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

Victoria Sherwood[1] and Irene M. Leigh[1]

The molecular mechanisms underlying cutaneous squamous cell carcinoma are less well established than those for other common skin cancers, but recent evidence has highlighted a potentially critical role for WNT signaling in both the development and progression of cutaneous squamous cell carcinoma. WNT pathways are aberrantly regulated in multiple tumor 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 cutaneous squamous cell carcinoma and discuss if WNT inhibition represents a realistic therapeutic strategy for the future.

Journal of Investigative Dermatology (2016) **136,** 1760–1767; doi:10.1016/j.jid.2016.05.108

INTRODUCTION

Patients developing metastatic cutaneous squamous cell carcinoma (cSCC) (5–10% of patients with cSCC), have a poor outcome, with a 25–50% 5-year survival rate (Epstein, 1984). Therapies targeted to immune checkpoint blockade or to pro-proliferative signaling pathways, such as the mitogen-activated protein kinase pathway, are providing 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 tumors based on in-depth molecular understanding of the disease. To progress this aim, expression array profiling of cSCC tumors 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 nonmelanoma 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. Here we examine this evidence and investigate the possibility of WNT inhibitors as a therapeutic opportunity for disease management of cSCC. To do this we will first summarize what is known about WNT signaling in cancer, briefly discuss the crucial role it plays in keratinocyte biology, and then focus on its activity in NMSCs, 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 multiprotein complex, termed the destruction complex (Dale, 1998), composed of scaffold proteins (axin and APC), kinases (glycogen synthase kinase-3β and casein kinase-1), and other enzymes. 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, Disheveled (DVL). DVL antagonizes destruction complex activity, causing β-catenin accumulation to allow nuclear translocation where, in consort with the T-cell factor/lymphoid enhancer factor 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 tumors, 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

[1]*Division of Cancer Research, School of Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK*

Correspondence: Victoria Sherwood, Division of Cancer Research, Jacqui Wood Cancer Centre, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, UK. E-mail: v.sherwood@dundee.ac.uk

Abbreviations: BCC, basal cell carcinoma; cSCC, cutaneous squamous cell carcinoma; DVL, Disheveled; EMT, epithelial-to-mesenchymal transition; FZD, Frizzled; HF, hair follicle; IFE, interfollicular epidermis; NMSC, nonmelanoma skin cancer; ROCK, Rho-associated protein kinase; SC, stem cell; SFRP, secreted Frizzled-related protein

Received 2 December 2015; revised 10 May 2016; accepted 21 May 2016; corrected proof published online 19 July 2016

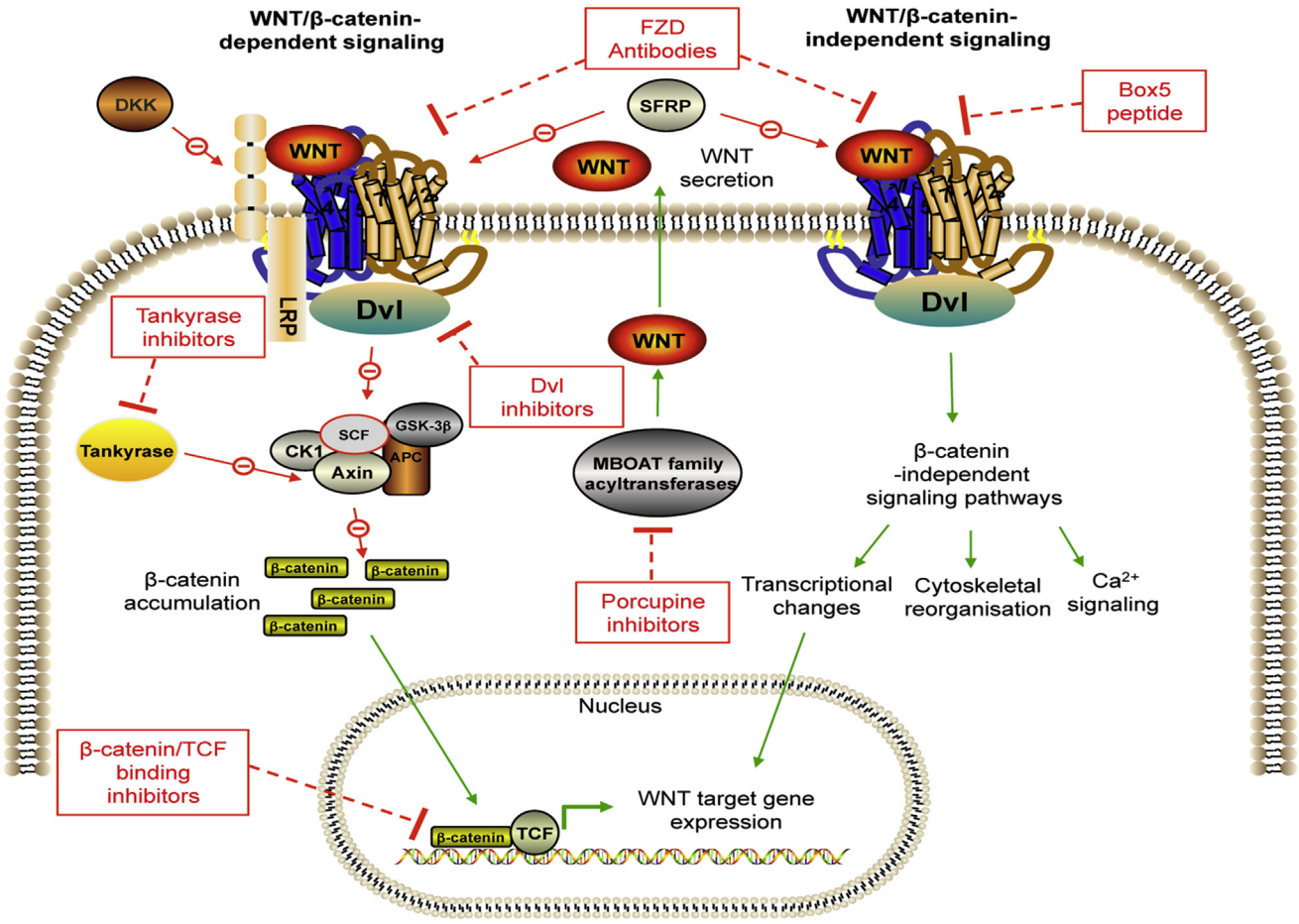

Figure 1. WNT signaling and associated inhibitors. WNT/β-catenin–dependent signaling (left): in the absence of WNT binding to the FZD/LRP5/6 receptors, the signaling pool of β-catenin is maintained at low levels, mediated by the multiprotein destruction complex (comprising APC, Axin, GSK3β, CK1, and the multiprotein E3 ubiquitin ligase complex SCF). Upon WNT-receptor interaction, DVL inactivates the destruction complex, stabilizing 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 stabilize 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/b-catenin–independent signaling ligand)especific inhibitory peptide Box5. Secreted WNT antagonists include Dickkopf proteins (DKKs) and secreted FZD-related proteins (SFRPs).

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 *APC* mutations were found in most colorectal cancers (Groden et al., 1991). Mutations in a number of WNT pathway genes have now been associated with a variety of tumor 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 tumors, showing that hyperactivation 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 summarized in Figure 1.

However, the concept that WNT signaling is always pro-oncogenic is too simplistic, because increased activity does not always 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 have tumor 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 tumors (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; it is involved in specification of the embryonic ectoderm to form the skin epithelium and in 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