A ZEB1-HDAC pathway enters the epithelial to mesenchymal transition world in pancreatic cancer

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Epithelial to mesenchymal transition (EMT) correlates with high-grade malignancy including the competence to form metastases. In addition, EMT has recently been linked to cellular self-renewal programmes of cancer stem cells and apoptosis/anoikis resistance, which are all features of therapeutic resistance. The EMT programme is driven by several transcription factors (TFs), such as the transcriptional regulators SNAIL, SLUG, ZEB1 and ZEB2 and the basic helix—loop—helix factors E47 and TWIST. These proteins target and repress the CDH1 gene, which encodes for E-cadherin, an important caretaker of the epithelial state. Expression studies in human pancreatic cancer showed expression of SNAIL in 78% and of SLUG in 50% of cases.[1] Although no or low levels of TWIST are expressed in pancreatic cancers, up-regulation of this gene under hypoxic condition may argue for a contribution towards tumour progression.[1 2] Expression of the ZEB2 gene was recently found to be silenced by promoter methylation in the majority of pancreatic cancers. This finding argues against ZEB2 as a major repressor of E-cadherin in pancreatic cancer.[3] In addition to such expression data, the functional relevance of SNAIL and SLUG for EMT and repression of the CDH1 gene has been described in various pancreatic cancer models in vitro and in vivo.[4–7]

Aghdassi et al (**see page 439**) present new compelling evidence that the zinc-finger TF ZEB1 is a repressor of E-cadherin expression.[8] Based on the observation that 40% of pancreatic cancers have reduced E-cadherin levels and that low E-cadherin expression correlates with a poor prognosis after pancreatic cancer resection, regulation of E-cadherin was investigated. Mutations of CDH1 and hypermethylation of CpG islands in the CDH1 promoter could be excluded as general mechanisms for reduced E-cadherin expression. When they compared the levels of E-cadherin and ZEB1, Aghdassi et al detected an inverse correlation in pancreatic cancer cell lines and tumour specimens.[8] Consequently, binding of ZEB1 to the CDH1 promoter was found specifically in cell lines lacking E-cadherin expression and inhibition of ZEB1 expression restored E-cadherin expression, grabbing ZEB1 into the row of EMT regulators and CDH1 repressors in pancreatic cancer. In accordance with these data, the Brabletz group has recently demonstrated that ZEB1 represses the expression of miR-200 family members in pancreatic cancer cells, which contributes to the activation of the tumour promoting NOTCH pathway.[9] ZEB1 expression was especially found in undifferentiated (G3 and G4) pancreatic cancers, restricted to invasive areas with signs of EMT,[9] which confirms the inverse correlation of E-cadherin with ZEB1 described by Aghdassi and colleagues.[8]

EMT-TFs can act in hierarchical cascades to cooperatively induce EMT. For example, TWIST can activate SNAIL[10] and SLUG[11] expression, whereas SNAIL[12] and SLUG[13] can drive ZEB1 expression. Consistent with this, Aghdassi et al observed that high SNAIL and ZEB1 expression was often directly correlated in pancreatic cancer cell lines. Furthermore, the IKK-NFκB signalling pathway, a well characterised inducer of EMT,[14] was recently shown to induce SNAIL and ZEB1 in pancreatic cancer cells.[15] While these observations provide some evidence for the existence of a SNAIL-ZEB1 axis, further studies are needed to precisely identify an EMT-TF hierarchy in pancreatic cancers. Considering the diverse features cells acquire during EMT, such as invasiveness, migration, stemness or anoikis resistance, it will be important to decipher where EMT-TFs possess unique competence and where and how the factors cooperate.

As a consequence of the fast progress in sequencing technologies, we know that pancreatic cancer is characterised by an extreme genetic heterogeneity. Therefore, it is important to find and distinguish molecular mechanisms, which are common in pancreatic cancer—the node concept. For the regulation of E-cadherin during EMT in pancreatic cancer cells, Aghdassi et al now demonstrate that histone deacetylases (HDACs), especially HDAC1 and HDAC2, are critically involved. HDACs deacetylate the ε-amino group of lysines located at the N-terminal tail of histones. This can lead to a repressive chromatin structure (heterochromatin) and altered gene transcription.[16] By modulating these epigenetic acetylation marks, HDACs can promote proliferation and confer therapeutic resistance.[16] HDAC1 and HDAC2 are both overexpressed in pancreatic cancer, and especially high HDAC2 expression was observed in poorly differentiated cancers.[17 18] Aghdassi et al demonstrate that ZEB1 can directly bind HDAC1 as well as HDAC2 and that ZEB1 can recruit a HDAC1/2 containing repressor complex to the CDH1 promoter in pancreatic cancer cells.[8] Accordingly, the repression of the CDH1 gene in mesenchymal murine pancreatic cancer cell lines derived from the genetic Kras[G12D] model, as well as the repression of E-cadherin in TGFβ-induced EMT of pancreatic cancer cells, rely on HDAC activity.[6] Furthermore, repression of E-cadherin by HDAC1/2 was recently demonstrated with in vivo selection models of pancreatic cancer EMT.[6] Recruitment of catalytically active HDAC1 or HDAC2 to the CDH1 promoter was demonstrated with chromatin immunoprecipitation assays in tissue sections of human pancreatic cancer, corroborating the importance of HDAC-dependent repression of E-cadherin in vivo.[8] The observation that Aghdassi et al detected exclusively HDAC1 or HDAC2 recruited to the CDH1 promoter in human pancreatic cancer tissue section is intriguing. Both enzymes are highly homologous, cooperatively act in co-repressor complexes, and share redundancy for many biological processes. Although the reasons for the specific recruitment of either HDAC remain unclear, an explanation might be the different ability of the

