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Non-canonical WNT5A signaling up-regulates the expression of the tumor suppressor 15-PGDH and induces differentiation of colon cancer cells

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ABSTRACT

The tumor suppressor 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is the key enzyme in prostaglandin E_2 catabolism and is down-regulated in colorectal cancer (CRC) tissue. Canonical Wnt signaling is frequently elevated in colon cancers and has been shown to down-regulate 15-PGDH expression. Therefore, we have in the current study investigated if the non-canonical ligand WNT5A relates to increased expression of 15-PGDH in colon cancer cells. In the same cohort of patients, we demonstrated a parallel and significant loss of 15-PGDH and WNT5A protein expression in CRC tissues compared with matched normal colon tissues. Furthermore, patients with low 15-PGDH/WNT5A expression in their tumors showed reduced survival compared with patients with high 15-PGDH/WNT5A expression. To investigate if WNT5A signaling directly affects 15-PGDH expression, we performed *in vitro* analyses of colon cancer cells (HT-29 and Caco-2). Both cell lines, when treated with recombinant WNT5A (rWNT5A) or Foxy-5, a WNT5A-mimicking peptide, responded by increasing their expression of 15-PGDH mRNA and protein. Our investigations showed that rWNT5A and Foxy-5 induced this increased expression of 15-PGDH through reduced β-catenin signaling as well as increased JNK/AP-1 signaling in colon cancer cells. WNT5A signaling also induced increased 15-PGDH expression in a breast cancer cell line both *in vitro* and *in vivo*. In agreement, WNT5A signaling also increased the expression of the differentiation markers sucrose-isomaltase and mucin-2 in colon cancer cells. Our results show that WNT5A signaling regulates 15-PGDH expression, thus uncovering a novel mechanism by which WNT5A acts as a tumor suppressor and suggests that increased 15-PGDH expression could be used as an indicator of a positive response to Foxy-5 in patients treated with this WNT5A agonist.

Abbreviations: rWNT-5a, recombinant WNT-5A; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; JNK, c-Jun N-terminal kinase.

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1. Introduction

Every year, more than one million new cases of colorectal cancer (CRC) are diagnosed worldwide (Tenesa and Dunlop, 2009). CRC is the third most common cancer in both men and women and the third most frequent cause of cancer-related deaths (Siegel et al., 2014). Etiological factors include the consumption of large quantities of alcohol and red meat, diets low in fiber and high in fat, and a lack of physical activity (Huxley et al., 2009). Inflammation, particularly protracted or chronic inflammation, including inflammatory bowel disease (IBD), is commonly associated with CRC (Triantafillidis et al., 2009). The development of CRC involves the accumulation of genetic and epigenetic alterations that cause the transformation of normal colonic epithelium into colon adenocarcinoma (Grady and Markowitz, 2002). These changes lead to the activation of various oncogenic pathways and the inactivation of tumor suppressor pathways (Markowitz and Bertagnolli, 2009). The up-regulation of cyclooxygenase-2 (COX-2), which occurs in the majority of colorectal tumors, plays a crucial role in colon cancer development (Sinicrope and Gill, 2004). The deregulation of COX-2 expression leads to increases in the levels of inflammatory lipids, including prostaglandins, particularly prostaglandin E2 (PGE_2) (Brown and DuBois, 2005). COX-2-derived PGE_2 is known to induce proliferation, neovascularization, cell death inhibition and motility of tumor cells (Turini and DuBois, 2002) and more recently to expand the number of colon cancer stem cells (Wang et al., 2015). 15-hydroxyprostaglandin dehydrogenase (15-PGDH), an important enzyme responsible for the degradation of PGE_2 (Ensor and Tai, 1995), has been shown to act as a colorectal tumor suppressor (Backlund et al., 2005; Yan et al., 2004). The loss of 15-PGDH is associated with CRC (Backlund et al., 2005), and its decreased expression has also been implicated in other cancers, including lung cancer, bladder cancer, pancreatic cancer and gastric cancer (Pham et al., 2010; Song et al., 2011; Tai et al., 2007; Tseng-Rogenski et al., 2010). Similarly, in the majority of breast cancer subtypes, the expression of 15-PGDH is reduced or lost, although it has been suggested that 15-PGDH might serve as a marker for the rare apocrine molecular subtype of breast cancer (Celis et al., 2008) possibly related to the finding that 15-PGDH tumor expression in a small sub-population of breast cancer patients is associated with a poor prognosis (Lehtinen et al., 2012).

