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FRIZZLED/WNT SIGNALLING: THE INSIDIOUS PROMOTER OF TUMOUR GROWTH AND PROGRESSION

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1. ABSTRACT

The recognition that the processes involved in tumour formation are strikingly similar to developmental morphogenetic processes, such as gastrulation, has refashioned our approach to cancer research. Wnt and its receptor Frizzled govern the morphogenetic processes of gastrulation. Directed cell movements during gastrulation require the cells to undergo transient epithelial to mesenchymal transitions, enabling the cells to dissociate and migrate. To do this, Frizzleds activate different intracellular signalling cascades that affect cellular processes such as differentiation, proliferation, cell motility and cell polarity. Cell dissociation and migration are also essential for tumour cell invasion and metastases and the frequent deregulation of Wnt and Frizzled in human cancers implicates them in this process. Indeed recent evidence links both canonical (Wnt/beta-catenin) and non-canonical (Wnt/Ca^{2+}) pathways to tumour invasion and metastases, emphasizing the importance of Frizzled in tumour growth and progression.

2. FRIZZLED/WNT SIGNALLING: THE INSIDIOUS PROMOTER OF TUMOUR GROWTH AND PROGRESSION

The hallmarks of malignant transformation are the capacity to invade and metastasise (1, 2). In order for these processes to occur, tumour cells must be able to dissociate from the primary tumour, migrate away and gain access to blood or lymphatic vessels to disseminate to distant sites in the body. It is assumed that a de-differentiation of the tumour cells in the invasive area is required for this process. Epithelial cells exhibit apical-basal polarity and close linkage to adjacent cells by cell adhesion molecules and junctions. In contrast, mesenchymal cells have a front end-back end polarity and have the ability to migrate through extracellular matrix. De-differentiation of tumour cells is characterised by a change from an epithelial to mesenchymal phenotype (3, 4), and is referred to as the cells undergoing epithelio-mesenchymal transition (EMT). EMT occurs physiologically during normal developmental morphogenetic processes that require cell migration and extracellular matrix invasion.

It is now becoming clear that tumour growth is also a morphogenetic process that is characterized by dynamic changes in structure and differentiation, which in many ways emulate morphogenetic processes in embryonic development (2, 5). Analysis of tumours *in situ* by immunohistochemistry indicates that EMT is associated with a decrease in cellular proliferative activity and an increase in the expression of mesenchymal genes that govern cell motility and invasion (5, 6). Interestingly, while tumour cells undergo an EMT allowing them to migrate away from the primary tumour, adenocarcinomas formed at the site of metastases recapitulate the differentiation status of the primary tumour (6). Thus, the disseminated mesenchymal tumour cells are thought to undergo a re-differentiation toward the primary tumour phenotype in order to proliferate and build up the metastases [reviewed in (2)]. Both cellular context and extracellular environmental cues are thought to regulate the interpretation and ultimate outcome of signals that are initiated by the interaction between receptors and ligands during morphogenesis. Similarly, cellular context and environmental cues are important regulators of tumour growth (7, 8). The analogies between developmental and neoplastic morphogenetic processes are further supported by the fact that the molecular pathways that govern early embryogenesis and organogenesis are directly or indirectly altered in most neoplasms (2, 9-11).

One such developmental molecular pathway is initiated by the Frizzled (FZD) receptors. FZD, as a family of receptors, are particularly amenable to modulation by environmental factors and the cellular context. The receptors can activate several signalling pathways that overlap, not only by shared components but also by cross-regulation. Transduction of signals down any of the signalling pathways is modulated by an ever-increasing number of both positive and negative regulators, including receptor co-factors and co-receptors (12). During development the ultimate outcome of FZD signalling affects cellular processes involved in cell differentiation, adhesion and motility (9): processes that are pivotal to tumour growth and invasion (2). The frequent deregulation of FZD receptors and their Wnt ligands in a rapidly increasing list of diverse human cancers and pathologies makes them compelling candidates to drive oncogenesis. The potential of FZD receptors as promoters of tumour growth and progression is the subject of this review.

3. THE FRIZZLED FAMILY OF RECEPTORS

FZD was first identified for its role in regulating planar cell polarity (front end-back end polarity) through genetic screens in *Drosophila* (13, 14) and was subsequently shown to serve as a receptor for Wnt ligands (15-17). A large number of FZD homologs have since been cloned from diverse organisms spanning the evolutionary tree from lower metazoans (18) to man (19). At present there are at least nineteen human Wnt ligands and ten FZDs that serve as their receptors (http://www.stanford.edu/%7Ernusse/wntwindow.html).
All FZD receptors have an N-terminal signal sequence and a highly conserved cysteine-rich extracellular domain. The cysteine-rich extracellular domain or CRD, now usually referred to as the Frizzled or Fz domain, includes ten conserved cysteine residues. This is followed by seven transmembrane helices separated by three cytoplasmic and three exofacial loops. The seventh transmembrane helix is followed by a C-terminal cytoplasmic tail that is essential for at least some aspects of receptor signalling (16, 20).

