Dkk3 as a putative candidate of PDAC heterogeneity
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Background/State of the art. Re-activation of genetic programs from early development is a hallmark of cancer. Developmental programs enable patterning events by cellular movements to specific embryo parts. “Stemness” gene networks and signaling cascades regulating embryonic patterning (e.g. Nodal, Hedgehog, ATM) are overexpressed/re-activated during pancreatic tumorigenesis and suggest a hierarchy within tumors (Russel et al. 2015). Indeed, a cellular hierarchy has been demonstrated by the identification of cancer stem cells (CSCs) (Hermann et al. 2007), a subpopulation of tumor cells with stem cell features that drive tumor initiation, metastasis, and therapy resistance (Hermann et al. 2007; Mueller et al. 2009; Lonardo et al. 2011). Targeting these CSCs by inhibiting key regulatory networks has been demonstrated to be a successful approach to improve therapy in pancreatic cancer (Hermann et al. 2013). This proposal aims to demonstrate a functional link between embryonic development, tumorigenesis, and CSCs via regulation of the Dickkopf family member Dkk3, discovered in a functional genomics approach during cellular reprogramming.

Preliminary work. We successfully established functional genomics during induced pluripotent stem cell formation. A subsequent shRNA screen led to the identification of genes that limit reprogramming, self-renewal and tumor formation (A. Kleger & K.L. Rudolph, unpublished). We validated the most potent candidate genes that increase iPSC formation, and here propose Dkk3 as very promising target in CSCs.

The Dkk family consists of four members (Dkk1-4) and soggy, a Dkk3-related protein. Unlike the other family members, Dkk3 does not inhibit Wnt but rather limits Tgf-signaling. Most interestingly its expression is lost in many human cancers, which maintains de-differentiation in pancreatic cancer cells. We have successfully generated lentiviral constructs mediating the knockdown or the overexpression of Dkk3 in iPS cells as well as in human and murine primary pancreatic cancer cells. Using these constructs, we have been able to generate preliminary data indicating a proliferation advantage of Dkk3-KD cells. More importantly, we have data suggesting a direct correlation between the expression level of “stemness genes” such as Nanog, Oct4, Sox2, and Klf4 and Dkk3 expression levels. In an RNA-SEQ screen comparing gene expression levels of “normal” differentiated cancer cells with cancer stem cell-enriched sphere cultures we have been able to demonstrate a variable downregulation of Dkk3 in spheres in a total of 6/6 investigated primary human pancreatic cancer cell lines. This evidence clearly points to a significant down-regulation of Dkk3 in a cancer stem cell context. For the successful investigation of Dkk3’s role in tumorigenesis in vivo, we have successfully crossed a genetically engineered mouse strain that lacks Dkk3 expression (Barrantes et al. 2006) with a genetically engineered strain of spontaneous pancreatic tumorigenesis mediated by an oncogenic K-Ras mutation under the control of the p48-PTF1A promoter (Hingorani et al. 2003, Wagner et al., 2001). The mice generated from these breeding will allow us the detailed investigation of the role of Dkk3 expression (or lack thereof) on tumor stage, grade, metastatic activity, and ultimately on therapeutic response in vivo.
**Overall hypothesis.** The loss of Dkk3 in pancreatic cancer mediates a more aggressive phenotype by expanding the cancer stem cell pool, thus promoting aggressiveness, therapy resistance, and metastasis.