Functional Evaluation of Smad4 Disruption in Mouse Intestinal Adenoma

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Abstract

Human colorectal cancer (CRC) is one of the most common types of cancer. Aberrant activation of the Wnt pathway, typically via *adenomatous polyposis coli (Apc)* loss of function, acts as the initiating event for adenoma formation. However, alterations in additional pathways are associated with progression towards CRC. For example, 10-15% of carcinomas downregulate the transforming growth factor-β (TGF-β) pathway due to SMAD4 mutations in either the MH1 domain, the linker or the MH2 domain. The growth advantages of Smad4 mutations and their contribution to the switch from a tumour suppressor effect of TGF-β to a tumour promoter are as yet poorly understood. The aim of this thesis was to evaluate the functional consequences of Smad4 loss of function on intestinal tumorigenesis in the context of active Wnt signalling in mouse conditional models of human CRC.

Apc$^{fl/fl}$*Smad4*$^{fl/fl}$*Lgr5-EGF-IRES-CreERT2* (*Smad4*$^{fl/fl}$) mouse models were generated by breeding, and tamoxifen mediated Cre-activation allowed conditional deletions to be made in the intestinal stem cell compartment. The functional consequences of Smad4 MH1 loss of function on adenoma growth were evaluated using intestinal adenoma culture. The effects of TGF-β1 on Smad4 disrupted organoids were also evaluated. Comprehensive gene expression was then performed to evaluate changes in transcriptome profiling according to Smad4 status in the presence or absence of TGF-β1.

Mice that had both *Apc* and *Smad4* genes disrupted in the Lgr5 compartment showed enhanced survival, reduced small intestinal adenoma burden, and large caecal tumours. Dysplastic histology with signs of inflammation were observed within tumours. *In vitro*, *Smad4*$^{fl/fl}$ adenomas displayed slow growth that was rescued with the Rock inhibitor, Y-27632. Gene set enrichment analysis (GSEA) of RNA-Seq showed that these adenomas might be autoregulating the TGF-β pathway by upregulating Tgfβ1 expression, differentially regulating genes associated with differentiation, inflammation, in addition to enhancing the Wnt pathway by upregulating β-catenin. The upregulation of Muc2 expression in these adenomas was validated from *Smad4*$^{fl/fl}$ tissue sections. Gene ontology analysis suggested that Smad4 disruption affects the cytostatic function of TGF- by differentially regulating genes involved in the cell cycle and proliferation. In *Smad4*$^{fl/fl}$ adenomas, resistance to TGF-β tumour suppressor effect was confirmed by a higher IC_{50}, higher fraction of proliferative cells, *c-Myc* upregulation, and *Survivin* modulation following TGF-β1 treatment. Smad4 disruption had no effect on epithelial-mesenchymal transition under the concentration of TGF-β1 used but was associated with enhanced migration in Matrigel.

In conclusion, Smad4 disruption in intestinal adenomas affects control of the tumour microenvironment and creates suitable conditions for inflammation. It alters tumour sensitivity to TGF-β1 and thus interferes with the tumour suppressor and tumour promoter effects of the TGF-β pathway. Further validation is required to determine whether the observed phenotypes are dependent on Smad2/3 transcriptional activity, concentration of TGF-β1 used, and can be rescued with Smad4 complementation.

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