FZD receptors bind Wnt signalling glycoproteins via the Fz domain (21, 22). This has been demonstrated by direct binding studies (23) and indirectly, since co-expression of the amino-terminal ectodomain of FZD receptors generally antagonizes Wnt-mediated FZD signalling (17, 24). Naturally occurring secreted frizzled-related proteins (sFRP), that contain the Fz domain but lack the remaining domains of FZD, have also been identified and shown to bind Wnt antagonising Wnt-mediated FZD signalling (25). The sFRPs are just one family of proteins that regulate the interaction between Wnt and FZD. Both positive and negative modulators (eg. Daam1, DKK, WIF) and co-receptors (LRP5/6) have been shown to regulate the coupling of Wnt to FZD (see http://www.stanford.edu/%7Ernusse/wntwindow.html and http://stke.sciencemag.org/cm/) and the frequent deregulation of these signal modulators in human cancers indicates strong selection for deregulated FZD signalling in oncogenesis.

4. FRIZZLED SIGNALLING PATHWAYS

4.1. Canonical Wnt/Frizzled Signalling

The FZD-dependent signalling cascade comprises several branches (Figure 1) whose differential activation depends on specific Wnt ligands, FZD isoforms and the cellular context (26-28). The best-known Wnt pathway is the Wnt/beta-catenin pathway (Figure 1a), also referred to as the canonical Wnt pathway (28). This pathway has been extensively studied in diverse vertebrate and invertebrate model systems. beta-Catenin is a central molecule in the canonical Wnt/FZD pathway. In the absence of a Wnt/FZD signal, beta-catenin is ubiquinated and rapidly degraded by the proteosome. Activation of the canonical pathway by FZD receptors leads to the phosphorylation of dishevelled (Dsh/Dvl) which, through its association with axin, prevents glycogen synthase kinase-3beta (GSK-3beta) and casein kinase 1alpha (CK1alpha) from phosphorylating critical substrates, including beta-catenin (29). Phosphorylation of beta-catenin at the N-terminus by CK1alpha and GSK-3beta is essential for its targeted degradation (30). Non-phosphorylated beta-catenin escapes recognition by beta-TRCP, a component of an E3 ubiquitin ligase complex, thereby avoiding degradation. This non-phosphorylated form of beta-catenin is then able to localise to the nucleus (31), form a complex with TCF/LEF transcription factors (32, 33) and induce the expression of downstream target genes (see web sites above). The core components of the canonical Wnt pathway are strongly conserved through evolution (28).

4.2. Planar Cell Polarity

In addition to the canonical pathway, recent developmental studies have identified alternative, beta-catenin-independent, Wnt/FZD signalling cascade branches. In the cell polarity pathway, referred to as the Planar Cell Polarity (PCP) pathway in *Drosophila* (Figure 1b), FZD functions to establish asymmetric cell polarities and coordinate cell shape changes and cellular movement. In this pathway FZD regulates the activity of the small GTPases Rho and Rac through different domains of Dsh. Rho and Rac in turn regulate the activity of Rock and Jun N-terminal kinase (JNK), respectively (34-36). Much of our current understanding of the molecules governing this pathway comes from studies in *Drosophila* as several clear effects of the PCP pathway, such as hair follicle and bristle orientation, can be readily assessed in this organism. In addition, the existence of mutant genes that affect planar polarized structures in *Drosophila* have been exploited to study the processes governing PCP. Planar cell polarity was recently demonstrated in a mammalian system. It was shown that in the mammalian cochlea, the PCP pathway initiated by Wnt-7a, governs the unidirectional orientation of sensory hair cells necessary for unimpaired hearing (37). The PCP pathway is also involved in regulating cell polarization during vertebrate gastrulation movements (24, 36, 38, 39) where activation of both Rho and Rac are required for convergent extension (36). Thus, although differences exist between vertebrate and invertebrate pathways, the core components of the pathway are conserved through evolution (34, 37).

