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Epigenetic regulation of the Hedgehog and Wnt pathways in cancer

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ABSTRACT

The Hedgehog (Hh) and wingless-Int1 (Wnt) pathways are important for tissue patterning in the developing embryo. In adult tissue, both pathways are typically dormant but are activated under certain conditions such as tissue damage. Aberrant activation of these pathways by mutations in key pathway regulators contributes to the genesis and progression of several cancer types. In addition, the impact of epigenetic regulation of the Hh and Wnt pathways on cancer is becoming increasingly clear. In this review, current knowledge on the epigenetic control of Hh and Wnt and the impact on tumor formation will be discussed. First, the role of epigenetic control on ligand production will be discussed, followed by the epigenetic regulation of the extra– and intracellular pathway members. Furthermore, the epigenetic control of pathway target genes will be highlighted. Lastly, an overview of current therapeutic strategies to target aberrant epigenetic control of the Hh and Wnt pathways is provided.

1. Introduction

Morphogens are molecules important for inducing cell fates during embryogenesis (Rogers and Schier, 2011). A morphogen signal produced by a producing group of cells typically forms a gradient in a larger field of surrounding cells. Receiving cells transduce the morphogen signal through dedicated pathways resulting in the expression of target genes in a morphogen concentration-dependent manner. This leads to the formation of distinct cell types and tissue patterns (Heemskerk and DiNardo, 1994; van den Heuvel et al., 1989; Briscoe and Thérond, 2013). A limited number of morphogen families are known and these include Notch, Netrin 1 (NTN1), transforming growth factor β (TGF-β), bone morphogenetic protein (BMP), Hh, and Wnt (Heemskerk and DiNardo, 1994; Ferguson and Anderson, 1992; Zecca et al., 1996; Artavanis-Tsakonas and Simpson, 1991; Mehlen et al., 2011). As an example of the many events that morphogens orchestrate, the Hh pathway controls digit patterning in developing limbs and organization of the central nervous system (Jiang and Hui, 2008). The Wnt signaling pathway controls body axis formation and the organization of several important organs such as the lungs and kidneys (Grigoryan et al., 2008). In adult tissue, the Hh and Wnt pathways are shown to regulate tissue homeostasis and repair. However, aberrant pathway activation may result in cancer, and this holds true in particular for the Hh and Wnt pathways (Beachy et al., 2004; Taipale and Beachy, 2001; Takebe et al., 2015; Duchartre et al., 2016; Wu et al., 2013). Loss-of-function and gain-of-function alterations lead to ligand-independent activation of the Hh and Wnt pathways and typically result in uncontrolled cell division.

Aberrant signaling in both pathways contributes to several cancer types. Activation of the Hh pathway is primarily observed in basal cell carcinomas (BCC) and medulloblastoma (MB), while Wnt activation is observed in colorectal cancer (CRC) and breast cancer (BC) (Briscoe and Thérond, 2013; Taipale and Beachy, 2001; Zhan et al., 2016; Berman et al., 2002; Johnson et al., 1996; Xie et al., 1998; Morin et al., 1996; Lin et al., 2000; Taylor et al., 2002). Traditionally, research has focused on DNA mutations as a cause of aberrant signaling. This resulted in the identification of several tumor-suppressive and (proto)oncogenic pathway components, some of which can be efficiently targeted (Gonnissen et al., 2016). However, Hh and Wnt driven tumors may eventually develop resistance against targeted compounds (Zahreddine et al., 2014; Yauch et al., 2009; Takebe et al., 2011). Recently, a shift of focus towards the epigenetic causes of aberrant activation has taken place, which may provide additional and possibly more effective targets for therapy (Di Magno et al., 2015; Serman et al., 2014).

