Pancreas Pathology in the Era of Whole Genome Sequencing

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In the late 1970s Frederick Sanger showed that chain-terminating dideoxynucleotide analogs that cause base-specific termination of primed DNA synthesis could be used to sequence DNA. The "Sanger Method" (also referred to as dideoxy sequencing or chain termination) quickly became the gold standard for sequencing, and ten years ago this month the nearly complete sequence of the human genome was published. In 2003 the 13-year long effort to sequence the human genome was completed at a cost of $2.7 billion. The hope, if not expectation, was that the unveiling of our genetic blueprint would dramatically impact the war against human diseases. Progress has, in fact, been relatively slow, and some large scale efforts to capitalize on the human genome project, such as DeCode Genetics in Iceland, have even gone into bankruptcy. In the entire field of pathology, with perhaps the exception of hematopathology and microbiology, it is particularly hard to point to specific examples where sequencing has entered our daily diagnostic practice in a substantial way.

In 1997 Shankar Balasubramanian and David Klenerman showed that individual DNA molecules attached to microspheres could be sequenced, and this advance formed the basis for new sequencing strategies. Massively parallel pyrosequencing platforms, referred to as "next generation sequencers," were commercially introduced in 2005, and these new machines can now be used to sequence entire human genomes in a matter of days for less than $10,000. This is a revolutionary technological advance; an advance that has the potential to fundamentally change our understanding of human diseases.

Cancer is particularly amendable to next generation sequencing because cancer is essentially a genetic disease- a disease caused by inherited and acquired mutations in specific cancer-causing genes. In addition, hundreds of tumor and normal biospecimens are removed from patients every day in thousands of hospitals across this country. These biosamples are waiting to be studied by the right people. Pathologists, as the keepers of the tissues, as the physicians with unique insight into the cellular compositions of cancer, and as physicians with a solid understanding of the relevant clinical issues, are in a truly unique position to apply next generation sequencing approaches to the study of human cancers, and to translate the discoveries made back to patient care.

This talk provides examples of how pathologists can play a key role in the sequencing of neoplasms, and how the resulting understanding of the genetics of these neoplasms can be used to improve patient care.

Genetic alterations in a variety of pancreatic neoplasms have been correlated with tumor histology and with patient clinical features and a new "molecular-pathologic classification" of pancreatic neoplasia has been developed. For example, ductal adenocarcinomas of the pancreas are genetically characterized by four "mountains"- the *KRAS*, *TP53*, *SMAD4* and *p16/CDKN2A*

genes are each mutated in >50% of ductal adenocarcinomas of the pancreas. By contrast, for example, solid-pseudopapillary neoplasms almost all have *CTNNB1* (beta-catenin) gene mutations. These genetic differences can be exploited diagnostically since immunostains are available for the protein products of many of these genes. For example, most solid-pseudopapillary neoplasms have an abnormal nuclear pattern of labeling for the beta-catenin protein, while most pancreatic neuroendocrine neoplasms have an intact membranous pattern of labeling.

An understanding of the genetic alterations in hypothesized non-invasive precancerous lesions can be used to establish that these lesions are, in fact, precursors to invasive cancer. The identification of the same cancer-causing mutation in a putative precursor lesion and in its associated invasive carcinoma is strong evidence that the precursor gave rise to the invasive cancer. Importantly, as the costs of sequencing drop, it is hoped that methods can be developed to detect the genetic alterations shed by precursor lesions before the lesions progress to invasive cancer.

At the other end of the spectrum, genetic sequencing has been used to understand the pathology of the genetic evolution of metastases. S. Yachida and C. Iacobuzio-Donahue sequenced multiple metastases and paired primary pancreatic cancers from patients who underwent a rapid autopsy, and used the sequence alterations identified as "molecular clocks" to define the timing and patterns of metastases in the pancreas. They discovered that, on average, the cell that gives rise to an invasive carcinoma genetically diverges from normal cells an average of 20 years before the patient dies of metastases. Studies such as this define the genetic heterogeneity of metastases, and highlight a huge window of opportunity for the early detection of pancreatic cancer.

Whole exome sequencing has also been used to discover new familial pancreatic cancer predisposition genes. Whole exome sequencing was used by S. Jones et al, to discover that *PALB2* (partner and localizer of BRCA2) is a familial pancreatic cancer predisposition gene. As more neoplasms and more patients are sequenced, it is clear that genes which slightly increase one's risk, as well as highly penetrant genes will be discovered. The challenge will be to integrate all of this knowledge into actionable clinical information.

As demonstrated by the EGFR stories in lung and colorectal neoplasia, specific genetic alterations are being discovered which can be used to select patients for the therapy that optimally exploits the genetic changes in their cancers. Pancreatic cancer patients whose cancers harbor genetic alterations in genes coding for proteins in the Fanconi anemia pathway may benefit from mitomycin C and from PARP inhibitors, and Villarroel et al have shown that pancreatic cancers with *PALB2* gene mutations are exquisitely sensitive to mitomycin C.

The exomes of ten pancreatic neuroendocrine neoplasms (PanNETs) were recently sequenced and a new cancer pathway (*DAXX/ATRX* gene mutations) and "targetable" mTOR pathway gene mutations were discovered.

It is clear that we have a very long way to go before the full genetic complexities of cancer are understood, and even longer before a large body of evidence-based medicine can be used to

establish the specific situations in which genetics can aid the practicing pathologist. Next generation sequencing, however, has greatly shortened the distance to the "goal line."

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