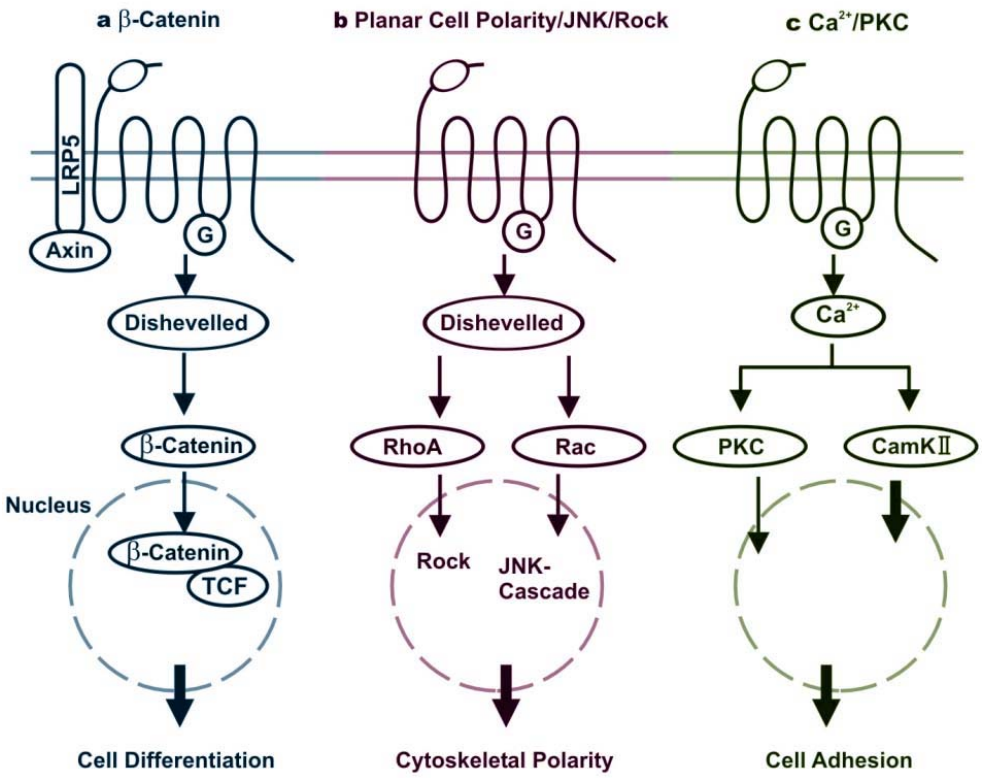

Figure 1. Signal transduction pathways activated by Frizzled receptors.

4.3. Wnt/Ca^{++} Signalling

In addition to the polarity pathway, Winklbauer *et al*. (40), provide compelling evidence that another branch of non-canonical Wnt/FZD signalling (the Wnt/Ca^{2+} pathway, Figure 1c), activated by the *Xenopus* homolog of FZD7 (Xfz7), is essential for tissue separation during vertebrate gastrulation. The Wnt/Ca^{2+} pathway involves signalling through the known Ca^{2+}-sensitive kinases PKC and CamKII (26, 27). Wnt-5a was the first Wnt ligand identified to signal down this pathway and, in a series of elegant studies, was shown to require coupling to G-proteins (41, 42). Interestingly, the Wnt/Ca^{2+} pathway activated by Wnt-5a, antagonizes the Wnt/beta-catenin pathway (38, 43-45). One mechanism of inhibition is via CamKII, where CamKII activates the MAP kinase-related Nemo-like kinase (NLK) to phosphorylate TCF/LEF and prevent beta-catenin/TCF/LEF-mediated transcription (43, 46, 47). In addition, PKC, activated by the Wnt/Ca^{2+} pathway, blocks the Wnt/beta-catenin pathway by phosphorylating Dsh (48).

5. WNT/FRIZZLED FUNCTION

5.1 Functional Classification of Wnt/Frizzled.

Since their discovery Wnt ligands have been classified into two functional groups. Ectopic expression of one group, referred to as the proto-oncogenic or transforming group (e.g. Wnt-1, -3A, -8 and –8B), induces a secondary axis in early *Xenopus* embryos (44) and transforms C57mg mammary epithelial cells (28, 49). Transforming Wnts also promote the stabilization of beta-catenin in some cultured mammalian cells (50) and thus, in general, can activate the Wnt/beta-catenin pathway. This initial link between beta-catenin stabilization and cellular transformation, and the association of the tumour suppressor gene, *APC* [*adenomatous polyposis coli* (51)], with regulation of beta-catenin, cemented interest in the oncogenic potential of the canonical or Wnt/beta-catenin pathway (52). Other Wnts (e.g. Wnt-4, -5a and –11) do not elicit axis duplication in *Xenopus* embryos (44) and cannot transform C57mg cells (49). However, these Wnts alter cell movements and reduce cell adhesion when over-expressed in *Xenopus* embryos (44, 53) and are referred to as morphogenetic Wnts. These Wnts can activate non-canonical Wnt pathways, including the Wnt/Ca^{2+} pathway and the Wnt/JNK/Rock pathway, and can antagonise the activity of the transforming Wnts (38, 43-48). Similarly, the FZD receptors can be classified into groups based on their basal signalling activity when ectopically expressed in *Xenopus*. In this context, in the absence of ectopic ligand, members of the FZD family preferentially activate either the beta-catenin pathway (induce the expression of beta-catenin target genes, *Xnr-3* and *siamois*) or the Ca^{2+} pathway (activate CamKII and PKC). R(Rat)fz-1, M(Mouse)fz-7 and Mfz-8 strongly activate expression of Xnr-3 and siamois, but do not activate either CamKII or PKC. Conversely, Rfz-2, Mfz-3, Mfz-4 and Mfz-6 activate both CamKII and PKC but do not appreciably induce expression of Xnr-3 and siamois (41).

