WNT Signaling in Cutaneous Squamous Cell Carcinoma

Sherwood, Victoria; Leigh, Irene

Published in:
Journal of Investigative Dermatology

DOI:
10.1016/j.jid.2016.05.108

Publication date:
2016

Document Version
Peer reviewed version

WNT Signaling in Cutaneous Squamous Cell Carcinoma: A Future Treatment Strategy?

Sherwood, Victoria; Leigh, Irene

Published in:
Journal of Investigative Dermatology

Publication date:
2016

Document Version
Peer reviewed version

WNT Signaling in Cutaneous Squamous Cell Carcinoma: A Future Treatment Strategy?

Victoria Sherwood* and Irene M. Leigh

Division of Cancer Sciences, School of Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD1 9SY, UK.

*Correspondence should be addressed to; Tel: +44 (0) 1382 383295; Email: v.sherwood@dundee.ac.uk

Running title: WNT and cutaneous squamous cell carcinoma

Abbreviations: cSCC, cutaneous squamous cell carcinoma; NMSC, non-melanoma skin cancer; BCC, basal cell carcinoma; DVL, Dishevelled; HF, hair follicle; IFE, interfolicular epidermis; FZD, Frizzled; ROCK, Rho-associated protein kinase; EMT, epithelial-to-mesenchymal transition; TCF, T-cell factor; LEF, lymphoid enhancer factor; SC, stem cell; SFRP, secreted Frizzled-related protein.

ABSTRACT

The molecular mechanisms underlying cutaneous squamous cell carcinoma are less well established than other common skin cancers, but recent evidence has highlighted a potentially critical role for WNT signaling in both the development and progression of cSCC. WNT pathways are aberrantly regulated in multiple tumour types (albeit in a context-dependent manner) and this has stimulated the development of WNT inhibitory compounds for cancer treatment. In this review, we examine existing evidence for a role of WNT signaling in cSCC and discuss if WNT inhibition represents a realistic therapeutic strategy for the future.

INTRODUCTION

Patients developing metastatic cutaneous squamous cell carcinoma (cSCC; 5-10% of cases), have a poor outcome with 25-50% 5-year survival (Epstein 1984). Therapies targeted to immune checkpoint blockade or to pro-proliferative signaling pathways such as the mitogen-activated protein kinase pathway, are providing novel treatments for metastatic melanoma patients (Flaherty, et al. 2010; Wolchok, et al. 2013). Moreover, identification of Hedgehog signaling as a molecular hallmark of basal cell carcinoma (BCC) (Epstein 2008; Hahn, et al. 1996; Johnson, et al. 1996) led to the development of Hedgehog antagonists for locally advanced or metastatic BCC. It would be desirable to adopt a similar approach for cSCC, where targeted therapies could be used to treat the most invasive and aggressive tumours, based on in-depth molecular understanding of the disease. To progress this aim, expression array profiling of cSCC tumours by our group and others has been used to identify the most dysregulated molecular pathways. Such studies have identified WNT signaling as significantly altered in cSCC (Haider, et al. 2006; Ra, et al. 2011; Watt, et al. 2011). Functional evidence also exists for a role of WNT signaling in cSCC and here we discuss these findings.

WNT signaling is composed of a group of signal transduction pathways, implicated in the development/progression of multiple cancers when aberrantly regulated. The role of WNT signaling in a number of non-melanoma skin cancers (NMSCs) including BCC (El-Bahrawy, et al. 2003; Salto-Tellez, et al. 2006; Yang, et al. 2008; Youssef, et al. 2012) is already defined, however evidence is also emerging for a role in the development and progression of cSCC too. Here we examine this evidence and investigate the possibility of WNT inhibitors as a novel therapeutic opportunity for disease management of cSCC. To do this we will first summarise what is known about WNT signaling in cancer, then briefly discuss the crucial role it plays in keratinocyte biology, before focusing on its activity in NMSCs and specifically cSCC.

WNT SIGNALING IN CANCER

Wnt genes encode for secreted glycolipoproteins that activate intracellular signaling pathways, which can be subdivided into two categories based on whether or not they signal through β-catenin (encoded by *ctnnb1*; referred to as the WNT/β-catenin-dependent or -independent pathways, respectively; Figure 1). There is significant crosstalk between the individual WNT signaling pathways (which is often antagonistic), leading researchers to view WNT pathways as a network of integrated signals, called the WNT signaling network (Kestler and Kuhl 2008; van Amerongen and Nusse 2009).

For WNT/β-catenin signaling; in the absence of WNT, a cytoplasmic pool of β-catenin is continuously degraded by a multi-protein complex (termed the destruction complex; (Dale 1998), comprised of scaffold proteins (Axin and Adenomatous polyposis coli), and kinases (Glycogen Synthase Kinase-3β and Casein Kinase-1). These kinases phosphorylate the amino terminus of β-catenin to allow subsequent ubiquitination and proteasomal-mediated degradation. Extracellular WNT ligands activate the pathway by binding to seven-pass transmembrane-containing Frizzled (FZD) receptors, plus the LRP5/6 co-receptor, which leads to recruitment of an intracellular scaffold, Dishevelled (DVL). DVL antagonises destruction complex activity, causing β-catenin accumulation to allow nuclear translocation where in consort with the T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors, β-catenin elicits activation of WNT target gene expression (Cadigan 2012). WNT/β-catenin-independent signaling commonly occurs through Ca^{2+} signaling in tumours, resulting in activation of Ca^{2+}-dependent enzymes to elicit transcriptional changes and increase small GTPase activity, causing cytoskeletal rearrangements and alteration of cell polarity/migration (Jenei, et al. 2009). WNT target genes direct a variety of fundamental cellular processes including cell proliferation, polarity, migration, angiogenesis and cellular metabolism in cancer cells (Brabletz, et al. 1999; He, et al. 1998; Sherwood 2015; Tetsu and McCormick 1999; Zhang, et al. 2001).

WNT signaling has been associated with cancer since the early 1990s when *Adenomatous polyposis coli* mutations were found in the majority of colorectal cancers (Groden, et al. 1991). Mutations in a number of WNT pathway genes have now been associated with a variety of tumour types (e.g. *Axin* loss-of-

function and constitutively activating *ctnnb1* mutations; (Herr, et al. 2012). Furthermore, epigenetic silencing of endogenous WNT inhibitors (Ekstrom, et al. 2011; Fukui, et al. 2005; Lee, et al. 2004; Suzuki, et al. 2004; Zou, et al. 2005), increased WNT ligand expression (Wong, et al. 2002) and up-regulation of WNT downstream effectors, such as DVL (Okino, et al. 2003) have also been identified in tumours, demonstrating that hyper-activation of WNT signaling in cancer is achieved through a variety of mechanisms. This work has prompted the development of WNT signaling antagonists (Anastas and Moon 2013), as summarised in Figure 1.

