduction in melanosphere formation, loss of cell rounding, lower Myosin II activity and inhibited invasion capability [44], highlighting an important role for FZD7 in melanoma, and warranting further research.

10. Cholangiocarcinoma

Cholangiocarcinoma (cancer of the bile ducts) (CCa) is an extremely aggressive disease with poor prognosis and currently radical surgical resection remains the only curative treatment option [87]. The expression of FZD7 has been shown to be upregulated when comparing tumours to patient matched healthy liver tissue [18]. Transcriptional upregulation of FZD7 within intrahepatic CCa has been associated with an upregulation of circACTN4 which co-localises with YBX1 to enhance FZD7 transcriptional expression [88]. Further in silico analysis has identified Receptor-Like Tyrosine Kinase (RYK) to be overexpressed in stage 4 CCa, significantly correlates with FZD7 expression and poor prognosis [89]. FZD7 has not been targeted as a therapeutic intervention for CCa in preclinical studies. However, the combination of these findings and the low rate of cytoplasmic Wnt component mutations observed in CCa [90], indicate FZD7 as an attractive therapeutic target for CCa in the near future.

11. Renal Cancer

Analysis of 53 human renal tumours revealed that FZD7 expression is upregulated when compared to healthy renal tissue [91]. Furthermore, manipulation of FZD7 expression presented a positive correlation between FZD7 expression and proliferation in renal cell carcinoma (RCC) cell lines [91]. Additionally, Wnt3a supplemented media was unable to rescue proliferation in FZD7 knockdown RCC cell lines, implicating FZD7 as a non-redundant transmitter of oncogenic Wnt signalling in RCC [91].

In RCC, miR-613 is downregulated, and has been reported to directly interact with FZD7, binding to the 3′ UTR region, which is associated with decreased FZD7 expression [22]. Overexpression of miR-613 significantly reduced proliferation and invasion in ACHN and 786-O RCC cells, whereas artificially inducing miR-613 overexpression appears to counteract FZD7-dependant RCC cells [22]. Although FZD7 is yet to be targeted pharmacologically in renal cancer, current research evidence suggests FZD7 may prove to be an attractive therapeutic target. However, additional work is needed to establish the true status and predictive value of FZD7 in renal cancer.

12. Discussion

FZD7 is highly expressed in several cancer types with current data suggesting it is an attractive target for therapy [24,39,40,42,44,68,82,84]. Moreover, specific targeting of FZD7 can inhibit many features of cancer from initiation to growth and metastasis [22,27,45-47,49,50,64,84].

FZD7's expression and role in the gastrointestinal tract has been extensively studied in both healthy tissue and cancer [14-17]. Although deletion of FZD7 in GI organoids results in organoid death, deletion of FZD7 *in vivo* is well tolerated as the tissue has evolved a mechanism to repopulate the epithelium following acute loss of critical genes, including FZD7 [15,16] or MYC [92]. This is supported by a recent systemic knockout of FZD7, which again demonstrates FZD7 loss is well tolerated in the GI epithelium with no adverse health effects to the mice [93]. Importantly, deletion of FZD7 in gastric tumours did not trigger repopulation (since these highly regulated evolutionary mechanisms are lost in the tumour), but rather the FZD7 deleted cells failed to proliferate, probably due to their lack of FZD7 that renders them unable to respond to the oncogenic Wnt signalling derived from the tumour microenvironment and niche [14-17]. FZD5 is also expressed in the intestinal epithelium, however genetic deletion results in widespread crypt atrophy in the small intestine and colon, associated with severe weight loss of the mice [13]. This data further suggests FZD7 is an attractive target for therapy, supported by recent work revealing treatment of humanised-FZD7 mice that are able to respond to the new FZD7-ADC show no signs of ill health or intestinal pathology [50].

Deletion of FZD7 in normal, non-tumour gastric epithelium resulted in perturbed differentiation and mis-localisation of differentiated cells, before repopulation was triggered in which homeostasis was restored within two weeks [15]. Together, these results support an evolutionarily conserved mechanism in the GI tract to tolerate and respond to deleterious genetic events such as loss of FZD7 and indicate FZD7 as an attractive target for therapy.

In the last eight years, research into targeting FZD7 has continued to be a promising approach to target Wnt-driven cancers. This is due to its differential and increased expression across many cancers compared to healthy tissues. Due to the superior specificity of novel humanised FZD7 antibodies currently being investigated (e.g., FZD7-ADC and SHH002-hu1), the on-target toxicity linked to the pan-FZD receptor inhibitor OMP-18R5

has potential to be mitigated if/when these compounds progress to clinical trials [49,50,70]. It will be important to further understand the molecular/cellular mechanism of how toxicity is avoided by these FZD7-directed therapies to help progress them towards clinical trials.

