The *cis*-regulatory switchboard of pancreatic ductal cancer

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Pancreatic ductal adenocarcinoma (PDAC) remains one of the most devastating human diseases. There is consequently a pressing need to understand its molecular underpinnings, which should enable new preventive and therapeutic strategies. A new study in *The EMBO Journal* (Diaferia *et al*, 2016) maps the transcriptome and epigenetic landscape associated with distinct PDAC grades and identifies *cis*- and *trans*-regulatory elements in tumour progression.

See also: **GR Diaferia *et al*** (March 2016)

P DAC originally acquired its name from its histological similarity with normal pancreatic ducts. However, recent work has shown that tumours with ductal morphology can arise upon activation of mutant *Kras* in duct-like embryonic pancreatic progenitors or in pancreatic acinar cells through a process known as acino-ductal metaplasia (Tuveson *et al*, 2004; Guerra *et al*, 2007; Pérez-Mancera *et al*, 2012). Acino-ductal metaplasia has thus been proposed to involve a process where acinar cells are reprogrammed to an embryonic duct cell progenitor state (Rooman & Real, 2012; Roy & Hebrok, 2015). PDAC tumours of low or moderate histological grade retain ductal features, while this phenotype is not seen in more aggressive high-grade tumours. The profound changes in cellular phenotypes that take place throughout pancreatic carcinogenesis are likely triggered by both somatic genetic alterations in tumour cells and environmental cues such as

stromal-derived signals, but they need to be executed by cell-specific transcriptional programmes. This means that there should be specific transcription factors that control regulatory programmes in each histological phenotype. On the other hand, it is now known that transcription factors control cell-specific programmes through the activation of tens of thousands of transcriptional regulatory elements, including long-range enhancers and proximal promoters, which in turn activate specific cohorts of cell-specific genes. Despite recent progress in understanding the epigenome of human embryonic pancreatic progenitors (Cebola *et al*, 2015), so far no study has systematically tackled the transcription factor and enhancer networks that underpin ductal differentiation in normal and tumour cells.

Gioacchino Natoli and colleagues have now examined the regulatory landscape of low- and high-grade human PDAC cell lines (Diaferia *et al*, 2016). They generated genomewide maps of active transcripts, enhancers and promoters in these cells and found that such regulatory features showed a striking clustering with histological grade (Fig 1). They identified sets of transcription factors that are selectively expressed in low-grade PDAC, including IRF1, ELF3, FOXA1, KLF5 and HNF1B, a core marker of embryonic and adult ducts. They also defined transcription factors that were selectively expressed in high-grade PDAC, such as ZEB1, IRF1, GATA2, ETV5 and TCF12. In parallel, they interrogated genomic sequences that are selectively used as transcriptional enhancers in low-grade

PDAC and found these to be highly enriched in recognition sequences for transcription factors that were found to be selectively expressed in PDAC. This finding was further corroborated by experimental analysis of genomewide binding of five of these factors. The analysis thus uncovered a core set of enhancer clusters that were specific for low-grade PDAC, several of which map to putative stage-specific regulatory genes, including *KLF5*. Importantly, the authors found that the transcriptional phenotype associated with stage-specific programmes could be largely explained by the activation of grade-specific enhancers.

Natoli and colleagues next tested the functional role of grade-specific transcription factors in PDAC. They used CRISPR-Cas9 to inactivate *KLF5* and *ELF3* in a low-grade PDAC cell line. The consequences of knocking out *ELF3* were comparatively mild, but inactivation of *KLF5* caused a dramatic epigenomic phenotype, with a reduction in H3K27ac and H3K4me levels (characteristic marks of active enhancers) in more than 20% of PDAC enhancers. Many of the affected enhancers were directly bound by *KLF5* in wt cells, and in *KLF5* knockout cells, they showed decreased binding of other transcription factors such as *ELF3* and *FOXA1*. This suggests that *KLF5* binding is required to nucleate the recruitment of transcription factors and to activate a large set of low-grade PDAC enhancers. *KLF5*-dependent enhancers were often located near genes that regulate proliferation and epithelial cell identity genes, and many such genes showed decreased expression in knockout

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Figure 1. Genomewide regulatory maps identified distinct enhancers that are active in low-grade versus high-grade PDAC.

KLF5 plays a key role in the activation of low-grade PDAC enhancers, which activates epithelial transcriptional programmes, and suppresses ZEB1, a regulator of the high-grade mesenchymal PDAC transcriptional programme, which in turn suppresses the low-grade epithelial programme.

cells. However, the authors also identified sub-programmes of epithelial identity that were only indirectly dependent on KLF5. In *KLF5* knockout cells, markers of epithelial–mesenchymal transition, such as vimentin and the transcriptional repressor ZEB1, were increased, and overexpression of ZEB1 partially recapitulated the changes observed in the *KLF5* knockouts. Not surprisingly, xenografts of *KLF5* knockout cells acquired a mesenchymal, rather than epithelial, cell morphology. These studies therefore delineated how the coordinated action of specific transcriptional regulators controls the enhancer programme that defines the phenotype of low-grade PDAC cells.

More generally, these elegant studies illustrate the power of combining epigenomics and genome editing to define critical programming events in cancer. They clearly pave the way to further studies of this nature in other tumour models, such as primary organoids (Boj *et al*, 2015), or in heterotypic systems (i.e. including inflammatory or stromal cells) to more precisely define transcriptional programming mechanisms that underlie the initiation of pancreatic carcinogenesis, or to define more clearly the exact transcriptional mechanisms that underlie specific PDAC subtypes.

The notion that deranged transcriptional programmes are central for carcinogenesis is supported by the observation that genes encoding chromatin regulatory proteins are frequently mutated in human cancers, including PDAC (Waddell *et al*, 2015). Interestingly, several transcriptional activators identified as regulators of the PDAC enhancer programme are mutated in other epithelial cancers (e.g. *ELF3* and *KLF5* in urothelial bladder cancer) (Cancer Genome Atlas Research Network, 2014) although somatic defects in these genes have so far not been reported in PDAC (Waddell *et al*, 2015). The importance of transcriptional programming mechanisms in pancreatic cancer is also in keeping with recent work showing that, in mice, PDAC can be treated with small-molecule inhibitors of proteins that interact with acetylated or methylated nucleosomal histones (Mazur *et al*, 2015). Moreover, chromatin regulators have been shown to affect pancreatic tumour initiation or progression (von Figura *et al*, 2014; Richart *et al*, 2016). It is thus reasonable to foresee that a more detailed understanding of the transcription factor and enhancer networks that control discrete stages of PDAC will provide further opportunities to develop precision therapies.

The maps of active regulatory elements in low- and high-grade PDAC are also relevant to efforts to decipher the mutational landscape of PDAC. The analysis of tumour exomes has uncovered multiple genes that are enriched in somatic mutations in PDAC, several of which are drivers of cancer initiation or progression. More recent efforts, such as Genome England's 100,000 genomes project, aim to interrogate whole-genome sequences in human tumours. Deciphering which non-coding mutations might be relevant to PDAC pathogenesis is a formidable challenge that requires *a priori* knowledge of which non-coding sequences are potentially relevant to this disease. The availability of PDAC regulatory maps should provide an opportunity to test the hypothesis that mutations in specific regulatory elements contribute to PDAC through dysregulation of transcription. The pioneering work from Natoli and colleagues (Diaferia *et al*, 2016) therefore opens diverse avenues to further our understanding of the pathogenesis and treatment of pancreatic cancer.

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