However, the concept that WNT signaling is always pro-oncogenic is too simplistic, as increased activity does not always necessarily correlate with worse prognosis. In melanoma for example, loss of nuclear β-catenin has (at least in some patient cohorts), been associated with poor survival (Chien, et al. 2009; Kageshita, et al. 2001; Maelandsmo, et al. 2003), raising the possibility that WNT/β-catenin signaling may also possess tumour suppressive functions in some contexts. Such findings highlight the potential hazards associated with intervention using WNT antagonists, without prior in-depth understanding of the complex nature of the signaling network within specific tumours (Kahn 2014). Here we review existing literature examining WNT signaling in cSCC and discuss its potential as a treatment target, but first we briefly review the well-defined role that the network plays in regulating keratinocyte biology.

WNT IN SKIN DEVELOPMENT AND HOMEOSTASIS

WNT signaling is fundamental in skin development; being involved in specification of the embryonic ectoderm to form the skin epithelium, through to blocking fibroblast growth factor signaling to induce keratin production in the nascent skin epithelia (keratinocyte specification), thereby forming the epidermis (Wilson, et al. 2001). WNT signaling is also necessary for the formation of skin appendages, in particular hair follicles (HFs), which involves crosstalk between the dermis and epidermis to form epidermal placodes at regularly spaced intervals in the skin. The initial signal to form these placodes is proposed to be dermally-derived WNT ligands (DasGupta and Fuchs 1999;

Huelsken, et al. 2001; Zhang, et al. 2009). Furthermore in response to this initial signal, WNT/β-catenin signaling is activated in the epidermis to promote placode fate (Zhang, et al. 2009) and is also required for signaling to the underlying mesenchymal cells to form dermal condensates that give rise to the dermal papillae (Huelsken, et al. 2001; Zhang, et al. 2009). The requirement of WNT/β-catenin signaling in HF development is further emphasised by the complete lack of placode formation in the presence of ectopic expression of the WNT/β-catenin-specific inhibitor, Dickkopf-1, in basal cells of developing murine epidermis (Andl, et al. 2002). WNT/β-catenin-independent signaling pathways are also involved in dermal development (Geetha-Loganathan, et al. 2006) and associated appendages (Bazzi, et al. 2007; Guo, et al. 2004). Hence the WNT signaling network is highly influential in integumentary system development.

The same signaling cues responsible for HF morphogenesis are also used for hair cycling in adult skin. In follicular stem cells (SCs) for example, β-catenin is restricted to nuclear/cytoplasmic expression during anagen (growth phase), but confined to the cell membrane during cycles of telogen (rest phase), suggesting that high levels of WNT/β-catenin signaling functions as an inducer of anagen onset (Greco, et al. 2009). In addition to regulating telogen-anagen transition, WNT signaling also controls cell fate determination in HFs, the direction of hair growth and sebocyte specification to form the pilosebaceous unit, as previously reviewed (Lim and Nusse 2013).

The role of the WNT/β-catenin pathway in the interfollicular epidermis (IFE) is more complex. For a long time it was generally considered that (at least in mouse skin) the WNT/β-catenin pathway controls epidermal SC fate (toward a HF lineage) rather than effect self-renewal properties (Andl, et al. 2002; Beronja, et al. 2013; Gat, et al. 1998; Huelsken, et al. 2001). However, other recent lineage tracing work in the murine plantar epidermis has identified a population of $Axin2^{+}$ basal cells, which represent IFE SCs continuously producing keratinocytes (Lim, et al. 2013). Interestingly, these $Axin2^{+}$ cells are themselves the source of WNT ligands in the IFE, where a high level of secreted WNT inhibitors in the suprabasal layers of the epidermis creates a gradient that restricts autocrine WNT signaling to the basal layer (Lim, et al. 2013), in effect creating spatial self-organization within the epidermis (Clevers, et al. 2014). This

is supported by additional *in vivo* work showing that β-catenin is required for epidermal proliferation and SC maintenance in the IFE (Choi, et al. 2013; Jensen, et al. 2009). Furthermore, the WNT/β-catenin pathway also maintains human IFE SC populations *in vitro* (Zhu and Watt 1999). Inducible reduction of WNT/β-catenin signaling in murine IFE inhibited proliferation under homeostatic conditions, but is not required for long-term maintenance of the IFE, nor needed for inflammatory-induced hyper-proliferation (Choi, et al. 2013). This work suggests that WNT signaling has highly specialised functions in murine IFE, but the precise role of the network within specific regions and SC populations of human skin, still remains to be determined. What is known however is that aberrant regulation of WNT/β-catenin signaling during wound healing causes keratinocyte proliferation and epidermal thickening during keloid scarring in humans (Chua, et al. 2011; Sato 2006).

WNT SIGNALING IN cSCC

Given the large body of work highlighting a crucial role for WNT signaling in regulating skin development/homeostasis, it is hardly surprising that when perturbations in activity occur, so too does the development of skin diseases, including NMSCs. In the HF for example aberrant WNT signaling can lead to the development of pilomatricoma (Chan, et al. 1999), trichofolliculoma (Sun, et al. 2014) and even sebaceous gland tumours (Takeda, et al. 2006). Unsurprisingly then, the WNT signaling network is also emerging as a dominant regulator in the development and progression of more common NMSCs too. In BCC, Hedgehog signaling up-regulates WNT/β-catenin activity (Yang, et al. 2008), particularly at the leading edge of invasive tumours (Youssef, et al. 2012) and stromally-derived WNT5A (signaling through the WNT/Ca^{2+} pathway) causes BCC tumours to differentiate and regress (Nitzki, et al. 2010).

