CRISPR/Cas9 engineering offers new opportunities to model pancreatic ductal adenocarcinoma development
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Pancreatic ductal adenocarcinoma (PDAC) is a dismal disease. The 5-year survival is below 5%, which is due to a combination of aggressive disease progression, late diagnosis and limited treatment options (1). Consequently, PDAC is predicted to be the second-largest contributor to cancer related deaths by 2020 (2). New approaches and models to accelerate therapeutic development and improve patient outcome are therefore urgently needed.

The common genetic aberrations of PDAC have been identified, where activating mutations in KRAS in combination with loss of function in the tumor suppressors SMAD4, CDKN2A and TP53 represent the most frequently occurring aberrations (3-6). Moreover, genomic rearrangements are typically observed in PDAC, where recent studies have grouped tumors dependent on the extent of rearrangements (4). Further, expression analysis in both primary cell lines as well as in tumors have identified a number of sub-classes with differing prognosis (7-9). Although there are still no targeted therapies clinically available for these aberrations, new opportunities may arise when less frequent mutations are categorized by their molecular function (6,10).

Our current understanding of the role whereby mutant KRAS drives PDAC development and how loss of function in SMAD4, CDKN2A and TP53 augment malignant progression stems from diligent analysis of preclinical models, where genetic engineered mouse models (GEMMs) have played an instrumental role (11,12). However, while several aspects of human PDAC are well recapitulated by GEMMs, including stromal desmoplasia, metastasis and genomic instability, other aspects are not recapitulated similarly. For example, in most GEMMs genetic inserts are driven by tissue specific promoters that induce expression in most cells during pancreas development. This results in multi-focal disease with short latency, which is in contrast to the human disease etiology that exhibits a stochastic development of disease with longer latency. Moreover, manipulation of multiple targets is cumbersome and requires large cohorts to derive sufficient high numbers of animals for studies. Consequently, animal studies of interplay between multiple co-occurring genetic aberrations have been limited.

To address these issues, Maresch and colleagues have developed an elegant method to introduce specific genetic aberrations in the adult pancreas using the CRISPR/Cas9 system for genome engineering (13).

Building on previous experience with in vivo delivery of plasmid DNA, the authors demonstrate that injection of DNA into the pancreas of adult mice, followed by electroporation, initially targets an average of 750 cells. While some cells undergo apoptosis and are removed by a local inflammatory response, an average of 120 cells survive long-term, demonstrated using the Cre reporter model Rosa26mT/mG. As such, electroporation of plasmid DNA only targets a small fraction of pancreatic cells, which may be a better approximation of the stochastic nature of the human disease.

To determine the effect of a number of tumor suppressor genes that display different frequency of loss of function in human PDAC, the authors generated individual guide RNA plasmids to target 13 tumor suppressor genes and 2 for the ‘neutral’ Rosa26 locus. When injected into the pancreas of animals bearing pancreas-specific expression of the oncogenic driver Kras\textsuperscript{G12D} (PK: Ptf1a\textsuperscript{Cre\textsuperscript{+}; Kras\textsuperscript{LSL-G12D/+}), the resulting animals developed tumors at accelerated rate with different histopathologic characteristics as well as overt liver
metastasis. Sequencing of the targeted alleles from isolated cell lines clearly demonstrated high frequency indels at multiple CRISPR/Cas9 sites where 7 to 14 of the 15 targets showed simultaneous mutations. Interestingly, the authors didn’t identify loss of function in Brca2, suggesting that the methodology may be used for negative selection screening.

Due to the relatively low frequency of cells targeted by electroporation, the authors hypothesized that the model also could be used for phylogenetic tracking of metastatic disease. Comparing the targeted allele frequency of the guide RNAs in tumor cell clones from 8 regions of the primary tumor and matching liver metastasis, the authors noted that while there was only a minor 5% contribution of clone 1 (of 2) in the primary tumor, the relative contribution was 50% between the clones at the metastatic sites.

Chromosomal rearrangements are a common feature of PDAC, where both intra-chromosomal and inter-chromosomal deletions, unbalanced translocations and chromothripsis are observed. Genome engineering by CRISPR/Cas9 has previously been shown to result in intra- and inter-chromosomal deletions and therefore an extensive analysis of these aberrations were undertaken across tumors and isolated cell lines. Inter-chromosomal deletions were frequently observed, with 3 out of 6 tumors displaying large deletions. Moreover it is noteworthy that these deletions frequently target tumor suppressors. Inter-chromosomal translocations were less common with only one event/tumor. Chromosomal aberrations have previously been observed in GEMMs of PDAC (11), albeit the nature and molecular understanding of these are still underexplored.

Taken together these data suggests this model recapitulates critical aspects of genome instability that may promote disease progression.

Overall the new model offers important complementarity to already available pre-clinical models and there are a plethora of unaddressed questions that can be now addressed. For example, it would be interesting to address whether tumors of different histopathology characteristics observed

Figure 1 Simultaneous injection of a pooled plasmid solution of different sgRNAs into the murine pancreas was followed by reverse electroporation. After tumor development, targeted sequencing was carried out for the sgRNA target sites. Efficiency of gene editing was determined, and different combinations of edited genes detected in different tumors. CRISPR/Cas9 induced some chromosomal rearrangements, with further work comparing a primary tumor to liver metastases for lineage tracking of the edited pancreatic cells. Negative selection was achieved, with detection of wild-type Brca2 gene but no edited genes despite inclusion of a high-efficiency Brca2 sgRNA. sgRNA, single-guide RNA; hSpCas9, codon-optimised S. pyogenes Cas9.
in the model display significant differences in their allele frequency of the targets and/or whether additional mutations have accumulated. It would also be highly informative to determine how well the overall mutational and neo-antigen burden in this model compares to other GEMMs as well as human PDAC. A subject not touched upon in this publication is the stromal reaction, where PDAC is characterized by a highly desmoplastic reaction. Whether some aspects of the human stromal reaction are better represented by this model remains to be described. Finally, there is now an unique opportunity to rapidly compare mutational spectrum with therapeutic response, a much-needed element to evaluate novel regimes for clinical translation.

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Footnote

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