provide a tool to better understand the pathogenesis of LGL and other MPNs reported to be reliant on STAT5 signaling, such as polycythemia vera, essential thrombocythemia, and myelofibrosis.

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Subtyping Pancreatic Cancer

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The complex tumor microenvironment can make molecularly subtyping cancer using mRNA expression challenging, particularly for cancers with low epithelial content, such as pancreatic cancer. In a recent edition of *Nature Genetics*, Moffitt and colleagues show that subtracting normal epithelial transcripts can provide insights into the molecular pathology of pancreatic cancer.

Pancreatic ductal adenocarcinoma (PDAC) continues to be the fourth leading cause of cancer death in our society, with little if any improvement in outcomes for over 40 years. Systemic therapies offer only incremental overall survival advantages but can be associated with significant responses in subgroups of patients that cannot be predicted prior to treatment. As a consequence, there is an urgent need to better define subgroups of patients that derive benefit from current treatment and identify novel therapeutic strategies. Recent large-scale cancer genomic studies demonstrate a heterogeneous mutational profile, with activating mutations of *KRAS* present in over 90%, mutations of *TP53*, *CDKN2A*, and *SMAD4* in over 50% of cases among a sea of diverse infrequently mutated genes mostly at a prevalence of less than 5% (Biankin et al., 2012; Waddell et al., 2015; Witkiewicz et al., 2015). Although some studies report potential prognostic and predictive genomic markers, few have been validated and none are used in the clinic. A recent issue of *Nature Genetics* presented an article by Moffitt et al. (2015), who assessed aberrant gene expression in pancreatic cancer to define molecular subtypes of tumor epithelium and stroma, with some features associated with patient outcome.

Expression profiling has defined subtypes in many cancer types, including breast and ovarian cancer, often with clinical relevance and usually the result of many studies that validated and refined initial findings. The density of mRNA expression information overcomes the relative diversity and sparseness of genomic mutation data to allow the modeling of differential expression relative to clinico-pathological attributes. A characteristic feature of PDAC is the abundant stroma, which, on average, constitutes over 70% of the tumor mass. In addition, PDAC's infiltrative nature means that the tumor can include normal pancreas (containing exocrine epithelium that secretes digestive enzymes and endocrine cells that secrete digestive hormones, including insulin and glucagon as well as a variety of others). These factors have made defining molecular subgroups and identifying carcinogenic mechanisms based on mRNA expression challenging.

Using mathematical approaches, Moffitt et al. (2015) subtracted the potential confounding effects of normal pancreas, allowing them to uncover candidate carcinogenic mechanisms of importance, particularly in the microenvironment. Our increasing appreciation of the tumor microenvironment in cancer has led to novel therapeutic strategies targeting the immune system. Although these immunotherapeutic strategies are showing promising results in melanoma and colon cancer, results in PDAC have, thus far, been disappointing. Moffitt et al. (2015) provide compelling evidence that the pancreatic cancer microenvironment differs substantially between tumors, heralding the likely necessity of patient selection based on molecular subtyping of the microenvironment in order to adequately assess immune and other therapies targeting related mechanisms in the clinic. They defined two subtypes of stroma, which they termed ''normal'' and ''activated''. While a number of genes differentiated the two subtypes, high expression of genes associated with macrophages were a distinct component of activated stroma. This dichotomy in stromal expression patterns might also explain the conflicting results that have been attributed to the PDAC stroma in the last few years, vis-à-vis its role in tumor progression (Gore and Korc, 2014), and is likely to inform how stroma-targeted therapies are applied in the clinic.

Previously, Collisson et al. (2011) physically microdissected neoplastic epithelium from PDAC and performed array-based transcript profiling. They uncovered three groups, which they termed ''classical,'' ''quasimesenchymal'' and ''exocrine-like'' based on unsupervised clustering approaches. In contrast, Moffitt et al. (2015) defined only two subgroups, which they termed ''classical'' and ''basal,'' the former being equivalent to Collisson's classical subtype and the latter overlapping with, but not exclusively, quasimesenchymal. Although subtracting transcripts associated with normal pancreas facilitated defining carcinogenic mechanisms, it was based on the assumption that cancers do not express mRNA that is present in the differentiated normal epithelium of that organ. Any ''exocrine-like'' subtype would be driven in part by transcripts defining differentiated exocrine pancreas and would not be apparent if these were removed as per the Moffitt et al. (2015) algorithm.

For many years, pathologists have used the expression of genes to classify cancers based on their organ-of-origin or the putative cell-of-origin within that organ. For example, a subset of neuroendocrine tumors of the pancreas expresses insulin, glucagon, or pancreatic polypeptide, which are also expressed by corresponding non-neoplastic islets (de Wilde et al., 2012). Comparable examples abound, such as the expression of Clara cell markers in pulmonary adenocarcinomas or glial fibrillary acidic protein (GFAP) in tumors of glial origin. In the clinic, carcinomas of unknown primary (CUP) are designated an organ-of-origin based on the expression of genes involved in the development or the differentiated state of that organ (Varadhachary, 2013). In the context of exocrine neoplasms of the pancreas, the most extreme example pertains to so-called acinar cell carcinomas, whose diagnostic sine qua non is expression of digestive enzymes native to non-neoplastic acinar cells, such as lipase or chymotrypsin. Studies conducted in genetically engineered mouse models (GEMMs) of pancreatic cancer strongly suggest that acinar cells are the most likely cells of origin for PDAC (Habbe et al., 2008; Kopp et al., 2012). These acinar cells undergo a process of acinar-to-ductal metaplasia, which are clearly evident in GEMMs, and while human PDAC do not overtly express markers of terminal acinar differentiation, it follows that these tumors are likely to retain such transcriptional networks at least early in their molecular evolution. From an ontogeny perspective, the expression of *PDX1*, a transcription factor that assigns pancreatic cell fate in the midgut, is shared between all pancreatic neoplasms in variable proportions (Park et al., 2011), confirming the persistence of tissue-specific transcriptional programs in neoplasms of otherwise distinct histology. Nonetheless, while making assumptions concerning the expression of transcripts native to the differentiated state of the organ may hamper the resolution of a holistic classification, Moffitt et al. (2015) elegantly demonstrate that meaningful data concerning pancreatic carcinogenesis can be uncovered. Further studies are urgently needed that overcome some of the technical challenges of analyzing genomic and transcriptomic data in tumors with highly variable and sometimes extremely low epithelial contents. Understanding the tumor as a whole, the epithelium and its microenvironment, and the complex biochemical processes and molecular mechanisms at play holds significant promise in advancing cancer therapeutics in the future.

An important element that has yet to be addressed is the clonal evolution of a tumor and the intra-tumoral heterogeneity that is manifest, particularly with respect to gene expression. Although we are beginning to understand clonal evolution in cancer based on mutations, we do not have meaningful data concerning differences in transcript, or protein, expression within a tumor. Limited studies in synchronous primary and metastases obtained from terminal PDAC patients suggest that the degree of heterogeneity in the actionable ''proteome'' is likely to be profound. It is conceivable that each tumor contains all particular subtypes with areas of active and inactive (normal) stroma, foci of inflammation, and variable epithelial cellularity. Elucidating how these levels of heterogeneity impact response to therapy is likely to be the next chapter in our evolving understanding of the PDAC molecular landscape.

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