These classifications are based on specific functional assays however; these classifications are not exclusive even within these functional assays. Although

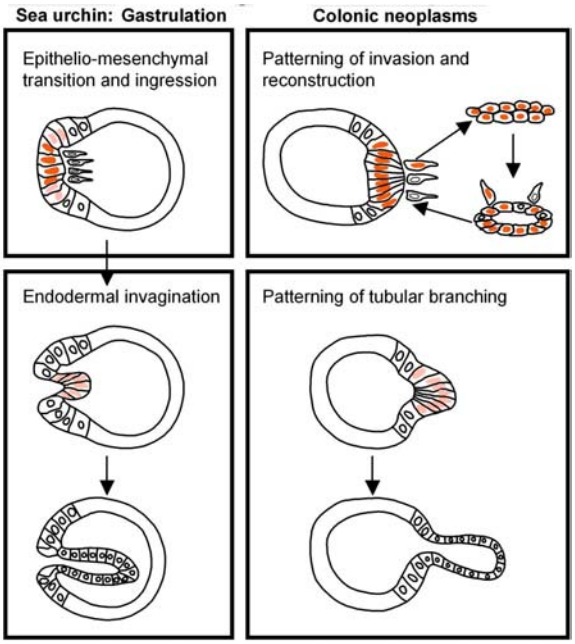

Figure 2. Schematic demonstration of the analogies of patterning and nuclear beta-catenin expression in sea urchin gastrulation and colonic neoplasms. The first phase of gastrulation with epithelio-mesenchymal transition corresponds with the patterning of invasion with tubular reconstruction in colonic adenocarcinomas. Strong nuclear beta-catenin expression (dark red) is found. The second phase of gastrulation is similar to the patterning of tubular branching. Here weaker nuclear beta-catenin expression (light red) is seen. Adapted from Kirchner and Brabletz (111).

Wnt-5a is classified as a morphogenetic/non-transforming Wnt, when co-expressed with a sub-group of FZDs [namely, h(human)fz-5, Mfz-8 and Xfz-8] it can induce axis duplication (17, 27). This is supported by the demonstration that Wnt-5a can interact with Xfz7 to induce the expression of beta-catenin target genes (20). Like Wnt-5a, Xfz7 has also been extensively studied and shown to activate multiple Wnt/FZD signalling pathways. It activates the Wnt/beta-catenin pathway (20, 54-57), and the non-canonical Wnt/Ca^{2+} (40, 41, 56, 58) and Wnt/JNK pathways (24, 39). Thus a given FZD or Wnt does not always activate one particular pathway. Instead, the pathway activated by a Wnt/FZD is context-defined and is likely to be influenced by co-expressed genes, for example cofactors (59), and is thus context-dependent.

Developmental studies in *Xenopus* and zebrafish suggest that efficient diverse signalling may underlie the essential role that FZD7 pays during gastrulation (39). Wnt-11 is also intimately involved in the regulation of gastrulation. Importantly, Wnt-5a can mimic the function of Wnt-11 in this process, thus both FZD7 and Wnt-5a have the functional capacity to direct morphogenetic processes during development. Another developmentally important morphogenetic process that is dependent on EMT is the formation of neural crest cells, an essential prerequisite for the formation of the peripheral nervous system (60). Wnt signalling initiated by Wnt-6 and FZD7 is necessary and sufficient to induce the mesenchymal neural crest cells in avian embryos. In this system, ectopic expression of FZD7 ectodomain inhibits neural crest cell induction (61). Thus, based on their role in embryonic morphogenesis, mechanistically Wnt and FZD, particularly Wnt-5a and FZD7, have the potential to direct morphogenetic processes during tumour growth and progression.