A number of genomic and transcriptomic analyses have identified the WNT signaling network as being potentially aberrantly regulated in cSCC. One of the earliest studies using comparative genomic hybridization found frequent amplification of chromosomes 7q, 8q, 11q and 17q in cSCC lines, which all contain *WNT* and/or *FZD* genes, suggesting that expression of these genes may

be increased in cSCC (Popp, et al. 2002). Direct evidence of this has subsequently been found by a number of gene expression array analyses in cSCC samples, which have identified increases in the mRNA levels of WNT ligands and their receptors, and in some samples also the down-regulation of endogenous secreted WNT inhibitors such as secreted FZD-related proteins (SFRPs) that antagonise WNT-FZD interactions (Haider, et al. 2006; Ra, et al. 2011; Watt, et al. 2011). For example, Haider and colleagues found that WNT5A and FZD6 were both upregulated in cSCC (Haider, et al. 2006), whilst a study by Ra *et al*. identified the WNT signaling network as the most significantly enriched set of molecular pathways in gene expression comparisons between cSCC and normal skin (Ra, et al. 2011). Evidence for enhanced β-catenin signaling in cSCC comes from a number of immunohistochemical staining studies on cSCC tumors, looking at β-catenin levels in tumours. Depending on the particular study, anywhere from 20-90% of human (Doglioni, et al. 2003; Lan, et al. 2014; Malanchi, et al. 2008; Papadavid, et al. 2002) and other mammalian (Bhatia and Spiegelman 2005; Bongiovanni, et al. 2011) cSCC tumours express high levels of nuclear β-catenin, whilst the membrane-bound pool of β-catenin is often found to be reduced (Bongiovanni, et al. 2011; Brasanac, et al. 2005; Fukumaru, et al. 2007; Papadavid, et al. 2002), the latter of which is commonly associated with loss of differentiation in carcinomas. In addition, elevated nuclear β-catenin has been identified in lymphatic metastases of human cSCC, suggesting it may also be associated with advanced stage disease (Toll, et al. 2013). Collectively these 'omic' and immunohistochemical studies have identified potentially altered WNT signaling activity in cSCC (in particular the WNT/β-catenin pathway), but what causes it and what effect this signaling has on the behaviour of cSCC cells is still under investigation. However, in addition to this circumstancial evidence there is also a body of work that has functionally investigated the role of WNT signaling in cSCC and here we summarise what is currently known.

Early studies identified a link between loss of the cell-cell signaling receptor, Notch and stabilised β-catenin, which results in β-catenin-TCF/LEF signaling in cSCC. Conditional *Notch1* deletion in murine keratinocytes increased suceptibility to chemically-induced skin carcinogenesis (including cSCC) and stabilised β-catenin (Nicolas, et al. 2003). In murine keratinocytes this Notch-

meidated repression of β-catenin was found to be (at least in part), dependent on p21-mediated transcriptional repression of *WNT* genes (Devgan, et al. 2005). Building on these findings, Parmacek and colleagues demonstrated that dominant negative repression of Notch signaling in mouse skin renders the animials susecptible to developing cSCC, with nuclear β-catenin accumulation and increased cyclinD1 expression, the latter of which is a common target of β-catenin signaling that drives tumour proliferation (Proweller, et al. 2006). p63 (a member of the p53 tumour suppressor gene family) has been found to be an upstream regulator of both the WNT/β-catenin and Notch signaling pathways modulating proliferation in immortalised human keratinocytes (Wu, et al. 2012). Presenilin-1 is part of the γ-secretase protease complex, required for Notch processing, where loss of Presenilin-1 in keratinocytes leads to elevated β-catenin signaling, cyclinD1 expression and cell proliferation, causing epidermal hyperplasia and cSCC in mice (Xia, et al. 2001). However, Presenilin-1-mediated β-catenin regulation appears to be independent of Notch processing (Xia, et al. 2001), suggesting that Notch-independent mechanisms also regulate β-catenin signaling in cSCC.

The concept that β-catenin signaling leads to the increased proliferation of cSCC cells is supported by more recent work, showing that knockdown of β-catenin in human cSCC cells in xenotransplantation models, reduced tumour volume and increased tumour-free survival (Beronja, et al. 2013). This paper also showed that conditional oncogeneic HRas expression in murine skin induced β-catenin signaling and importantly, that short-hairpin knockdown of *ctnnb1* was found to be a selective inhibitor for oncogenic yet not normal epidermal growth in the mouse (Beronja, et al. 2013).

Additional signaling mechanisms have also been identified that stabilise β-catenin in cSCC. For example, Rho-associated protein kinase (ROCK) is activated in cSCC tumours, where the development of a genetically engineered mouse model of conditional ROCK over-expression in the skin was used to identify the mechanism of tumour development. This model showed that ROCK activity led to increased β-catenin signaling and subsequent hyperproliferation/thickening of the IFE (Samuel, et al. 2011). This was later found to involve a mechanotransduction pathway, triggered by ROCK-mediated

increased expression of extracellular matrix proteins (such as collagen and fibronectin) in invasive human cSCC, to elicit integrin signaling resulting in GSK3β inactivation, and subsequent stabilisation of β-catenin thereby initiating tumor progression, and invasion (Ibbetson, et al. 2013). Interestingly, *ROCK1/2* genes are negatively regulated by Notch in human keratinocytes (Lefort, et al. 2007), which may potentially represent another mechanism by which loss of Notch can promote β-catenin signaling in the skin.

Epithelial-to-mesenchymal transition (EMT) events have been suggested as another mechanism that can lead to the accumulation of nuclear β-catenin in cSCC, through loss of E-cadherin-β-catenin binding to facilitate increased β-catenin signaling (Margulis, et al. 2005), which is linked with metastatic spread in human cSCC (Toll, et al. 2013). More recently, the receptor tyrosine-kinase, Axl, which is over-expressed in cSCC biopsies (Green, et al. 2006), was shown to promote expression of EMT markers, nuclear β-catenin and stem-like properties that conferred increased sphere formation, tumor initiation, and drug resistance in human cSCC cells (Cichon, et al. 2014). Activation of EMT promotes cancer SC phenotypes (Mani, et al. 2008), so importantly active β-catenin signaling has not only been shown to be induced by EMT events, but crucially was also required to sustain cancer SC phenotypes in a murine cSCC model (Malanchi, et al. 2008). However, it remains to be determined if this is also true for tumor-inititating populations in human cSCC.

From these studies it is reasonable to conclude that β-catenin signaling, can promote both the development and progression of cSCC, and although this can be initiated by several signaling pathways, WNT ligands that activate WNT/β-catenin signaling are also pro-oncogenic in cSCC. An important recent study has found that focal activation of WNT3-β-catenin signaling occurs in cSCC tumours, but not in normal human skin (Sobel, et al. 2015). Interesting this work also found that WNT3 expression is restricted to the tumour-stroma and that it signals in a paracrine fashion in the tumour microenvironment to increase epithelial cell proliferation and stromal remodelling, suggesting WNT signaling may also promote skin carcinogenesis in a paracrine manner (Sobel, et al. 2015). WNT/β-catenin-independent signaling also regulates cSCC cells, where WNT5A leads to chemotactic migration at the leading edge of human cSCC tumours

(Pourreyron, et al. 2012). Furthermore, epigenetic profiling of metastatic cSCC compared to non-metastatic tumours, identified the *FrzB* gene, which encodes for the secreted WNT inhibitor, SFRP3, as having the most hypermethylated promoter in human cSCC, suggesting that loss of SFRP3 expression and subsequent WNT activation is a critical step in the development of metastatic tumours (Darr, et al. 2015). Indeed, hypermethylation of a number of other *SFRP* genes (*SFRP1*, *2*, *4* and *5*) have also been identified in cSCC tumours compared to normal human skin (Liang, et al. 2015). Further work is needed to fully understand the effects of the WNT signaling network in the metastatic progression of cSCC.

