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Inactivation of *Itf2* promotes intestinal tumorigenesis in $Apc^{Min/+}$ mice

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ABSTRACT

Deregulation of Wnt/β-catenin signaling following inactivation of the adenomatous polyposis coli (*APC*) tumor suppressor gene is frequently found in colorectal cancer. We have previously shown that levels of ITF-2B, encoded by the β-catenin target gene *ITF2* that is located on the tumor suppressor gene locus 18q21, are increased in colonic adenomas with deregulated β-catenin activity. However, during tumor progression ITF-2B levels are reduced, suggesting that ITF-2B interferes with tumor development. To investigate the role of *ITF2* in intestinal tumorigenesis, we specifically inactivated *Itf2* in the intestinal epithelium of $Apc^{Min/+}$ mice. We found that genetic disruption of *Itf2* on the $Apc^{Min/+}$ background results in earlier death and a significant increase in tumor number and size in the small intestine. Based on these data *Itf2* acts as a tumor suppressor gene of the intestinal tract that inhibits tumor initiation and growth.

1. Introduction

Colorectal cancer is a genetic disease that develops from adenomatous precursor lesions by the accumulation of multiple independent somatic alterations in oncogenes and tumor suppressor genes [1]. Inactivating mutations of the *APC* tumor suppressor gene are viewed as an early event in up to 80% of human sporadic colorectal cancers and have been implicated in the development of hundreds of adenomas mainly in the colon in hereditary familial adenomatous polyposis (FAP) [2–4]. Loss of APC function leads to deregulation of the Wnt/β-catenin signaling cascade, resulting in stabilization and nuclear translocation of the protein β-catenin (reviewed in Ref. [1]). Subsequently, β-catenin modulates the transcription of its target genes. The gene *ITF2* alias *TCF4* encoding the basic helix-loop-helix protein (bHLH) and transcription factor ITF-2B has been identified as a β-catenin target gene [5]. Previously, we have demonstrated that deregulated activity of the protein β-catenin induces the transcription of the *ITF2* gene in colonic adenomas. However, during tumor progression, ITF-2B protein levels are frequently reduced due to loss of heterozygosity on chromosome 18q as well as deacetylation of the *ITF2* promoter, suggesting that loss of *ITF2* function is necessary for tumor development [6].

The $Apc^{Min/+}$ mouse is a widely used mouse model for the study of colorectal carcinogenesis. $Apc^{Min/+}$ mice carry an inactivating mutation in one allele of the *Apc* tumor suppressor gene [7]. Somatic loss of the remaining *Apc* wild-type allele leads to deregulation of the Wnt signaling pathway, resulting in the development of a multitude of tumors throughout the whole intestinal tract [8]. The majority of these neoplastic lesions is distributed to the small intestine and only few occur in the colon [9–11]. In $Apc^{Min/+}$ mice all intestinal tumors are benign adenomas. Progression to adenocarcinoma is very rare and may occasionally be observed in older animals. $Apc^{Min/+}$ mice die as a consequence of secondary effects of tumor growth, mainly intestinal bleeding and obstruction caused by tumors [9,11].

The aim of the present study was to investigate the role of *Itf2* in intestinal tumorigenesis *in vivo*. Therefore, we inactivated *Itf2*

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specifically in the intestinal epithelium of $Apc^{Min/+}$ mice and analyzed consequences for tumor formation.

2. Material and methods

2.1. Animals

$Itf2^{fl/fl}$ mice [12] were crossed with *vil-Cre*$^{+}$ mice [13]. $Itf2^{fl/fl}$;*vil-Cre*$^{+}$ mice heterozygous for the *vil-Cre* transgene were bred into the $Apc^{Min/+}$ background to obtain $Itf2^{fl/fl}$;$Apc^{Min/+}$ and $Itf2^{fl/fl}$;*vil-Cre*$^{+}$;$Apc^{Min/+}$ mice, respectively. In all experiments, mice on the $Itf2^{fl/fl}$ background were used as controls. $Apc^{Min/+}$ mice were purchased from the Jackson Laboratory. All mouse strains were maintained on a C57BL/6 background.

Mice were inspected on a daily basis and sacrificed when moribund. Animals were housed under specific pathogen free conditions in a closed barrier system. Experiments were carried out in accordance with the German Animal Welfare Act and with permission of the Government of Upper Bavaria.

2.2. Tissue processing, tumor scoring and histology

Mice were sacrificed by cervical dislocation, the intestine was excised and rinsed with PBS to remove fecal material. The small intestine was cut into 3 equal segments and each intestinal section was placed on a piece of filter paper, opened longitudinally, laid open and fixed in 4% buffered formaldehyde solution. Tumor number and their maximum diameter were determined under a dissecting microscope at 10x magnification. The colon and rectum were scored as “colon”. A quantity of small intestinal lesions was resected including adjacent normal tissue. In case no polyps were found, the small intestine and colon were processed as “Swiss rolls” [14]. The material was dehydrated and embedded in paraffin. 4 μm tissue sections were cut in parallel with the mucosal surface and stained with H&E or Periodic acid-Schiff reagent (PAS) according to standard protocols. Histopathological analysis of neoplastic lesions was performed in a blinded manner using standard criteria [15].