5.2 Developmental morphogenesis and the neo-morphogenesis of tumours.

There is little evidence to support either loss of function mutations or gain of function mutations of Wnt and FZD genes, suggesting that genetic mutation is an unlikely causative mechanism for Wnt/FZD-mediated tumourigenesis (11). Nonetheless, Wnt and FZD are over-expressed in diverse cancers (Table 1) and given that Wnt and FZD govern morphogenetic developmetal processes, such as gastrulation and neural crest cell induction (2), it is highly likely that they have a similar tissue-remodelling function in cancer.

Each branch of the Wnt/FZD signalling cascade (Figure 1) regulates cellular processes that are essential for normal vertebrate dorsoventral patterning and gastrulation. During vertebrate gastrulation the early embryo is reorganised into three germ layers: the outer ectoderm, the inner endoderm and the mesoderm located between the ectoderm and endoderm. This process is accompanied by remarkable changes in cell adhesion, cell morphology and cell migration, it is thus not surprising then that Wnt/FZD signalling is critical during gastrulation (9). Importantly, these same cellular processes are now recognised as pivotal to tumour growth and progression, and striking analogies between embryogenesis and tumorigenesis have been identified (2, 5).

The shared patterns identified are cell dissociation, reassembly, tubular reconstruction and branching of neoplastic cells as the tumour tissue mass grows (Figure 2). The epithelial cells at the 'invasive front' or leading edge undergo an EMT, which is characterised by remodelling of the cytoskeleton to change cell shape and is accompanied by a shift in gene expression from an epithelial to a mesenchymal repertoire. This is characterised by a switch from cytokeratin to vimentin intermediate filament expression and a reduction in the expression of cell adhesion molecules, particularly E-cadherin (2). Cell division is either down regulated or shut off before the initiation of morphogenetic changes during development, and indeed, this is also observed in tumour tissues. In colon adenocarcinoma, cancer cells at the invasive front of tumours are less proliferative than the cells at the core of the tumour (6). Detection of EMT markers, rather than proliferative index, in cells at the invasive front is now a well-recognised hallmark of cancer progression characterising invasive and metastatic carcinomas (2, 4).

The analogy between tumorigenic and developmental processes has provided new prognostic

Table 1. FZD Expression in Human Cancers

Frizzled	Cancer	Reference
FZD1/2	Breast, colon, gastric-intestinal, head and neck, mesothelioma, renal	Tothill *et al*., Peter MacCallum Cancer Centre, (95-97).
FZD3	Colon, ovarian, melanoma	http://genome-www5.stanford.edu, (98-101).
FZD4	Melanoma	http://genome-www5.stanford.edu, (98-101)
FZD5	Melanoma, head and neck, colon, ovarian	http://genome-www5.stanford.edu, (86), (95), (98-101).
FZD6	Melanoma, renal	http://genome-www5.stanford.edu, (98-101).
FZD7	Bladder/ureter, breast, colon, chondrosarcoma, gastric, lung (squamous and adenoma), oesophageal, melanoma, mesothelioma, ovarian, pancreatic, prostate	http://genome-www5.stanford.edu, (98-106), Tothill *et al*., Peter MacCallum Cancer Centre.
FZD8	Glioblastoma, ovarian	http://genome-www5.stanford.edu, (98-101).
FZD9	Squamous cell carcinoma, melanoma	http://genome-www5.stanford.edu, (98-101).
FZD10	Colon, ovarian, juvenile granulosa tumour	http://genome-www5.stanford.edu, (98-101), (107).
Other		
Wnt-5a	Bladder/ureter, melanoma, breast, colon, gastric, lung (squamous and adenocarcinoma), ovarian, pancreas, prostate	http://genome-www5.stanford.edu, (86), (96), (98-101), (105), (108), (109), Tothill *et al*., Peter MacCallum Cancer Centre.
sFRP4	Colon, gastric, prostate.	http://genome-www5.stanford.edu, (97-101), Tothill *et al*., Peter MacCallum Cancer Centre.