To advance the field of FZD7-targeted cancer therapies, a key next step is to investigate FZD7 protein expression in patient tissue samples using tissue microarray (TMA) studies. Many current investigations focus predominantly on mRNA expression, potentially overlooking important post-translational regulation by components such as RNF43 which is deregulated in several cancers resulting in increased FZD proteins on the cell surface [94]. Protein studies via TMA can offer more direct insights into FZD7's role in cancer progression and metastasis and potentially help identify which patients may benefit from FZD7-directed therapies. Moreover, the validation of a universally accepted, commercially available, FZD7-specific antibody would significantly enhance our ability to study FZD7's functionality, not only within cancer cells but also within the metastatic niche, the extracellular matrix (ECM) and stromal cells, all critical in tumorigenesis and metastasis [95].

Antibody-drug conjugates are a novel cancer therapy comprised of a monoclonal antibody aimed to target cancer cells linked to a cytotoxic payload by a chemical linker [96-98]. Such antibody-conjugated designs result in specific targeting of cancer cells, limiting the cytotoxicity to normal cells that have lower expression of the antibody of interest [98]. FZD7 is an ideal candidate for an ADC target as it has several attractive features [96].

Firstly, FZD7 is highly expressed throughout embryonic development and then its expression is reduced in normal tissues [96]. In contrast, FZD7 expression is highly upregulated in a diverse range of cancer types, making it a good candidate to target cancer cells [96]. Secondly, FZD7 is a surface receptor, and not a secreted protein from the cancer cells, thus providing specific cancer cell targeting [96]. Finally, when the ADC compound binds FZD7 in the cancer cells, it is internalized in lysosomes of FZD7-expressing cells, resulting in apoptosis of FZD7 high cancer cells [50]. Indeed, our recently published FZD7-ADC compound shows promise to be an effective treatment for FZD7 expressing cancer types, opening a new window for cancer therapy [50].

Monoclonal antibody based therapeutic approaches currently appear to be the most attractive method of targeting the differential expression of FZD7 in multiple cancer types [49,50]. However, the cost to produce these compounds could continue to limit the research field [99]. A possible solution to this approach would be adopting scFVs antibodies, which are significantly cheaper and easier to produce [100]. Research on the efficacy of scFVs ability to inhibit FZD7 shows promising results however requires further investigation [46]. A potential avenue for harnessing scFVs' could be using them to deliver cytotoxic agents such as MMAE or MMAF as this has been shown to be efficacious using monoclonal antibodies [49,50,99,100]. This approach could reduce the cost of compound production and help progress on this approach to target FZD7 expressing cancer cells.

Nanoparticles also offer significant value in cancer therapy due to their ability to enhance drug delivery precision and minimize off-target effects. In the context of targeting FZD7, nanoparticles have been effectively engineered to deliver chemotherapeutic agents like doxorubicin directly to tumour cells [64]. By conjugating FZD7-specific antibodies to nanoparticles, researchers have achieved targeted drug delivery, reducing systemic toxicity. Furthermore, combining nanoparticles with siRNA targeting β-catenin has demonstrated improved inhibition of tumour growth and metastasis [35]. These approaches underline nanoparticles' potential to revolutionize cancer treatment by enhancing therapeutic efficacy while minimizing adverse effects [35,64].

Targeting FZD7 specifically has the potential to be less toxic than pan-Wnt/FZD inhibitors. Indeed, our promising new FZD7-ADC displayed no toxicity in humanised-FZD7-mice [50], although it will be important to understand how toxicity is avoided to progress this new therapy along the translational pipeline. The FZD7 blocking antibody, SHH002-hu1, shows good anti-tumour effects in NSCLC xenografts, and no cytotoxicity of bronchial epithelial cells *in vitro,* however it is not clear if there was any toxicity *in vivo* [49], which will be important to identify to inform clinical trials. Similarly, the promising Fz7-21, a Fzd7 binding peptide, will need to be investigated in preclinical *in vivo* models of cancer to establish efficacy and toxicity for them to progress to clinic [51]. As FZD7 promotes cancer stem cell activity [17,31], future research should also focus on co-treatments with chemotherapy to target chemo-resistant cancer stem cell populations. Similarly, checkpoint inhibitor response can be improved with co-treatment with OMP-18R5 [101], suggesting FZD7 specific inhibitors may also be attractive to combine with modifiers of the tumour immune response for future research.

13. Conclusion

This review highlights the increasing body of work that strongly suggests that FZD7 is an attractive target for therapeutic intervention, due to its high expression in several cancers, its role in promoting tumour initiation, growth and progression, and also the recent evidence that targeting FZD7 itself, or FZD7 expressing cells, is well tolerated, with no adverse health signs. Together this information should encourage researchers and industrial partners to continue to develop new FZD7-directed therapies and propel current FZD7 targeting agents along the translational pipeline, to help improve cancer patient health whilst avoiding toxicity [50].

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