MUC4 is negatively regulated through the Wnt/β-catenin pathway via the Notch effector Hath1 in colorectal cancer

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Keywords: MUC4, Wnt, colorectal cancer, β-catenin, Hath1

***Received**: May 16, 2016* ***Accepted**: June 18, 2016* ***Published**: June 23, 2016*

ABSTRACT

MUC4 is a transmembrane mucin lining the normal colonic epithelium. The aberrant/*de novo* over-expression of MUC4 is well documented in malignancies of the pancreas, ovary and breast. However, studies have reported the loss of MUC4 expression in the majority of colorectal cancers (CRCs). A *MUC4* promoter analysis showed the presence of three putative TCF/LEF sites, implying a possible regulation by the Wnt/β-catenin pathway, which has been shown to drive CRC progression. Thus, the objective of our study was to determine whether *MUC4* is regulated by β-catenin in CRC. We first knocked down (KD) β-catenin in three CRC cell lines; LS180, HCT-8 and HCT116, which resulted in increased *MUC4* transcript and MUC4 protein. Additionally, the overexpression of stabilized mutant β-catenin in LS180 and HCT-8 resulted in a decrease in MUC4 expression. Immunohistochemistry (IHC) of mouse colon tissue harboring tubular adenomas and high grade dysplasia showed dramatically reduced Muc4 in lesions relative to adjacent normal tissue, with increased cytosolic/nuclear β-catenin. Luciferase assays with the complete *MUC4* promoter construct p3778 showed increased *MUC4* promoter luciferase activity in the absence of β-catenin (KD). Mutation of all three putative TCF/LEF sites showed that *MUC4* promoter luciferase activity was increased relative to the un-mutated promoter. Interestingly, it was observed that MUC4 expressing CRC cell lines also expressed high levels of *Hath1*, a transcription factor repressed by both active Wnt/β-catenin and Notch signaling. The KD of β-catenin and/or treatment with a Notch γ-secretase inhibitor, Dibenzazepine (DBZ) resulted in increased *Hath1* and MUC4 in LS180, HCT-8 and HCT116. Furthermore, overexpression of Hath1 in HCT-8 and LS180 caused increased *MUC4* transcript and MUC4 protein. Taken together, our results indicate that the Wnt/β-catenin pathway suppresses the Notch pathway effector Hath1, resulting in reduced *MUC4* in CRC.

INTRODUCTION

Colorectal cancer (CRC) is the third leading cause of cancer deaths in the United States, accounting for 49,700 estimated total deaths in the year 2015 alone [1]. CRC is characterized by the mutational inactivation of tumor suppressor genes such as Adenomatous polyposis coli gene (APC), p53 and components of the TGF-β pathway as well as activation of oncogenes such as *KRAS* [2]. Most frequently, tumors possess mutations in the APC gene, causing the activation of the canonical Wnt pathway [2]. A small subset of patients possess activating