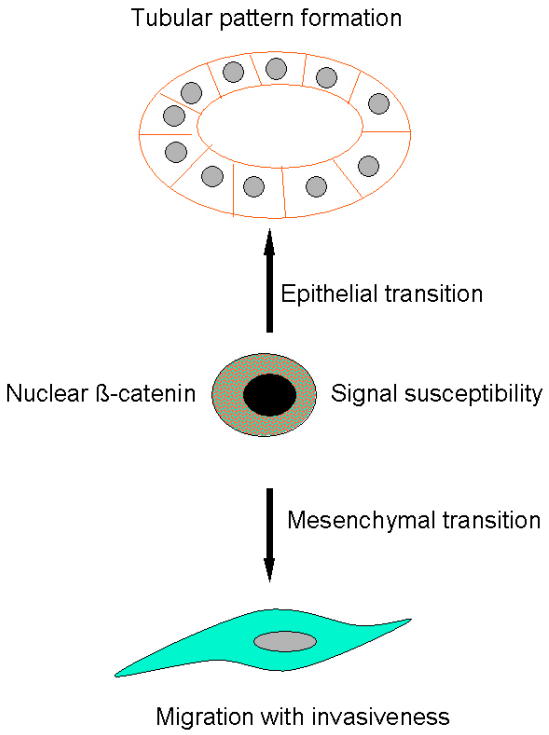

Figure 3. A hypothetical model for the morphogenesis of colonic adenocarcinoma. Cells are primed to receive signals to undergo transition to an epithelial or a mesenchymal phenotype. Adapted from Kirchner and Brabletz (111).

markers and predictors of disease outcome. For example, co-localization of nuclear beta-catenin and matrilysin in colon tumour sections is a strong indicator for tumour recurrence and these patients segregate to a group with less favourable survival rates (62). Indeed, several genes that are associated with EMT and/or cell migration and invasion [e.g. matrilysin (MMP7) (63, 64), fibronectin (65), vimentin (66)] are increased at the invasive front of colon tumours and are targets of beta-catenin/TCF-mediated transcription. Interestingly many of the genes disrupted in colon cancer that have been extensively studied in terms of cell proliferation, differentiation and apoptosis, are now often assigned properties associated with 'neo-morphogenesis' such as cell placement (tissue patterning) and movement (cell-cell contact, cell polarity, cell-matrix adhesion, migration and invasion). For example, c-src is a potent regulator of cell proliferative responses, however, elevated c-src is linked to altered cell-matrix adhesion rather than proliferation in colon cancer cells, suggesting a role for c-src in cell motility (67). This is corroborated by the demonstration that c-src acts synergistically with LEF-1 to induce matrilysin (MMP7) expression and thus has the potential to affect cellular invasion (68). Similarly, expression of c-myc, another well-characterised regulator of proliferation that is a beta–catenin target gene, correlates with colon adenocarcinoma tumour size rather than cell proliferative capacity (69).

One aspect of tumour metastases that has not been appreciated until recently, and again stems from analogies with developmental processes, is the requirement for the re-instalment of epithelial characteristics for cell re-assembly and reconstruction of the tumour at the site of metastases [reviewed in (2, 5)]. Colon adenocarcinoma metastases, which presumably arise from mesenchymal tumour cells that dissociate from the well-differentiated colon tumours, are also well differentiated. Thus the histology of the metastases is similar to that of the primary tumour (6) and indicates that a mesenchymal to epithelial transition (MET) has occurred. This is supported by recent microarray studies where the genetic profile of the metastases mirrored that of the primary tumour (70, 71). Thus the gene clusters that predict the ability to metastasise to distant sites are an early and inherent genetic property of the primary tumour. These findings argue against the widely accepted idea that metastatic potential is acquired relatively late during

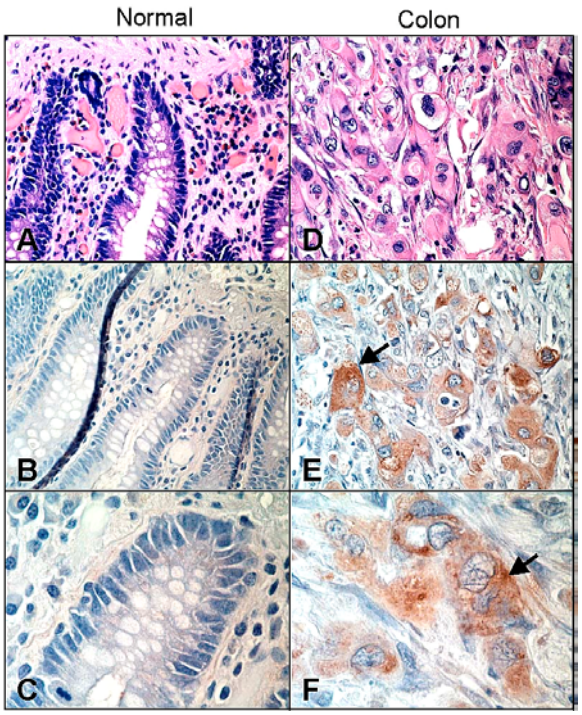

Figure 4. Expression of FZD1/2 in colon cancer (E, F) but not normal (B, C) colon tissue, as detected by immunohistochemical staining (brown). A and D are haematoxylin and eosin stained sections. Adapted from Holcombe *et al*., (96).

