The impact of methylation signatures on cancer cell aggressiveness and cancer-testis antigen specific immunity in head and neck squamous cell carcinoma

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Background/State of the art. Cancer-testis antigen (CTA) expression is restricted to germline cells. However, in cancer these embryologic antigens are often re-expressed by promoter demethylation. CTA are immunogenic and attractive targets for specific immunotherapy. MAGE-antigens, PRAME and NY-ESO-1 are the most frequently expressed CTAs in head and neck squamous cell carcinoma (HNSCC) and associated with impaired survival (Laban et al. 2014, Veit et al. 2016). Demethylating agents such as decitabine have been shown to increase CTA expression (Srivastava et al. 2016), which has been implied to improve CTA-specific immunotherapy. On the other hand, decitabine-induced CTA expression may result in increased aggressiveness and reduced prognosis (Zhang et al. 2015). Details of CTA function and their regulation by hypomethylation are poorly characterized to date. Thus it is necessary to evaluate the impact of methylation signatures on the malignant phenotype and CTA specific immunity in HNSCC.

Preliminary work. In a large cohort of 453 HNSCC patients, different CTA expression patterns were noted and had a major impact on the prognosis. The expression pattern, namely simultaneous cytoplasmic and nuclear expression (cyt+nuc) of pan-MAGE, MAGE-A3/A4 and NY-ESO-1, were identified as independent prognostic markers indicating poor OS (Laban et al. 2014). We hypothesize that combined cyt+nuc expression may be a sign of increased biologic activity supporting an aggressive phenotype (compare objective 1). Additionally, we suppose that CTA-specific immunity is dysfunctional in HNSCC (compare objective 2). To test the latter hypothesis we have collected tumor tissue (fresh frozen tissue [FFT] and formalin-fixed paraffin embedded [FFPE] tissue) at diagnosis as well as blood and serum samples longitudinally for 12 months (before, during, after treatment) in a prospective observational trial. Finally, the use of demethylating agents resulting in increased CTA expression may affect T cell mediated CTA-specific immune responses (objective 3).

Overall hypothesis. CTA expression (pattern) contributes to the malignant phenotype and can be changed by differential methylation of CTA genes. At the same time, CTA-specific immune responses are impaired by the tumor microenvironment. Demethylation treatment may increase the expressed CTA repertoire as targets for specific T cells.

Role of Protein Kinase D (PKD) isoforms in the evolution of ductal pancreatic adenocarcinoma (PDAC)

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Background/State of the art. Overexpression or loss of distinct proteins is a hallmark of tumor evolution and drives this process. Members of the Protein kinase D (PKD) family of serine/threonine kinases have been implicated in cancer cell motility, angiogenesis, proliferation and metastasis (Eiseler et al. 2009; Azoitei et al., 2010; Eiseler et al. 2012; Wille et al. 2014). PKD1 and PKD2 are differentially expressed in
various tumors, including pancreatic ductal adenocarcinoma (PDAC), where PKD2 expression is elevated whereas PKD1 expression is reduced (Wille et al. 2014). However, the functional consequences of these differences in expression so far are incompletely understood. Recently, PKD1 has been proposed as a novel target for PDAC (Harikumar et al., 2010; Liou et al., 2015).

**Preliminary work.** Our data support an isoform-specific role for PKD1 in the inhibition of tumor cell motility and invasion of various cancer cell lines, including pancreatic cancer cells (Eiseler et al. 2009; Eiseler et al. 2010; Wille et al. 2014). This is mediated by phosphorylation of actin regulatory substrates such as Slingshot1L (SSH1L) and Cortactin (Eiseler et al. 2009; Eiseler et al. 2010). SSH1L is selectively phosphorylated by PKD1 at dynamic peripheral actin structures (Eiseler et al. 2009; Wille et al. 2014). By contrast, PKD2 enhances expression and secretion of vascular endothelial growth factor (VEGF-A) from pancreatic cancer cells driving tumor angiogenesis (Wille et al. 2014). Our recent data indicate that PKD2 also selectively enhances the expression and secretion of matrix-metalloproteinases (MMPs) 7 and 9 from PDAC cells. These MMPs in turn promote invasion and angiogenesis by liberating VEGF-A from the extracellular matrix (ECM) *in-vitro* and *in-vivo* (Wille et al. 2014; Eiseler et al., 2016). Our study now aims at characterizing the biological consequences of a loss of PKD1 or overexpression of PKD2, respectively, for pancreatic tumor evolution *in vivo*. We have generated a pancreas-restricted PKD1 knockout mouse model with mutated K-Ras (p48Cre-KRasG12D/PKD1−/−). Likewise, we have successfully generated a PKD2 overexpressing mouse model. Our preliminary analyses of the PKD1 knockout model indicate that this isoform acts as a novel tumor suppressor in PDAC. Knockout of PKD1 impairs median survival of (p48Cre-KRasG12D/PKD1−/−) animals and mice develop more PanIN lesions of all stages as well as full carcinomas after 5 and 9 months, respectively. However, the underlying molecular mechanisms still need to be clarified. Thus, the aim of this proposal is to investigate how and to which extent PKD1 acts as a novel tumor suppressor during PDAC formation, whereas PKD2 drives tumor progression. We will identify molecular mechanisms and test *in-vivo* functions of PKD1 and -2 in our mouse model systems.

**Overall hypothesis.** PKD1 and PKD2 exhibit different expression in human cancers including PDAC and fulfill opposing functions in carcinogenesis and metastasis. The characterization of the precise mechanisms of these kinases will help to understand tumor heterogeneity and to design specific targeting agents for PDAC.