Overall this body of work provides evidence that (as in other NMSCs), aberrant regulation of the WNT signaling network is pro-tumourigenic in cSCC (Figure 2a), where the findings summarised have been obtained from functional studies in human tissues/cells and mouse models (Figure 2b). Currently however, a comprehensive list of WNT target genes is not available for human cSCC cells, which is needed to help delineate the mechanisms that facilitate these WNT-dependent oncogenic effects.

Interestingly, mutations in WNT/β-catenin signaling components are generally rare in SCC tumours, including cSCC (Doglioni, et al. 2003; Li, et al. 2015; Pickering, et al. 2014). For example in oesophageal SCC, the frequency of *ctnnb1* mutations is only 1.1%, but yet 86.4% of oesophageal SCC tumours contain mutated WNT pathway genes (Song, et al. 2014), suggesting the mechanisms by which WNT signaling function is peterbed in SCC tumours, are potentially diverse. Epigenetic inactivation of WNT signaling antagonists such as the *SFRP* genes is not restricted to cSCC, but rather is detected in a variety of other types of SCC tumours, including oral (Paluszczak, et al. 2015; Pannone, et al. 2010; Sogabe, et al. 2008), oesphageal (Kishino, et al. 2016; Liu, et al. 2011; Meng, et al. 2011; Saito, et al. 2014; Yang, et al. 2012), cervical (Delmas, et al. 2011; Siegel, et al. 2015) and head & neck (HN) SCCs (Marsit, et al. 2006), and thus could represent a universal mechanism of WNT activation in SCC tumours.

TARGETING WNT SIGNALING IN cSCC; A THERAPEUTIC OPPORTUNITY?

The identification that WNT pathways are hyper-activated and pro-tumourigenic in cSCC, raises the distinct possibility that WNT targeting compounds may represent a pertinent therapeutic strategy. A number of compounds that antagonise WNT signaling are currently in oncology clinical trials (or are completed and awaiting study results; NCT trials; 02278133, 01351103, 02413853, 01606579, 02521844, 01469975, 01764477 and 01608867). WNT/β-catenin inhibitors work to block extracellular WNT-receptor interactions, antagonise DVL signal transduction, stabilise the β-catenin destruction complex, interfere with β-catenin binding in the nucleus or inhibit Porcupine (a membrane bound O-acetyltransferase that is needed for the post-translational modification of WNT proteins to facilitate secretion; Figure 1). It is difficult without prior testing, to predict which (if any) of these inhbitors are likely to be effective in cSCC. However a small molecule Porcupine inhibitor, LGK974, is well-tollerated, potent and highly efficatious in human HNSCC cells (Liu, et al. 2013), suggesting it may prove a lead therapeutic in other SCCs where WNT/β-catenin signaling drives carcinogenesis. Consistent with this is the interesting finding that another Porcupine inhibitor, IWP2, can induce tumour regression in chemically induced murine keratoacanthomas (a HF-derived benign variant of cSCC), which highlights WNT/β-catenin signaling as a key regulator to sustain cutaneous tumour growth (Zito, et al. 2014). cSCC is one of the most heterogenous cancers (South, et al. 2014), meaning that patient stratification will likely prove important for intervening with effective therapies, particularly where drugs, such as WNT inhibitors, target context-dependent signaling pathways. For example, the identification that a loss of Notch in cSCC results in activation of β-catenin signaling, suggests that cSCC patients with *Notch* loss-of-function mutations may benefit from intervention with WNT/β-catenin pathway inhibitors. Consistant with this hypothesis, is the finding that loss of Notch1 activity in HNSCC cells correlates with LGK974 responsiveness (Liu, et al. 2013).

WNT/β-catenin-independent signaling also promotes cSCC progression (Pourreyron, et al. 2012), suggesting that intervention at this arm of the WNT network also has therapeutic potential, albeit there are few inhibitors currently targeted to this pathway. However a WNT5A-derived hexapeptide, termed Box5,

developed to inhibit WNT/Ca^{2+} signaling in melanoma (Jenei, et al. 2009), has recently been shown to antagonise WNT5A/Ca^{2+} signaling in HNSCC (Prgomet, et al. 2015). Future efforts should be focused on identifying which WNT network inhibitors could provide potent therapeutic activity in cSCC.

Mounting evidence is being generated that highlights a key role for the WNT signaling network in cSCC, providing a tantalising argument for the use of WNT antagonists as a novel therapeutic approach. Although caution should be exercised when intervening with WNT inhibitors (Kahn 2014), robust investigations using pre-clinical models of cSCC will help to identify if this represents a suitable treatment approach for patients in the future.

ACKNOWLEDGEMENTS

This work was supported by Cancer Research UK and Tenovus Scotland.

CONFLICTS OF INTEREST

The authors state no conflict of interest.

Figure Legends

Figure 1| WNT signaling and associated inhibitors. WNT/β-catenin-dependent signaling (left); in the absence of WNT binding to the FZD/LRP5/6 receptors, the signaling pool of β-catenin is maintained at low levels, mediated by the multi-protein destruction complex (comprising; APC, Axin, GSK3β, CK1 and the multi-protein E3 ubiquitin ligase complex, SCF). Upon WNT-receptor interaction, DVL inactivates the destruction complex, stabilising β-catenin to promote TCF/LEF transcriptional activity. WNT/β-catenin-independent signaling (right; details not shown); this results in transcriptional changes, cytoskeletal rearrangement, changes in cell polarity/migration and Ca^{2+} signaling. Inhibitors (red) include; tankyrase inhibitors that stabilise the destruction complex through Axin degradation, DVL inhibitors, antagonists of β-catenin/TCF interactions, inhibitors that block Porcupine (required for WNT ligand secretion), FZD antibodies that block WNT binding, and the WNT5A (commonly a WNT/β-catenin-independent signaling ligand)-specific inhibitory peptide, Box5.

