Abstract 4137: Clonal evolution defines the natural history of metastatic pancreatic cancer

Alvin P. Makohon-Moore, Ming Zhang, Johannes G. Reiter, Ivana Bozic, Fay Wong, Yuchen Jiao, Krishnendu Chatterjee, Martin A. Nowak, Nickolas Papadopoulos, Bert Vogelstein, Kenneth W. Kinzler, and Christine A. Iacobuzio-Donahue

Author Affiliations

1Johns Hopkins Medical Institutions, Baltimore, MD; 2Institute of Science and Technology Austria, Klosterneuburg, Austria; 3Harvard University, Cambridge, MA; 4Memorial Sloan Kettering Cancer Center, New York, NY.

Proceedings: AACR 106th Annual Meeting 2015; April 18-22, 2015; Philadelphia, PA

Abstract

INTRO: Pancreatic adenocarcinoma (PDAC) patients are often diagnosed with metastatic disease, yet the extent to which metastasis is determined by genetic evolution is unknown. A better understanding of this lethal process would have great clinical significance.

METHODS: We selected four patients from the Johns Hopkins Rapid Medical Donation Program using strict criteria that included diagnosis with Stage IV disease and no treatment prior to death. Tissues from each patient were macrodissected to reduce stromal cell contamination and genomic DNA was extracted and quantified. A set of high quality primary or metastatic samples from these four patients (n = 35, range 4-11 per patient) were whole genome sequenced using the Illumina Hi-Seq 2000 platform, ultimately generating a raw list of 165,815 potential variants. After alignment to the hg19 human reference genome, the variants were filtered for single nucleotide polymorphisms, sequence directionality, and allele frequency in order to limit sequencing artifacts while maximizing detection of clonal changes. Subsequently, we selected 3256 somatic variants for targeted resequencing of the original 35 samples and in an additional 146 samples of the primary carcinoma or metastases (range 5-53 additional samples per patient).

RESULTS: Whole genome sequencing resulted in a median high quality distinct coverage of 63X per genome and a median effective coverage of 97.9%. Point mutations in KRAS were identified at allele frequencies ranging from 8-88%. Targeted sequencing resulted in a median distinct coverage depth of 189X across all samples. We developed a robust statistical method to determine the mutation status across the samples of a patient based on an estimated false positive rate of the targeted sequencing of 0.23%. After discarding samples with low purity or low coverage, we found high homogeneity in the mutation profiles across samples from the primary tumor and samples from different metastases in each patient. We show that for many metastases there exists a corresponding sample from the primary tumor differing only in a few positions. The majority of mutations were either present in all or in only one sample of a patient. The low number of remaining parsimony-informative mutations prevents the generation of precise evolutionary trees. However, using hierarchical clustering and our newly developed phylogenetic method, we were able to infer basic evolutionary relationships among the samples.

CONCLUSIONS: The natural history of metastatic PDAC is defined by the accumulation of a large number of mutations within the primary tumor, including most of the driver mutations. Indeed, evolutionary analyses indicate that metastases have relatively few unique mutations and are by-products of clonal evolution within the primary tumor over a prolonged period.


©2015 American Association for Cancer Research.