Molecular pathology selected abstracts

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Diagnosing and targeting pancreatic cancer at the single-cell level

May 2019—Pancreatic ductal adenocarcinoma is a highly aggressive cancer that is often unresectable at the time of diagnosis. It also is frequently metastatic and associated with a high mortality rate. One approach to reduce the poor outcomes associated with this disease could involve early detection and treatment of the cancer before it develops the ability to invade and spread to other organs. A known precursor lesion to pancreatic ductal adenocarcinoma (PDAC) is intraductal papillary mucinous neoplasia (IPMN), with high-grade dysplasia in this setting harboring the greatest risk of developing into PDAC. IPMN has been shown to contain mutations in important cancer-associated genes, such as *KRAS, GNAS,* and *TP53,* that can distinguish them from benign pancreatic lesions. However, the genetic alterations that can identify the IPMN subtypes that will progress to cancer are not known, and no methods exist to detect lesions that contain such alterations in patients who are suspected of having a pancreatic abnormality and are undergoing evaluation for pancreatic cancer. The authors sought to address this problem by developing a method using 5,400 cells obtained from surgically resected pancreatic tissue from six patients. To ensure that the changes they identified were specific to predicting progression to PDAC, they recruited two IPMN patients with low-grade dysplasia, which has a low risk of progression; two patients with high-grade dysplasia; and two patients with invasive PDAC. Using droplet-based single-cell RNA sequencing to study the transcriptional profiles of epithelial cells derived from the patients' specimens, the authors found specific clusters of transcribed genes that were associated with various stages of cancer progression. Patients with low-grade dysplasia expressed genes previously described in this category, such as the apomucin-encoding gene *MUC5AC* and the *RAP1GAP* gene that can suppress invasion and metastasis. Transition to high-grade dysplasia resulted in pathway changes, including regulation of cell death and activation of cell-signaling pathways, such as Wnt/β-catenin pathway activity and small GTPase expression, whereas transition to PDAC showed increased cell proliferation, changes in TGF-β signaling, and increased oxidative phosphorylation, among other alterations. The authors also examined changes in the cells surrounding the epithelial lesion, which can include stroma (supporting cells), inflammatory cells, and blood vessels. Progression to PDAC resulted in a reduction in cytotoxic T cells, which have been reported to monitor and attack cancer cells in some settings, as well as a reduction in pro-inflammatory stromal molecules. These findings suggest that progression to pancreatic cancer may involve evasion of immune cell recognition of the cancer. The results of this study also suggest that early identification of pancreatic cancer may be possible, which is relevant given that IPMN lesions with low- and high-grade dysplasia are often present years before the development of pancreatic cancer. In many cases, patients undergo cytopathology evaluation in which a low number of cells have been obtained for microscopic analysis. Single-cell analysis could expand opportunities to more definitively diagnose precursor lesions and treat patients definitively before the onset of invasive disease. This study has identified numerous known and new molecular alterations that could be applied in the context of targeted therapy in early disease. Additional applied research in this area could be valuable in reducing the mortality associated with pancreatic cancer.

Bernard V, Semaan A, Huang J, et al. Single-cell transcriptomics of pancreatic cancer precursors demonstrates epithelial and microenvironmental heterogeneity as an early event in neoplastic progression. *Clin Cancer Res.* 2019;25(7):2194–2205.

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Use of a plasmid-based approach to protect against Zika virus infection

Zika virus infection has raised public health concerns given its prevalence in many parts of the world, including

areas of the United States, and its detrimental effects on fetal development. Fetal infection can result in microcephaly, or a smaller than normal head size, and severe defects in brain function. Zika virus infection can also impact adults, causing a loss of the nerve covering myelin and, potentially, paralysis. The virus can survive in the brain and the testis, leading to persistent infection, and spread through sexual contact and mosquito-borne transmission when it is present in the blood. Given the potential severity of the disease and its transmission risk, much effort has been devoted to investigating ways to prevent Zika virus infection. One area of study involves immune system recognition of the Zika virus, such as developing vaccines and other methods of enhancing immune response. Patients who have recovered from Zika virus infection show a persistence of antibodies that can protect against new infection, and antibodies against Zika virus proteins can prevent infection in animal models that have not been exposed to the virus. Using this knowledge, the authors developed a method of manufacturing DNA constructs, called plasmids, that can produce protective antibodies once injected into mice and nonhuman primates and mimic protection due to a prior infection. This method avoids the problems related to antibody storage, such as rigorous temperature control due to the less stable nature of proteins. The authors developed synthetic plasmid DNA that could produce a human monoclonal antibody that has been shown to generate protection against the Zika virus (mAb ZK190) or a mutated variant of this antibody as a control against nonspecific binding effects (mAb ZK190-LALA). The plasmids were injected into the muscle of mice or rhesus macaque monkeys and an electrical current was used to help transfer the DNA into the cells. Analysis of antibody status showed that active antibody against the Zika virus was present in the blood within the first week and lasted several months. All animals injected with the engineered plasmids showed protection against symptoms of the Zika virus, in contrast to animals that were injected with control plasmids. In the laboratory, the antibodies produced from these engineered plasmids could bind to a protein derived from the Zika virus, showing that the antibodies produced were specific to this virus. This study represents the first time DNA constructs that generate protective antibodies have been shown to work in nonhuman primate models, which may lead to clinical trials using DNA plasmids encoding antibodies against the Zika virus in humans. This may also represent a preventative approach against other types of viral infections. Importantly, the rapid onset of antibody titers and effectiveness of the antibodies produced may be crucial in an outbreak, in which short-term protection is critical until the longer term immunity derived from vaccinations takes effect. While human clinical trials are necessary to demonstrate the safety and viability of this approach, these early findings may help in developing short-term interventions in high-risk viral infections.

Esquivel RN, Patel A, Kudchodkar SB, et al. In vivo delivery of a DNA-encoded monoclonal antibody protects non-human primates against Zika virus. Mol Ther. 2019;27(5). https://doi.org/10.1016/j.ymthe.2019.03.005.

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