Figure 2| WNT signaling in cSCC. (**a**) WNT/β-catenin signaling is proposed to promote cell proliferation. Aside from WNT activation of β-catenin, ROCK-mediated inactivation of GSK3β has also been shown to increase β-catenin signaling. β-catenin also binds cadherin at adherens junctions, where reduced E-cadherin expression through EMT increases β-catenin signaling. Axl (a tyrosine-kinase) promotes β-catenin signaling by increasing expression of EMT drivers (not shown). Loss of Notch also promotes β-catenin signaling (by a currently undefined mechanism). Stromal paracrine WNT/β-catenin signaling is also proposed to increase proliferation. WNT/β-catenin-independent signaling, initiated by WNT5A, promotes pro-invasive behaviour in cSCC cells (through an undefined mechanism). Epigenetic loss of *SFRP* expression, results in increased WNT signaling, but it is currently unknown whether this will affect both arms of the WNT network. (**b**) Summary of literature identifying how the WNT signaling network affects cSCC cells (as outlined in a). Mechanistic studies investigating the effects in human disease were used as a focus for this summary, but also highlighted is work generated from mouse models that supports the molecular functions identified in human tissue. See text for detailed discussion.

REFERENCES

Anastas, J. N., and R. T. Moon
2013 WNT signalling pathways as therapeutic targets in cancer. Nature reviews. Cancer 13(1):11-26.

Andl, T., S. T. Reddy, T. Gaddapara, and S. E. Millar
2002 WNT signals are required for the initiation of hair follicle development. Developmental cell 2(5):643-653.

Bazzi, H., K. A. Fantauzzo, G. D. Richardson, C. A. Jahoda, and A. M. Christiano
2007 The Wnt inhibitor, Dickkopf 4, is induced by canonical Wnt signaling during ectodermal appendage morphogenesis. Dev Biol 305(2):498-507.

Beronja, S., P. Janki, E. Heller, W. H. Lien, B. E. Keyes, N. Oshimori, et al.
2013 RNAi screens in mice identify physiological regulators of oncogenic growth. Nature 501(7466):185-190.

Bhatia, N., and V. S. Spiegelman
2005 Activation of Wnt/beta-catenin/Tcf signaling in mouse skin carcinogenesis. Molecular carcinogenesis 42(4):213-221.

Bongiovanni, L., D. Malatesta, C. Brachelente, S. D'Egidio, and L. Della Salda
2011 beta-catenin in canine skin: immunohistochemical pattern of expression in normal skin and cutaneous epithelial tumours. J Comp Pathol 145(2-3):138-147.

Brabletz, T., A. Jung, S. Dag, F. Hlubek, and T. Kirchner
1999 beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. Am J Pathol 155(4):1033-1038.

Brasanac, D., I. Boricic, V. Todorovic, N. Tomanovic, and S. Radojevic
2005 Cyclin A and beta-catenin expression in actinic keratosis, Bowen's disease and invasive squamous cell carcinoma of the skin. The British journal of dermatology 153(6):1166-1175.

Cadigan, K. M.
2012 TCFs and Wnt/beta-catenin signaling: more than one way to throw the switch. Curr Top Dev Biol 98:1-34.

Chan, E. F., U. Gat, J. M. McNiff, and E. Fuchs
1999 A common human skin tumour is caused by activating mutations in beta-catenin. Nature genetics 21(4):410-413.

Chien, A. J., E. C. Moore, A. S. Lonsdorf, R. M. Kulikauskas, B. G. Rothberg, A. J. Berger, et al.
2009 Activated Wnt/beta-catenin signaling in melanoma is associated with decreased proliferation in patient tumors and a murine melanoma model. Proceedings of the National Academy of Sciences of the United States of America 106(4):1193-1198.

Choi, Y. S., Y. Zhang, M. Xu, Y. Yang, M. Ito, T. Peng, et al.
2013 Distinct functions for Wnt/beta-catenin in hair follicle stem cell proliferation and survival and interfollicular epidermal homeostasis. Cell Stem Cell 13(6):720-733.

Chua, A. W., D. Ma, S. U. Gan, Z. Fu, H. C. Han, C. Song, et al.

2011 The role of R-spondin2 in keratinocyte proliferation and epidermal thickening in keloid scarring. The Journal of investigative dermatology 131(3):644-654.

Cichon, M. A., Z. Szentpetery, M. P. Caley, E. S. Papadakis, I. C. Mackenzie, C. H. Brennan, et al.
2014 The receptor tyrosine kinase Axl regulates cell-cell adhesion and stemness in cutaneous squamous cell carcinoma. Oncogene 33(32):4185-4192.

Clevers, H., K. M. Loh, and R. Nusse
2014 Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science 346(6205):1248012.

Dale, T. C.
1998 Signal transduction by the Wnt family of ligands. The Biochemical journal 329 (Pt 2):209-223.

Darr, O. A., J. A. Colacino, A. L. Tang, J. B. McHugh, E. L. Bellile, C. R. Bradford, et al.
2015 Epigenetic alterations in metastatic cutaneous carcinoma. Head Neck 37(7):994-1001.

DasGupta, R., and E. Fuchs
1999 Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. Development 126(20):4557-4568.

Delmas, A. L., B. M. Riggs, C. E. Pardo, L. M. Dyer, R. P. Darst, E. G. Izumchenko, et al.
2011 WIF1 is a frequent target for epigenetic silencing in squamous cell carcinoma of the cervix. Carcinogenesis 32(11):1625-1633.

Devgan, V., C. Mammucari, S. E. Millar, C. Brisken, and G. P. Dotto
2005 p21WAF1/Cip1 is a negative transcriptional regulator of Wnt4 expression downstream of Notch1 activation. Genes & development 19(12):1485-1495.

Doglioni, C., S. Piccinin, S. Demontis, M. G. Cangi, L. Pecciarini, C. Chiarelli, et al.
2003 Alterations of beta-catenin pathway in non-melanoma skin tumors: loss of alpha-ABC nuclear reactivity correlates with the presence of beta-catenin gene mutation. Am J Pathol 163(6):2277-2287.

Ekstrom, E. J., V. Sherwood, and T. Andersson
2011 Methylation and loss of Secreted Frizzled-Related Protein 3 enhances melanoma cell migration and invasion. PloS one 6(4):e18674.

El-Bahrawy, M., N. El-Masry, M. Alison, R. Poulsom, and M. Fallowfield
2003 Expression of beta-catenin in basal cell carcinoma. The British journal of dermatology 148(5):964-970.

Epstein, E. H.
2008 Basal cell carcinomas: attack of the hedgehog. Nature reviews. Cancer 8(10):743-754.

Epstein, E., Sr.
1984 Metastases of sun-induced SCC. J Dermatol Surg Oncol 10(6):418.