Epigenetics are heritable changes in gene expression, occurring without changing the DNA sequence. Grossly speaking, three distinct epigenetic mechanisms are known: DNA methylation, histone modification, and interference by noncoding strands of RNA (ncR) of different lengths, such as micro-RNA (miRNA or miR) and long non-coding RNA (lncR) (Dawson and Kouzarides, 2012). Several such epigenetic factors that act on the Hh and Wnt signaling pathways have been associated with cancer initiation and progression. The aim of this review

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is to provide a comprehensive overview of the epigenetic mechanisms that affect the Hh and Wnt signaling pathways in cancer. Epigenetic control of the production of Hh and Wnt ligands will be explored, followed by a discussion of the extra- and intracellular components of both pathways in the framework of associated tumor types. The changes in epigenetic control of pathway target genes will be described, as well as possible treatment options that target this.

2. Epigenetic regulation of the Hedgehog and Wnt pathways and implications for respective typically-associated tumors

2.1. Ligand production

Production of ligands in secreting cells is a complicated, but roughly similar process for both the Hh and Wnt pathways. Ligands are produced as precursors in the endoplasmic reticulum (ER) (Kadowaki et al., 1996; Chen et al., 2011). Inside of the ER, the Hh ligands are modified by the addition of cholesterol group following autoproteolytic cleavage of the precursors protein, followed by the addition of a palmitate moiety (Chamoun et al., 2001; Porter et al., 1996). The ligands are transported to the plasma membrane, where the added groups promote retention, and prevent diffusion (Tukachinsky et al., 2012; Burke et al., 1999). Following translocation by dedicated mechanisms, Hh ligands are released from the plasma membrane. The ligands may for instance be secreted as monomers with the help of transmembrane proteins Dispatched/Scube (DISP/SCUBE) (Tukachinsky et al., 2012), ligand oligomers can associate with lipoproteins to form lipoprotein particles (Panáková et al., 2005), and ligands may be released on exosomes (Liégeois et al., 2006). In addition, Hh monomers can self-associate and form soluble particles (Tukachinsky et al., 2012). Wnt ligand production and secretion follows a quite similar path, however with the aid of different proteins. While inside of the ER, Wnt ligands also undergo a palmitate modification (Zhai et al., 2004). The ligands are then translocated to and retained at the plasma membrane (Bänziger et al., 2006; Bartscherer et al., 2006). Wnt ligands may be secreted as monomers aided by WLS/EVI and cofactor secreted Wnt-interacting molecule (Swim) (Bänziger et al., 2006; Bartscherer et al., 2006; Mulligan et al., 2012). Moreover, Wnt ligands may be secreted in association with lipoproteins or on exosomes3 (Panáková et al., 2005; Neumann et al., 2009; Gross et al., 2012).

Several tumor types depend on aberrant Hh and Wnt ligand production, and Table 1 summarizes the epigenetic mechanisms that control this. Hh and Wnt ligands can act on the tumor cells themselves (autocrine signaling) or the surrounding non-transformed cells (paracrine signaling) depending on the tumor type (Briscoe and Thérond, 2013; Zhan et al., 2016). Autocrine Hh ligand-dependent activation has been observed in gastric cancers (GC), while paracrine signaling has been shown in pancreatic cancers (PC) (Yauch et al., 2008; Berman et al., 2003). Autocrine Wnt ligand activation has been proposed to drive the invasive behavior of BC cells through exosomes (Luga et al., 2012). Additionally, Wnt ligand production in circulating tumor cells may assist in the distal spread of PC (Yu et al., 2012).

While these studies provide evidence for ligand-dependent oncogenic signaling, only limited research has been done on the epigenetic factors that drive ligand production. Nuclear factor-κB (NF-κB) activity is known to drive expression of the main Hh paralog, Sonic Hedgehog (SHH) (Nakashima et al., 2006). Hypomethylation of the promoter region of SHH containing the binding site for NF-κB has been linked to upregulation of SHH in GC and BC. Hypomethylation is likely to cause a higher affinity for NF-κB, thereby resulting in increased SHH production (Wang et al., 2006; Duan et al., 2015; Cui et al., 2010). However, aberrant SHH ligand production can also occur when its promoter is heavily methylated, as shown *in vitro* in BC cell lines (ten Haaf et al., 2011). These contradictory results may be explained by the two transcription start sites (TSS) in the promoter region of the SHH gene. Promoter methylation of the predominant TSS leads to a shift of the transcription machinery, resulting in an elongated version of the SHH ligand precursor. Additionally, the SHH gene can be further epigenetically regulated by histone modifications (ten Haaf et al., 2011).