The numbers of PAS-positive cells and enteroendocrine cells, respectively, were counted in 20 crypts each in the small intestine

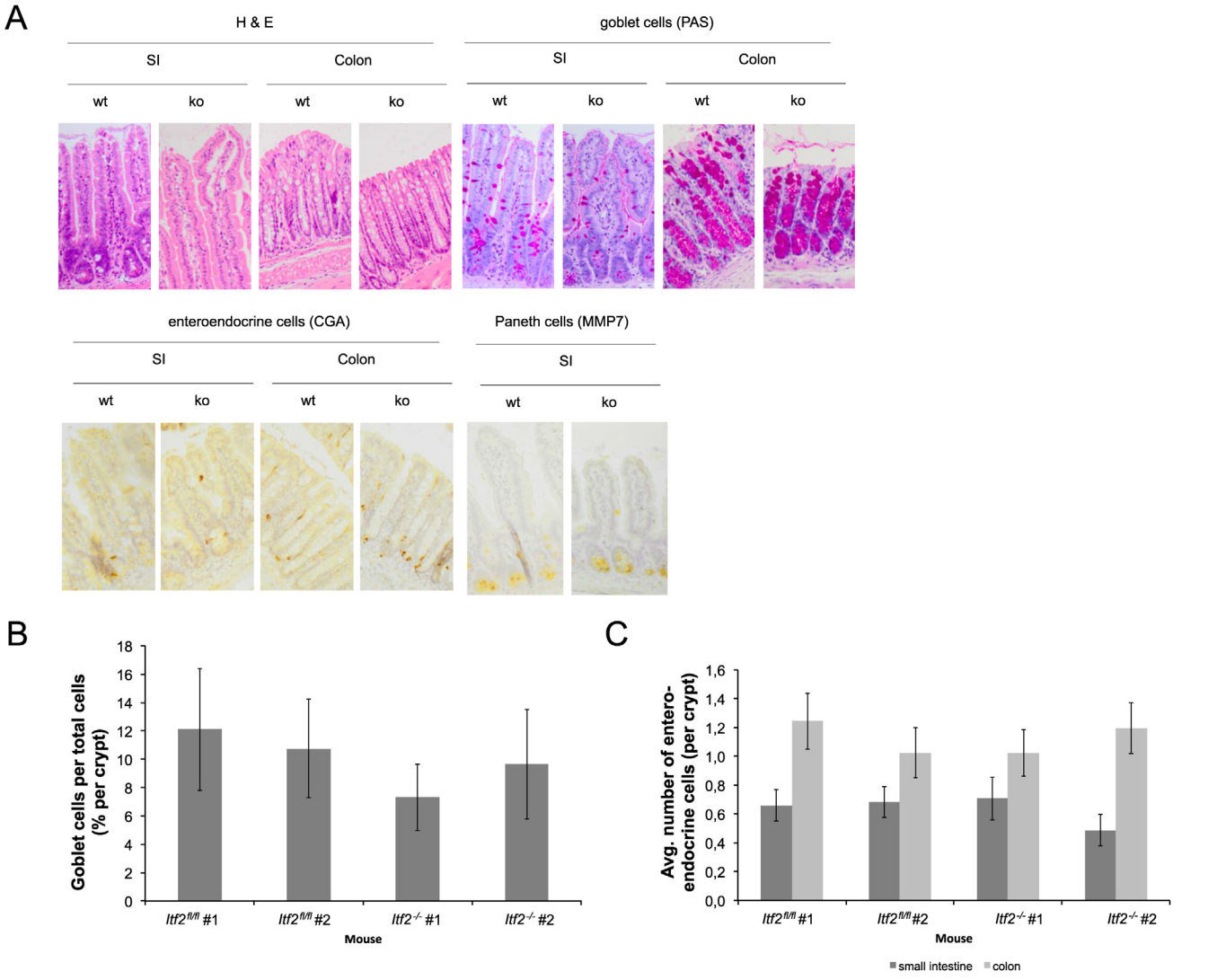

Fig. 1. Genetic disruption of *Itf2* has no effect on the maintenance of the intestinal epithelium. (A) H&E-staining of small intestine and colon of an $Itf2^{fl/fl}$ and an $Itf2^{-/-}$ mouse. PAS staining (goblet cells) in the small intestine and colon of an $Itf2^{fl/fl}$ and an $Itf2^{-/-}$ mouse. Immunohistochemical staining for chromogranin A (enteroendocrine cells) in the small intestine and colon of an $Itf2^{fl/fl}$ and an $Itf2^{-/-}$ mouse. Immunohistochemical staining for MMP7 (Paneth cells) in the small intestine of an $Itf2^{fl/fl}$ and an $Itf2^{-/-}$ mouse. (B) The number of PAS-positive cells was counted in 20 crypts in the small intestine of $Itf2^{fl/fl}$ and $Itf2^{-/-}$ mice. Error bars indicate standard deviations. (C) The number of enteroendocrine cells was counted in 20 crypts in the small intestine of $Itf2^{fl/fl}$ and $Itf2^{-/-}$ mice. Error bars indicate standard deviations.