multistep tumorigenesis but support the notion that the changes in tumour cell phenotype at the invasive front are transient and reversible (Figure 3). Inducible transient expression of mesenchymal gene in metastasising cells is supported by the finding that vimentin-positive cervical cancer tumours are more likely to have lymph node metastases, but only a small percentage of the cells in the primary tumour and the metastases are vimentin positive. This suggests that the induction of vimentin expression is reversed as the migrating cells re-establish in the invasive clusters (72). Identifying the genes that facilitate or regulate these phenotype transitions is the next important challenge. Our current understanding of Wnt/FZD function in early embryogenesis and organogenesis and their frequent deregulation in cancer, make these molecules prime candidates.

5.3. Wnt and Frizzled in human cancers

Genome profiling and expression studies are powerful tools that continue to identify the FZDs and Wnts that are aberrantly expressed in human cancers (Table 1). However, translating aberrant expression into pathological function has been a slow process to date. The proto-oncogenic effects of Wnt were discovered some twenty years ago (73), yet it was only recently demonstrated in a transgenic mouse model that tumour growth is dependent on Wnt-1 expression *per se* (74). The over-expressed Wnt-1 is the wild-type molecule and is presumably signalling through FZD receptor/s that remain/s to be identified. Despite the slow beginning, this next phase is rapidly gaining momentum and is propelled by genome profiling of human cancers and experimentation in cell culture and animal models systems.

Expression studies have uncovered some intriguing surprises. Inactivation of APC appears to be an obligatory step for the initiation of colon cancer as inactivation of this tumour suppressor is an early event in a vast majority of all colon adenocarcinomas (51). The tumour suppressor function of APC has been largely attributed to the role APC has in facilitating the degradation of beta-catenin. Thus loss of APC in colon cancer has been equated with loss of beta-catenin regulation (52). It may seem surprising then that re-activation of ligands, receptors and receptor inhibitors putatively up-stream of beta-catenin are implicated by expression studies, in the progression of colon cancer. However, several Wnts, FZDs (including FZD1/2 shown in Figure 4) and sFRPs are indeed expressed in colon tumours and colon adenocarcinoma cell lines (references in Table 1) and recent evidence suggests that it may in fact be the loss of functional APC that induces their expression in colon cancer. For example, several studies have demonstrated that colon cancer cells express FZD7 (references in Table 1 and Vincan *et al*., unpublished data). The FZD7 gene promoter contains TCF binding sites and FZD7 expression is elevated in response to activation of beta-catenin/TCF-mediated transcription (75). In addition to FZD, several FZD/Wnt pathway molecules are also targets of beta-catenin/TCF-mediated-transcription and are expressed in cancers with deregulated Wnt/FZD signalling. These include transcriptional factors [e.g. TCF-1 (76), LEF-1 (77), TLE/Groucho (75)] and regulators/transducers [e.g. Axin-2 (78-80), DVL (81, 82)]. In this regard, the mutational inactivation of APC may contribute to establishing the genetic makeup that facilitates cell phenotypic changes induced by FZDs and other factors (Figure 3). The recent observation that LEF-1-induced EMT was dependent on inactive mutant APC (83) strongly supports this notion. LEF-1 is known to initiate EMT during normal development of several organs (84), however in the context of cancer, both LEF-1 and loss of APC are required for EMT. Interestingly, loss of APC tumour suppressor function is also a precursor to invasive adenocarcinoma of the oesophagus and thus, presumably EMT in this cancer (85). APC is mutated in several other cancers (11); hence APC inactivation may be a pre-requisite for phenotype modulation in cancers other than gastrointestinal cancers.

Although, the initial focus on the Wnt/FZD pathway in oncogenesis stemmed from the recognition of the oncogenic potential of beta-catenin and the canonical Wnt/beta-catenin pathway, more recent evidence illustrates the oncogenic potential of the non-canonical pathways. A recent report has shown that Wnt-5a expression in melanoma correlates with progression to metastatic disease (86). In addition, *in vitro* studies revealed that over-expression of Wnt-5a in melanoma cells increased cell motility and invasion. No increase in nuclear beta-catenin was detected but PKC activity was dramatically increased, indicating that the Wnt/Ca^{2+} pathway, and not the Wnt/beta-catenin pathway, was activated during this phenotype transition. Furthermore, FZD5 was required for

the Wnt-5a effects *in vitro* (86). Thus in this context, the combination of Wnt-5a and FZD5 activates the Wnt/Ca^{2+} pathway and directly affects cell motility and invasion. The identification of Wnt/FZD signalling pairs, and the signalling pathways they activate, will help define the role of FZDs in oncogenesis. Given the roles of the small GTPases Rac, Rho and Cdc42, in the regulation of actin-membrane interactions that are implicated in cell motility, and the impact of these GTPases on the canonical Wnt/beta-catenin pathway (87), direct evidence for the involvement of the planar polarity branch of FZD signalling in cancer is imminent.