Canonical Wnt/β-catenin pathway signaling is one of the crucial signaling pathways controlling the proliferation, differentiation and morphogenesis of cells during development, and several mutations in this pathway have been positively linked to CRC (White et al., 2012). In addition to canonical Wnt signaling, there also exists a non-canonical arm of Wnt signaling, and one of the most extensively studied non-canonical ligands is WNT5A (Kikuchi et al., 2012). We previously showed that WNT5A significantly reduces the migration of colon and breast cancer cells (Dejmek et al., 2005a; Jonsson and Andersson, 2001). Immunohistochemical data from both colon and breast cancer patients demonstrated that high WNT5A expression is a good prognostic marker (Dejmek et al., 2005a, 2005b). Similar findings have also been presented for other cancers, including prostate cancer, lymphoma, thyroid carcinoma and neuroblastoma (Blanc et al., 2005; Kremenevskaja et al., 2005; Liang et al., 2003; Syed Khaja et al., 2011). Foxy-5, a formylated hexapeptide derived from WNT5A, has been shown to mimic the inhibitory effect of WNT5A on cancer cell migration and tumor metastasis (Säfholm et al., 2006, 2008), making it an attractive molecule for future anti-metastatic cancer therapy. Foxy-5 has been tested in a completed phase-I clinical trial of patients with breast, colon and prostate cancer (www.clinicalTrials.gov; NCT02020291).

Several studies have shown that the expression of 15-PGDH is associated with the suppression of colon carcinogenesis and invasiveness (Choi et al., 2014; Li et al., 2008; Myung et al., 2006). This has resulted in attempts to re-express 15-PGDH in this type of cancer as a strategic area of therapeutic research. In this context, canonical Wnt/β-catenin signaling has been shown to suppress 15-PGDH expression in colorectal cancer cells (Smartt et al., 2012b). Based on demonstrations in different studies that non-canonical WNT5A signaling has the ability to oppose canonical Wnt/β-catenin stabilization in colon cancer cells (Cheng et al., 2014; Topol et al., 2003), we decided to investigate whether the WNT5A ligand has the ability to regulate the expression of 15-PGDH, thus mechanistically linking these two tumor suppressors.

The present results reveal for the first time that WNT5A signaling, induced by either recombinant WNT5A or the WNT5A-mimicking peptide Foxy-5, can positively regulate the expression of 15-PGDH mRNA and protein in colon cancer cells. These data reveal a novel mechanism by which WNT5A acts as a tumor suppressor and indicate that increased 15-PGDH expression might serve as a marker of a positive response to Foxy-5 in patients treated with this WNT5A agonist.

2. Materials and methods

2.1. Antibodies and reagents

The antibodies used were as follows: rabbit polyclonal against 15-PGDH (dilution 1:5000 for western blotting and dilution 1:500 for immunohistochemistry; Novus Biologicals, Cambridge, UK), goat polyclonal against WNT5A (dilution 1:200 for western blotting and immunohistochemistry; R&D Systems, Inc. Minneapolis, MN, USA), mouse monoclonal against phospho-JNK (p-JNK dilution 1:1000) or JNK (dilution 1:1000; Santa Cruz Biotechnology Inc. CA, USA). The p-JNK antibody is raised against an epitope from JNK1, however it recognizes both JNK1 and JNK2 and therefore we use JNK when referring to our results on this kinase. Rabbit polyclonal against sucrase-isomaltase (SI) (dilution 1:1000; Sigma Life Science, St. Louis, MO, USA), mouse monoclonal against β-catenin (dilution 1:500 for immunofluorescence and 1:1000 for western blotting; BD Transduction Laboratories, Franklin Lakes, NJ, USA), Rabbit monoclonal against non-phospho active β-catenin (Ser33/37/Thr41, D13A1; dilution 1:1000), rabbit monoclonal against phospho-c-JUN/AP-1 or c-JUN/AP-1 (both diluted 1:1000; Cell Signaling Technology, Danvers, MA, USA). Rabbit monoclonal against c-Myc (dilution 1:1000;

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