Dissecting Hedgehog signaling in the mesenchymal and epithelial compartment in K-Ras$^{G12D}$ induced pancreatic neoplasia

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Background/State of the art. The oncogenic K-Ras mutation represents one of the decisive driver mutations in human pancreatic cancer. Mouse models based on the expression of oncogenic K-Ras$^{G12D}$ recapitulate major features of human pancreatic cancer such as progressive PanIN lesions and expansion of a stromal niche. However, lessons learned from both p48-PTF1a and PDX1 constitutive und inducible driven K-Ras$^{G12D}$ expression clearly indicated that most pancreatic cells seem to be refractory to K-Ras$^{G12D}$ driven transformation despite a small, putative cancer stem cell population. The canonical Hedgehog (Hh) pathway comprises para- and autocrine signals (e.g. sonic hedgehog), cell autonomous negative (e.g. patched) and positive (e.g. smo) regulatory elements merging at Gli transcriptions factors that directly and indirectly activate negative and positive feedback loops (Borggrefe et al., 2016). This network is further complicated due to direct, Ras dependent activation of Gli downstream effectors in the non-canonical Hh-pathway. The Hh-signaling network regulates stemness, epithelial–mesenchymal interactions and pancreatic development and contributes to the initiation and propagation of pancreatic cancer as shown in numerous studies. However, recent findings challenged the underlying mechanisms especially with respect to differential effects of the Hh pathway on the epithelial and mesenchymal compartment and comprehensive morphological studies indicated that only a subpopulation of pancreatic duct cells might be permissive for Hh signaling (Tia et al., 2009; Nolan-Stevaux et al., 2010). Recent evidence further indicates, that the mesenchymal niche might rather suppress pancreatic cancer progression by hitherto unknown mechanisms after epithelial deletion of the Hh-pathway secretory component Shh (Rhim et al., 2014). This highly interesting model clearly contradicts a longstanding paradigm that tumor stroma only supports and promotes growth of pancreatic cancer and offers one explanation of clinical failure of stroma-targeting therapies. However, these biological effects are not mutually exclusive and the nature of cellular crosstalk between different components of the microenvironment and the different roles of the members of the Hh signaling network remain to be elucidated.

Preliminary work. We studied the effects of conditional deletion of patched in the PTF1a/p48 expression domain. Our data indicate patched-dependent, negative regulation of Hh-signaling in PTF1a/p48 positive pancreatic precursors is dispensable for pancreatic development. However, morphological examination of d40 pancreata revealed small foci of proliferating ductal cells, strongly positive for activated NF-κB signaling as shown by immunohistochemistry for phosphorylated p65. Furthermore, comprehensive expression analysis revealed upregulation of Hh target genes in p48-cre x patched$^{fl/fl}$ mice. Early lethality of p48-cre x patched$^{6/6}$ mice at the age of two month prevented further investigation of the fate of these foci. Lethality is due to malignant medulloblastoma. p48-PTF1a and Atho1 are well recognized regulators of cerebellum development and mutual exclusive expression domains of these transcription factors directed cerebellar cell populations in two different lineages. We found a miss location of PTF1a/p48 positive precursors in the Atho1 expression domain between e11.5 and e12.5 due to haploinsufficiency of p48-cre mice as bona fide explanation of this phenotype, since the development of medulloblastomas is well recognized after deletion of patched in Atho1 positive cells.
Although further analysis of the pancreatic phenotype is not possible in this mouse model, important conclusions can be drawn: (I) Deletion of patched is able to induce neoplasia (cerebellum) and early, potentially preneoplastic lesions (pancreas) even in the absence of mutated K-Ras. (II) This capability is tightly temporally and spatially controlled in both organs.

**Overall hypothesis.** In this project, we will combine defined mouse models of pancreatic cancer with cell autonomous genetic activation (conditional deletion of patched) and inactivation (conditional deletion of smo) of the Hh-signaling pathway in both the epithelial and the mesenchymal compartment. In response to this unique spatial and temporal Hh titration system, we hope to shed light on different histological PDAC subtypes (e.g. dedifferentiated stroma-poor vs. rich tumors) to explain heterogeneity but also various clinical responses to Hh targeting strategies.