Flaherty, K. T., I. Puzanov, K. B. Kim, A. Ribas, G. A. McArthur, J. A. Sosman, et al.
2010 Inhibition of mutated, activated BRAF in metastatic melanoma. The New England journal of medicine 363(9):809-819.

Fukui, T., M. Kondo, G. Ito, O. Maeda, N. Sato, H. Yoshioka, et al.

2005 Transcriptional silencing of secreted frizzled related protein 1 (SFRP 1) by promoter hypermethylation in non-small-cell lung cancer. Oncogene 24(41):6323-6327.

Fukumaru, K., N. Yoshii, T. Kanzaki, and T. Kanekura
2007 Immunohistochemical comparison of beta-catenin expression by human normal epidermis and epidermal tumors. J Dermatol 34(11):746-753.

Gat, U., R. DasGupta, L. Degenstein, and E. Fuchs
1998 De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 95(5):605-614.

Geetha-Loganathan, P., S. Nimmagadda, R. Huang, B. Christ, and M. Scaal
2006 Regulation of ectodermal Wnt6 expression by the neural tube is transduced by dermomyotomal Wnt11: a mechanism of dermomyotomal lip sustainment. Development 133(15):2897-2904.

Greco, V., T. Chen, M. Rendl, M. Schober, H. A. Pasolli, N. Stokes, et al.
2009 A two-step mechanism for stem cell activation during hair regeneration. Cell Stem Cell 4(2):155-169.

Green, J., M. Ikram, J. Vyas, N. Patel, C. M. Proby, L. Ghali, et al.
2006 Overexpression of the Axl tyrosine kinase receptor in cutaneous SCC-derived cell lines and tumours. British journal of cancer 94(10):1446-1451.

Groden, J., A. Thliveris, W. Samowitz, M. Carlson, L. Gelbert, H. Albertsen, et al.
1991 Identification and characterization of the familial adenomatous polyposis coli gene. Cell 66(3):589-600.

Guo, N., C. Hawkins, and J. Nathans
2004 Frizzled6 controls hair patterning in mice. Proceedings of the National Academy of Sciences of the United States of America 101(25):9277-9281.

Hahn, H., C. Wicking, P. G. Zaphiropoulous, M. R. Gailani, S. Shanley, A. Chidambaram, et al.
1996 Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell 85(6):841-851.

Haider, A. S., S. B. Peters, H. Kaporis, I. Cardinale, J. Fei, J. Ott, et al.
2006 Genomic analysis defines a cancer-specific gene expression signature for human squamous cell carcinoma and distinguishes malignant hyperproliferation from benign hyperplasia. The Journal of investigative dermatology 126(4):869-881.

He, T. C., A. B. Sparks, C. Rago, H. Hermeking, L. Zawel, L. T. da Costa, et al.
1998 Identification of c-MYC as a target of the APC pathway. Science 281(5382):1509-1512.

Herr, P., G. Hausmann, and K. Basler
2012 WNT secretion and signalling in human disease. Trends Mol Med 18(8):483-493.

Huelsken, J., R. Vogel, B. Erdmann, G. Cotsarelis, and W. Birchmeier
2001 beta-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell 105(4):533-545.

Ibbetson, S. J., N. T. Pyne, A. N. Pollard, M. F. Olson, and M. S. Samuel

2013 Mechanotransduction pathways promoting tumor progression are activated in invasive human squamous cell carcinoma. Am J Pathol 183(3):930-937.

Jenei, V., V. Sherwood, J. Howlin, R. Linnskog, A. Safholm, L. Axelsson, et al.
2009 A t-butyloxycarbonyl-modified Wnt5a-derived hexapeptide functions as a potent antagonist of Wnt5a-dependent melanoma cell invasion. Proceedings of the National Academy of Sciences of the United States of America 106(46):19473-19478.

Jensen, K. B., C. A. Collins, E. Nascimento, D. W. Tan, M. Frye, S. Itami, et al.
2009 Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. Cell Stem Cell 4(5):427-439.

Johnson, R. L., A. L. Rothman, J. Xie, L. V. Goodrich, J. W. Bare, J. M. Bonifas, et al.
1996 Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 272(5268):1668-1671.

Kageshita, T., C. V. Hamby, T. Ishihara, K. Matsumoto, T. Saida, and T. Ono
2001 Loss of beta-catenin expression associated with disease progression in malignant melanoma. The British journal of dermatology 145(2):210-216.

Kahn, M.
2014 Can we safely target the WNT pathway? Nature reviews. Drug discovery 13(7):513-532.

Kestler, H. A., and M. Kuhl
2008 From individual Wnt pathways towards a Wnt signalling network. Philos Trans R Soc Lond B Biol Sci 363(1495):1333-1347.

Kishino, T., T. Niwa, S. Yamashita, T. Takahashi, H. Nakazato, T. Nakajima, et al.
2016 Integrated analysis of DNA methylation and mutations in esophageal squamous cell carcinoma. Molecular carcinogenesis.

Lan, Y. J., H. Chen, J. Q. Chen, Q. H. Lei, M. Zheng, and Z. R. Shao
2014 Immunolocalization of vimentin, keratin 17, Ki-67, involucrin, beta-catenin and E-cadherin in cutaneous squamous cell carcinoma. Pathol Oncol Res 20(2):263-266.

Lee, A. Y., B. He, L. You, S. Dadfarmay, Z. Xu, J. Mazieres, et al.
2004 Expression of the secreted frizzled-related protein gene family is downregulated in human mesothelioma. Oncogene 23(39):6672-6676.

Lefort, K., A. Mandinova, P. Ostano, V. Kolev, V. Calpini, I. Kolfschoten, et al.
2007 Notch1 is a p53 target gene involved in human keratinocyte tumor suppression through negative regulation of ROCK1/2 and MRCKalpha kinases. Genes & development 21(5):562-577.

Li, Y. Y., G. J. Hanna, A. C. Laga, R. I. Haddad, J. H. Lorch, and P. S. Hammerman
2015 Genomic analysis of metastatic cutaneous squamous cell carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research 21(6):1447-1456.

Liang, J., X. Kang, Y. Halifu, X. Zeng, T. Jin, M. Zhang, et al.
2015 Secreted frizzled-related protein promotors are hypermethylated in cutaneous squamous carcinoma compared with normal epidermis. BMC Cancer 15(1):641.

Lim, X., and R. Nusse
2013 Wnt signaling in skin development, homeostasis, and disease. Cold Spring Harb Perspect Biol 5(2).