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EMT-TFs to interact with certain HDACs in specific cellular contexts. The authors report that ZEB1 interacts with HDAC1 and HDAC2 in PaTu-8988T cells but only with HDAC2 in MiaPaCa2 cells.[8] Moreover, SNAIL preferentially interacts with HDAC2 in an in vivo selection model of pancreatic cancer EMT.[6] Here, it will be important to decipher determinants of these context-specific interactions of EMT-TFs. It may also be relevant to test a role for HDAC3 as the authors used the HDACi MS-275 which also blocks this class I HDAC in addition to HDAC1/2. Differences in HDAC protein stability at specific genetic loci and during transcription cycles may also be a critical issue. Aghdassi et al found induction of E-cadherin in cells exposed to apoptosis inducing concentrations of HDACi, which might point to protein stability as a possible regulator. Furthermore, in light of the fact that EMT-TFs as well as HDACs become post-translationally modified, such structural alterations might be the key for a better understanding of EMT in pancreatic cancer. Although many questions regarding EMT of pancreatic cancer cells remain unresolved, the demonstration of an important ZEB1-HDAC axis (figure 1) influences further preclinical studies. These are needed to dissect the EMT program in molecular detail. For example, it will be important to determine if ectopic expression of ZEB1 in the genetically engineered $Kras^{G12D}$-dependent mouse model of pancreatic cancer drives tumour progression by inducing EMT, invasion and metastasis in vivo. This will also allow deciphering modulators of EMT which are potentially suitable for therapeutic intervention. In an analogous manner, HDAC functions should be addressed in vivo. Considering the present data revealing that HDAC1 and/or HDAC2 are essentially involved in the regulation of the $CDH1$ gene, it will be important to see whether one or both enzymes are needed for EMT and metastasis. According to the node concept, inhibiting HDACs rather than blocking individual EMT-TF might be an attractive and promising anti-metastatic treatment strategy in the future. Since HDAC inhibitors are currently evaluated in clinical phase II and III studies in a wide variety of solid cancers, this might be a straight forward approach for anti-EMT directed chemotherapies. Altogether, Aghdassi et al provide new insights into the molecular mechanisms by which EMT-TFs act in concert with HDACs to direct EMT and tumour progression. This will direct the way to novel therapeutic approaches in the future.
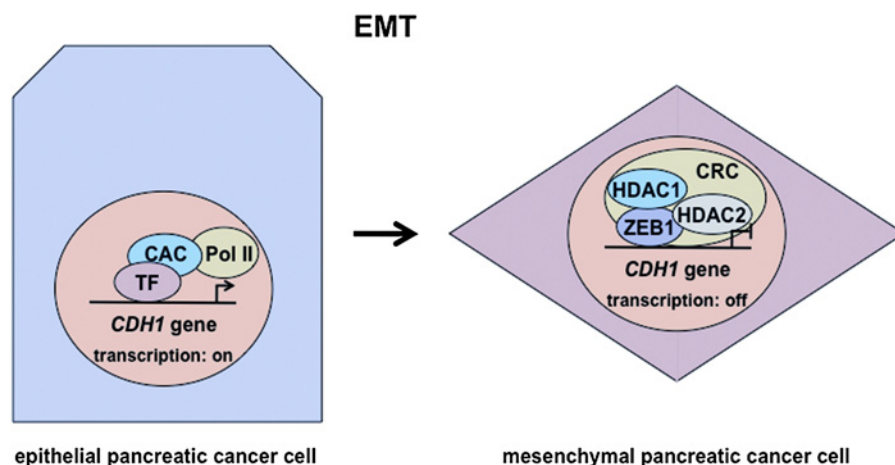

Figure 1 ZEB1 is an important repressor of the $CDH1$ gene in pancreatic cancer. In epithelial pancreatic cancer cells, transcription of the $CDH1$ gene, encoding E-cadherin, is activated by a multiprotein complex containing transcription factors (TF) which recruit RNA polymerase II (Pol II) via an coactivator complex (CAC) to the $CDH1$ promoter. During the dynamic process of epithelial to mesenchymal transition (EMT), the $CDH1$ gene is epigenetically silenced. One pathway involves the transcriptional repressor ZEB1, which recruits HDAC1 and/or HDAC2 containing corepressor complexes (CRC) to inhibit transcription of the $CDH1$ gene.

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