Promoter methylation may also affect the production of Wnt ligands; however, this is confounded by the specific Wnt paralogs that exist and that are differentially regulated. The WNT5A, WNT9A and WNT10 B promoters are frequently hypermethylated in CRC cell lines and tumors, suggesting a tumor suppressive action of these ligands in cancer (Ying et al., 2008; Shu et al., 2006; Yoshikawa et al., 2007; Galamb et al., 2016). Furthermore, WNT5A is silenced by histone modification in CRC and miR-374a in BC (Cai et al., 2013; Li and Chen, 2012). Additionally, WNT2 is upregulated in CRC due to loss of repressive histone marks (Jung et al., 2015). However, the downstream effects of these modifications and resulting Wnt ligand levels are still under debate and may depend on cancer type studied, the available receptors, and other factors (Yoshikawa et al., 2007; Asem et al., 2016). Although ligand-dependent signaling plays a clear role in many cancer types, and epigenetic regulation of ligand production has been observed, the main part of this review will focus on epigenetic regulation of ligand-independent activation of the Hh and Wnt signaling pathways (Figs. 1 and 2).

2.2. Extracellular signaling

Both (canonical) Hh and Wnt signaling pathways rely on membrane receptors and co-receptors to perceive ligand and transduce the signal to the nucleus. Co-receptors can either bind ligands directly or affect ligand binding to other receptors. In addition to membrane bound receptors, cells can secrete proteins that sequester ligands, typically preventing pathway activation. An overview of epigenetic regulation of such pathway members is presented in Table 1.

The primary receptor for Hh ligands is Patched-1 (PTCH1), which inhibits pathway activation in the absence of Hh ligand (Marigo et al., 1996; van den Heuvel and Ingham, 1996). In the presence of ligand, PTCH1 is internalized and degraded, followed by translocation of Smoothened (SMO) to the cell surface on the primary cilium. SMO is then able to activate the glioma-associated oncogene (GLI) transcription factors (Corbit et al., 2005; Yang et al., 2012). Four different isoforms of PTCH1, PTCH-1 PTCH-1A, PTCH–1 B and PTCH-1C, have been discovered. These isoforms are generated by alternative use of first exons, with a distinct CpG island in the promoters for PTCH-1, PTCH-1A and PTCH–1 B and a distinct CpG island in the promoter for PTCH-1C (Kogerman et al., 2002; Nagao et al., 2005; Shimokawa et al., 2004). The promoters for isoforms PTCH–1 B and PTCH-1C contain the GLI binding sites of the PTCH1 gene, and are therefore targets of the Hh signaling pathway itself. Activation of the Hh cascade leads to upregulation of PTCH–1 B and PTCH-1C isoforms, thus resulting in a negative feedback loop (Shimokawa et al., 2007). Loss-of-function of the PTCH1 gene relieves inhibition of SMO, and consequently leads to ligand-independent activation of the Hh pathway. Given its important role in regulating the Hh pathway and thereby directly preventing uncontrolled cell division, PTCH1 is considered a tumor suppressor and is often mutated in Hh pathway-driven cancers. Its downregulation through epigenetic mechanisms would be a likely oncogenic event, but early research did not identify epigenetic silencing of the PTCH1 gene in MB and BCC, even when near-absent PTCH1 mRNA expression was measured (Pritchard and Olson, 2008; Cretnik et al., 2007). However, these studies focused on the PTCH–1 B isoform because of the high CG content of its promoter. More recent research on the methylation status of the PTCH1 gene did find promoter methylation for the PTCH-1C isoform (Diede et al., 2010). Furthermore, one study using more sensitive methods for the detection of methylation did show low levels of PTCH–1 B promoter methylation in BCC (Heitzer et al., 2010). No clear consensus has been reached on the contributions of the two isoforms yet. However, as both isoforms are differentially expressed during embryogenesis (Shimokawa et al., 2007), this may also account for