Lim, X., S. H. Tan, W. L. Koh, R. M. Chau, K. S. Yan, C. J. Kuo, et al.
2013 Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling. Science 342(6163):1226-1230.
Liu, J. B., F. L. Qiang, J. Dong, J. Cai, S. H. Zhou, M. X. Shi, et al.
2011 Plasma DNA methylation of Wnt antagonists predicts recurrence of esophageal squamous cell carcinoma. World J Gastroenterol 17(44):4917-4921.
Liu, J., S. Pan, M. H. Hsieh, N. Ng, F. Sun, T. Wang, et al.
2013 Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. Proceedings of the National Academy of Sciences of the United States of America 110(50):20224-20229.
Maelandsmo, G. M., R. Holm, J. M. Nesland, O. Fodstad, and V. A. Florenes
2003 Reduced beta-catenin expression in the cytoplasm of advanced-stage superficial spreading malignant melanoma. Clinical cancer research : an official journal of the American Association for Cancer Research 9(9):3383-3388.
Malanchi, I., H. Peinado, D. Kassen, T. Hussenet, D. Metzger, P. Chambon, et al.
2008 Cutaneous cancer stem cell maintenance is dependent on beta-catenin signalling. Nature 452(7187):650-653.
Mani, S. A., W. Guo, M. J. Liao, E. N. Eaton, A. Ayyanan, A. Y. Zhou, et al.
2008 The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133(4):704-715.
Margulis, A., W. Zhang, A. Alt-Holland, H. C. Crawford, N. E. Fusenig, and J. A. Garlick
2005 E-cadherin suppression accelerates squamous cell carcinoma progression in three-dimensional, human tissue constructs. Cancer research 65(5):1783-1791.
Marsit, C. J., M. D. McClean, C. S. Furniss, and K. T. Kelsey
2006 Epigenetic inactivation of the SFRP genes is associated with drinking, smoking and HPV in head and neck squamous cell carcinoma. International journal of cancer. Journal international du cancer 119(8):1761-1766.
Meng, Y., Q. G. Wang, J. X. Wang, S. T. Zhu, Y. Jiao, P. Li, et al.
2011 Epigenetic inactivation of the SFRP1 gene in esophageal squamous cell carcinoma. Dig Dis Sci 56(11):3195-3203.
Nicolas, M., A. Wolfer, K. Raj, J. A. Kummer, P. Mill, M. van Noort, et al.
2003 Notch1 functions as a tumor suppressor in mouse skin. Nature genetics 33(3):416-421.
Nitzki, F., A. Zibat, S. Konig, M. Wijgerde, A. Rosenberger, F. H. Brembeck, et al.
2010 Tumor stroma-derived Wnt5a induces differentiation of basal cell carcinoma of Ptch-mutant mice via CaMKII. Cancer research 70(7):2739-2748.
Okino, K., H. Nagai, M. Hatta, T. Nagahata, K. Yoneyama, Y. Ohta, et al.
2003 Up-regulation and overproduction of DVL-1, the human counterpart of the Drosophila dishevelled gene, in cervical squamous cell carcinoma. Oncol Rep 10(5):1219-1223.
Paluszczak, J., J. Sarbak, M. Kostrzewska-Poczekaj, K. Kiwerska, M. Jarmuz-Szymczak, R. Grenman, et al.

2015 The negative regulators of Wnt pathway-DACH1, DKK1, and WIF1 are methylated in oral and oropharyngeal cancer and WIF1 methylation predicts shorter survival. Tumour Biol 36(4):2855-2861.

Pannone, G., P. Bufo, A. Santoro, R. Franco, G. Aquino, F. Longo, et al.
2010 WNT pathway in oral cancer: epigenetic inactivation of WNT-inhibitors. Oncol Rep 24(4):1035-1041.

Papadavid, E., M. Pignatelli, S. Zakynthinos, T. Krausz, and A. C. Chu
2002 Abnormal immunoreactivity of the E-cadherin/catenin (alpha-, beta-, and gamma-) complex in premalignant and malignant non-melanocytic skin tumours. J Pathol 196(2):154-162.

Pickering, C. R., J. H. Zhou, J. J. Lee, J. A. Drummond, S. A. Peng, R. E. Saade, et al.
2014 Mutational landscape of aggressive cutaneous squamous cell carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research 20(24):6582-6592.

Popp, S., S. Waltering, C. Herbst, I. Moll, and P. Boukamp
2002 UV-B-type mutations and chromosomal imbalances indicate common pathways for the development of Merkel and skin squamous cell carcinomas. International journal of cancer. Journal international du cancer 99(3):352-360.

Pourreyron, C., L. Reilly, C. Proby, A. Panteleyev, C. Fleming, K. McLean, et al.
2012 Wnt5a is strongly expressed at the leading edge in non-melanoma skin cancer, forming active gradients, while canonical Wnt signalling is repressed. PloS one 7(2):e31827.

Prgomet, Z., L. Axelsson, P. Lindberg, and T. Andersson
2015 Migration and invasion of oral squamous carcinoma cells is promoted by WNT5A, a regulator of cancer progression. J Oral Pathol Med 44(10):776-784.

Proweller, A., L. Tu, J. J. Lepore, L. Cheng, M. M. Lu, J. Seykora, et al.
2006 Impaired notch signaling promotes de novo squamous cell carcinoma formation. Cancer research 66(15):7438-7444.

Ra, S. H., X. Li, and S. Binder
2011 Molecular discrimination of cutaneous squamous cell carcinoma from actinic keratosis and normal skin. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 24(7):963-973.

Saito, T., H. Mitomi, A. Imamhasan, T. Hayashi, K. Mitani, M. Takahashi, et al.
2014 Downregulation of sFRP-2 by epigenetic silencing activates the beta-catenin/Wnt signaling pathway in esophageal basaloid squamous cell carcinoma. Virchows Arch 464(2):135-143.

Salto-Tellez, M., B. K. Peh, K. Ito, S. H. Tan, P. Y. Chong, H. C. Han, et al.
2006 RUNX3 protein is overexpressed in human basal cell carcinomas. Oncogene 25(58):7646-7649.

Samuel, M. S., J. I. Lopez, E. J. McGhee, D. R. Croft, D. Strachan, P. Timpson, et al.
2011 Actomyosin-mediated cellular tension drives increased tissue stiffness and beta-catenin activation to induce epidermal hyperplasia and tumor growth. Cancer cell 19(6):776-791.

Sato, M.

2006 Upregulation of the Wnt/beta-catenin pathway induced by transforming growth factor-beta in hypertrophic scars and keloids. Acta Derm Venereol 86(4):300-307.