The frequent detection of Wnts and FZDs in diffuse or poorly differentiated cancers or cancers with a high metastatic potential (references in Table 1), suggests a role for Wnt/FZD in cell de-differentiation or induction of a mesenchymal phenotype. This is supported by the 'mesenchyme' inducing role of FZD during development (61) and is further supported by the tumour suppressive effects of sFRP proteins in some cancers (25). For example, membranous expression of sFRP4 is associated with a good prognosis in pancreatic cancer, while ectopic expression of sFRP4 in a prostate cancer cell line, induced a more epithelial phenotype (88, 89). Several Wnt, FZD and sFRP, including sFRP4, are expressed during foregut development and are thought to govern epithelial-mesenchymal interactions during pancreatic morphogenesis (90). The dramatic over-expression of sFRP4 in pancreatic cancer and the link to clinical outcome (88) suggests that sFRP4 may have a similar role in regulating epithelial-mesenchymal interactions during prostate cancer morphogenesis.

It is also conceivable that FZDs may promote tumor growth by invoking more epithelial characteristics so that tumor cells can form cell junctions, reduce their potential to migrate and remain cohesive for initiation of tumor growth to occur. Several lines of evidence support a role for FZD7, in particular, in promoting an epithelial phenotype in 'oncogenic' gastrointestinal epithelial cells. FZD7 is the only FZD family member conserved in developing gut systems through evolution from *Hydra* (18) to man (90, 106) where it is thought to contribute to the establishment/maintenance of the epithelial lining. Furthermore, the cytoplasmic tail of FZD7 (and FZD1, 2 and 4) interacts with molecules that govern apical-basal cell polarity, the hallmark of the epithelial phenotype (91, 92). The versatility of FZD7 as a signal transducing receptor and the pivotal role it plays in developmental processes (39) make this receptor and its Wnt ligands, such as Wnt-5a, compelling candidates to contribute to the regulation of the dynamic remodeling required for tumor morphogenesis.

The frequent detection of several Wnts and FZDs in a particular cancer (Table 1) may at first imply extensive redundancy. However, functionally distinct Wnts induce different responses and have distinct effects on gene expression in one cell type (93). Ectopic expression of Wnt-1 but loss of function of Wnt-5a transforms C57mg mammary epithelial cells. Microarray analysis of Wnt-1 and Wnt-5a (and/or Rfz-2) expressing C57mg cells revealed that, in addition to several Wnt pathway target genes that were up-regulated by either Wnt, expression of two genes, mesothelin and stromelysin-1, was increased exclusively in Wnt-1 and Wnt-5a/Rfz-2 C57mg cells, respectively. In the Wnt-5a/Rfz-2 C57mg cells, there was a moderate reduction in cell proliferation and phenotypically, the cells were more spread out (93). Both these characteristics are consistent with induction of a 'relaxed epithelial' phenotype or partial EMT. Stromelysin-1 is a matrix metalloproteinase known to induce EMT in mammary epithelial cells (94) and the demonstration that Wnt-5a/Rfz-2 can induce its expression provides a possible mechanism for Wnt-5a function in some breast cancers. Unravelling the function of each FZD in the context of each cancer will no doubt reveal complex mechanisms that are likely to be dependent on the environment of the cancer *in situ*.

6. CONCLUSIONS

Developmental studies have demonstrated that FZD receptors trigger signal transduction pathways that govern cellular processes that are essential for cell placement, adhesion and motility. The recognition that these processes also govern tumour growth, invasion and metastases, together with the frequent deregulation of FZD in human cancers, is set to catapult FZD to the forefront of cancer research. Indeed, both canonical and non-canonical Wnt/FZD signal transduction pathways have been directly linked to tumour invasion and metastases (5, 86). The potential to block or manipulate the Wnt/FZD pathway offers novel avenues to prevent the progression of cancer and tumour recurrence.

7. ACKNOWLEDGMENTS

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Abbreviations: casein kinase-1alpha, CK1alpha; EMT, dishevelled, Dsh/Dvl; epithelio-mesenchymal-transition; FZD, Frizzled; glycogen synthase kinase-3beta, GSK-3beta; Jun N-terminal kinase (JNK); nemo-like kinase (NLK); plana cell polarity, PCP.

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