Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance

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The Hippo signaling pathway was discovered in the fruit fly Drosophila and functions primarily to phosphorylate and inhibit Yorkie, a coactivator of gene transcription that promotes cell proliferation and inhibits cell death (1). Phosphorylation is the addition of a phosphate group to a molecule such as a protein, and the enzyme Hippo is a protein kinase that phosphorylates an intermediate protein kinase that in turn phosphorylates Yorkie. When components of the Hippo pathway upstream of Yorkie are functionally inactivated, Yorkie becomes underphosphorylated, its abundance increases because of diminished degradation, and it shifts from residing in the cytoplasm to the nucleus, a shift that is accompanied by massive organ overgrowth. Therefore the Hippo pathway is considered essential to the determination of proper organ size. The components of the pathway are conserved and duplicated in mammals, and their functions have been diversified. Thus, the protein kinases Mst1 and Mst2, the mammalian orthologs of the Hippo kinase, act as redundant tumor suppressors in the liver through inhibition of Yes-associated protein 1 (Yap1), the Yorkie ortholog; the combined inactivation of Mst1 and Mst2 in the liver results in diffuse liver overgrowth and in the rapid development of hepatocellular carcinomas (2). In contrast, Mst1 in T lymphocytes, a type of immune cell, acts to suppress proliferation and promote cell adhesion and migration (3) through Yap1-independent mechanisms. The role of the Hippo pathway in the development and cell turnover of most other mammalian tissues remains poorly understood, as is the contribution of the Yap1 oncogene to other common human cancers.

Hepatocytes turn over roughly once per year by replication of differentiated cells; in contrast, the epithelial cells of the intestinal lining turn over every 4 or 5 d, renewed from a stem cell compartment. Mst1, Mst2, and Yap1 are expressed in mouse intestinal epithelium, with Yap1 localized predominantly in the cytoplasm. Previously, when a non-phosphorylatable Yap1 mutant was overexpressed in the intestinal epithelium of transgenic (i.e., genetically modified) mice, it was localized largely in the nucleus and resulted in the expansion of the stem cell compartment and the loss of differentiated (mature) cell types (4). This finding, however, did not reveal the normal function of the pathway in intestinal homeostasis. Therefore, we examined the impact of inactivating the genes encoding the Mst1, Mst2, and Yap1 proteins in the intestinal epithelium. Global inactivation of either Mst1 or Mst2 had no effect on the intestinal epithelium; however, combined elimination of both Mst1 and Mst2, specifically in the intestine, resulted in a marked expansion of undifferentiated stem-like cells and a complete loss of all intestinal secretory cell types, defects that strongly resembled those elicited by the overexpression of mutant non-phosphorylatable Yap1. Loss of Mst1 and Mst2 was accompanied by greatly diminished Yap1 phosphorylation and a marked increase in the abundance of Yap1, now predominantly located in the nucleus. The proliferation of intestinal stem cells is driven primarily by a pathway called the “Wnt” pathway, acting synergistically with another pathway, the Notch pathway. The Wnt pathway controls intestinal stem cell proliferation through β-catenin, a protein that regulates gene transcription (5). The Mst1/Mst2-deficient intestinal epithelium exhibited evidence of strong activation of gene expression as directed by β-catenin but without altered β-catenin abundance. Increased levels of the Notch intracellular domain and activation of Notch transcriptional responses also were evident. Thus, the active inhibition of Yap1 through Mst1 and Mst2 is required to prevent overproliferation of intestinal stem cells.

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activation of Wnt and Notch signaling, so as to enable proper epithelial development (Fig. P1 A and B).

Surprisingly, inactivating both copies of the Yap1 gene in the intestinal epithelium caused no detectable aberration in intestinal development, indicating that Yap1 is dispensable for normal intestinal stem cell proliferation and development. Nevertheless, elimination of a single copy of the Yap1 gene from the Mst1/Mst2-deficient intestine, which reduces Yap1 protein expression to nearly wild-type or normal levels, completely restored the morphology and development of the Mst1/Mst2-deficient epithelium to normal. Although overall Yap1 protein levels in this circumstance were near those of wild-type, the Yap1 protein in the Mst1/Mst2-deficient intestinal epithelium was hypophosphorylated (under-phosphorylated) and predominantly within the nucleus; nevertheless, excessive proliferation and failed development in the Mst1/Mst2-deficient intestinal epithelium was reversed completely (Fig. P1C). This outcome indicates that wild-type levels of Yap1, even when the protein is fully within the nucleus, are insufficient to promote intestinal stem cell overproliferation and loss of differentiation. In essence, increased abundance of the Yap1 protein beyond endogenous levels is necessary to engage these responses. Moreover, Mst1 and Mst2, through their ability to promote Yap1 phosphorylation, actively inhibit both Yap1 abundance and Yap1 nuclear residence in the normal intestinal epithelium.

Yap1 polypeptide was found to be overabundant in more than 95% of 71 human colon cancers examined by a standard technique, immunohistochemistry. Yap1 mRNA levels in these cancers were increased consistently, although to a variable extent and often markedly less than Yap1 protein levels. Yap1 polypeptide also was overabundant in 30 of the 36 colonic adenoma and cancer cell lines examined, although Yap1 phosphorylation usually was preserved. In the colon cancer cell lines SW480 and HCT116, which exhibit moderate Yap1 overabundance, depletion of Yap1 by shRNA-induced silencing of gene expression strongly inhibited gene transcription directed by β-catenin, without altering the abundance of total or nuclear β-catenin. Yap1 depletion in HCT116 colon cancer cells also strongly reduced the abundance of the Notch intracellular domain and Notch-directed transcription. Concurrently, in these and other colon cancer lines that overexpress Yap1, Yap1 depletion greatly inhibited proliferation in culture (Fig. P1D).

In conclusion, Yap1, when overexpressed beyond the levels normally present in intestinal epithelium, drives the proliferation of intestinal stem and/or transiently amplifying cells and interferes normal epithelial differentiation. The overabundance and nuclear localization of Yap1 normally is prevented by the activity of the Mst1 and Mst2 kinases. At a molecular level, the effects of Yap1 likely result from the ability of high levels of Yap1 within the nucleus to activate β-catenin transcriptional activity synergistically. Nevertheless, Yap1 is entirely dispensable for normal intestinal epithelial development, and the circumstances that reduce Mst1/Mst2 activity so as to recruit Yap1 in intestinal epithelia remain to be defined. Overabundance of Yap1 is a ubiquitous feature of human colon cancer, where it serves to augment substantially the already inappropriately high levels of β-catenin signaling to drive proliferation and promote survival. Given the dispensability of Yap1 for normal intestinal homeostasis, interference with Yap1 expression and/or outputs may provide attractive therapeutic targets for this common cancer.