Sherwood, V.
2015 WNT Signaling: an Emerging Mediator of Cancer Cell Metabolism? Molecular and cellular biology 35(1):2-10.

Siegel, E. M., B. M. Riggs, A. L. Delmas, A. Koch, A. Hakam, and K. D. Brown
2015 Quantitative DNA methylation analysis of candidate genes in cervical cancer. PloS one 10(3):e0122495.

Sobel, K., M. Tham, H. J. Stark, H. Stammer, S. Pratzel-Wunder, J. R. Bickenbach, et al.
2015 Wnt-3a-activated human fibroblasts promote human keratinocyte proliferation and matrix destruction. International journal of cancer. Journal international du cancer 136(12):2786-2798.

Sogabe, Y., H. Suzuki, M. Toyota, K. Ogi, T. Imai, M. Nojima, et al.
2008 Epigenetic inactivation of SFRP genes in oral squamous cell carcinoma. International journal of oncology 32(6):1253-1261.

Song, Y., L. Li, Y. Ou, Z. Gao, E. Li, X. Li, et al.
2014 Identification of genomic alterations in oesophageal squamous cell cancer. Nature 509(7498):91-95.

South, A. P., K. J. Purdie, S. A. Watt, S. Haldenby, N. Y. den Breems, M. Dimon, et al.
2014 NOTCH1 mutations occur early during cutaneous squamous cell carcinogenesis. The Journal of investigative dermatology 134(10):2630-2638.

Sun, P., K. Watanabe, M. Fallahi, B. Lee, M. E. Afetian, C. Rheaume, et al.
2014 Pygo2 regulates beta-catenin-induced activation of hair follicle stem/progenitor cells and skin hyperplasia. Proceedings of the National Academy of Sciences of the United States of America 111(28):10215-10220.

Suzuki, H., D. N. Watkins, K. W. Jair, K. E. Schuebel, S. D. Markowitz, W. D. Chen, et al.
2004 Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. Nature genetics 36(4):417-422.

Takeda, H., S. Lyle, A. J. Lazar, C. C. Zouboulis, I. Smyth, and F. M. Watt
2006 Human sebaceous tumors harbor inactivating mutations in LEF1. Nat Med 12(4):395-397.

Tetsu, O., and F. McCormick
1999 Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature 398(6726):422-426.

Toll, A., E. Masferrer, M. E. Hernandez-Ruiz, C. Ferrandiz-Pulido, M. Yebenes, A. Jaka, et al.
2013 Epithelial to mesenchymal transition markers are associated with an increased metastatic risk in primary cutaneous squamous cell carcinomas but are attenuated in lymph node metastases. J Dermatol Sci 72(2):93-102.

van Amerongen, R., and R. Nusse
2009 Towards an integrated view of Wnt signaling in development. Development 136(19):3205-3214.

Watt, S. A., C. Pourreyron, K. Purdie, C. Hogan, C. L. Cole, N. Foster, et al.

2011 Integrative mRNA profiling comparing cultured primary cells with clinical samples reveals PLK1 and C20orf20 as therapeutic targets in cutaneous squamous cell carcinoma. Oncogene 30(46):4666-4677.
Wilson, S. I., A. Rydstrom, T. Trimborn, K. Willert, R. Nusse, T. M. Jessell, et al.
2001 The status of Wnt signalling regulates neural and epidermal fates in the chick embryo. Nature 411(6835):325-330.
Wolchok, J. D., H. Kluger, M. K. Callahan, M. A. Postow, N. A. Rizvi, A. M. Lesokhin, et al.
2013 Nivolumab plus ipilimumab in advanced melanoma. The New England journal of medicine 369(2):122-133.
Wong, S. C., S. F. Lo, K. C. Lee, J. W. Yam, J. K. Chan, and W. L. Wendy Hsiao
2002 Expression of frizzled-related protein and Wnt-signalling molecules in invasive human breast tumours. J Pathol 196(2):145-153.
Wu, N., J. Rollin, I. Masse, J. Lamartine, and X. Gidrol
2012 p63 regulates human keratinocyte proliferation via MYC-regulated gene network and differentiation commitment through cell adhesion-related gene network. The Journal of biological chemistry 287(8):5627-5638.
Xia, X., S. Qian, S. Soriano, Y. Wu, A. M. Fletcher, X. J. Wang, et al.
2001 Loss of presenilin 1 is associated with enhanced beta-catenin signaling and skin tumorigenesis. Proceedings of the National Academy of Sciences of the United States of America 98(19):10863-10868.
Yang, S. H., T. Andl, V. Grachtchouk, A. Wang, J. Liu, L. J. Syu, et al.
2008 Pathological responses to oncogenic Hedgehog signaling in skin are dependent on canonical Wnt/beta3-catenin signaling. Nature genetics 40(9):1130-1135.
Yang, S. H., S. L. Li, Z. M. Dong, and Q. C. Kan
2012 Epigenetic inactivation of Wnt inhibitory factor-1 in human esophageal squamous cell carcinoma. Oncol Res 20(2-3):123-130.
Youssef, K. K., G. Lapouge, K. Bouvree, S. Rorive, S. Brohee, O. Appelstein, et al.
2012 Adult interfollicular tumour-initiating cells are reprogrammed into an embryonic hair follicle progenitor-like fate during basal cell carcinoma initiation. Nature Cell Biology 14(12):1282-1294.
Zhang, X., J. P. Gaspard, and D. C. Chung
2001 Regulation of vascular endothelial growth factor by the Wnt and K-ras pathways in colonic neoplasia. Cancer research 61(16):6050-6054.
Zhang, Y., P. Tomann, T. Andl, N. M. Gallant, J. Huelsken, B. Jerchow, et al.
2009 Reciprocal requirements for EDA/EDAR/NF-kappaB and Wnt/beta-catenin signaling pathways in hair follicle induction. Developmental cell 17(1):49-61.
Zhu, A. J., and F. M. Watt
1999 beta-catenin signalling modulates proliferative potential of human epidermal keratinocytes independently of intercellular adhesion. Development 126(10):2285-2298.
Zito, G., I. Saotome, Z. Liu, E. G. Ferro, T. Y. Sun, D. X. Nguyen, et al.
2014 Spontaneous tumour regression in keratoacanthomas is driven by Wnt/retinoic acid signalling cross-talk. Nat Commun 5:3543.
Zou, H., J. R. Molina, J. J. Harrington, N. K. Osborn, K. K. Klatt, Y. Romero, et al.

2005 Aberrant methylation of secreted frizzled-related protein genes in esophageal adenocarcinoma and Barrett's esophagus. International journal of cancer. Journal international du cancer 116(4):584-591.