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The canonical Wnt pathway regulates the metastasis-promoting mucin MUC4 in pancreatic ductal adenocarcinoma

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ABSTRACT

Aberrant Wnt signaling frequently occurs in pancreatic cancer (PC) and contributes to disease progression/metastases. Likewise, the transmembrane-mucin MUC4 is expressed *de novo* in early pancreatic intraepithelial neoplasia (PanINs) and incrementally increases with PC progression, contributing to metastasis. To determine the mechanism of MUC4 upregulation in PC, we examined factors deregulated in early PC progression, such as Wnt/β-catenin signaling. *MUC4* promoter analysis revealed the presence of three putative TCF/LEF-binding sites, leading us to hypothesize that *MUC4* can be regulated by β-catenin. Immunohistochemical (IHC) analysis of rapid autopsy PC tissues showed a correlation between MUC4 and cytosolic/nuclear β-catenin expression. Knock down (KD) of β-catenin in CD18/HPAF and T3M4 cell lines resulted in decreased *MUC4* transcript and protein. Three *MUC4* promoter luciferase constructs, p3778, p3000, and p2700, were generated. The construct p3778, encompassing the entire *MUC4* promoter, elicited increased luciferase activity in the presence of stabilized β-catenin. Mutation of the TCF/LEF site closest to the transcription start site (i.e., −2629/−2612) and furthest from the start site (i.e., −3425/−3408) reduced *MUC4* promoter luciferase activity. Transfection with dominant negative TCF4 decreased MUC4 transcript and protein levels. Chromatin immunoprecipitation confirmed enrichment of β-catenin on −2629/−2612 and −3425/−3408 of the *MUC4* promoter in CD18/HPAF. Functionally, CD18/HPAF and T3M4 β-catenin KD cells showed decreased migration and decreased Vimentin, N-cadherin, and pERK1/2 expression. Tumorigenicity studies in athymic nude mice showed CD18/HPAF β-catenin KD cells significantly reduced primary tumor sizes and metastases compared to scrambled control cells. We show for the first time that β-catenin directly governs MUC4 in PC.

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Abbreviations: KD, knock down; PanIN, Pancreatic intraepithelial neoplasia; PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; TMA, tissue microarray; CM, Conditioned medium.

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