Molecular Pathology Selected Abstracts, 1/17

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Tumor profiling and patient outcomes in genotype-matched clinical trials

Molecular profiling of tumors with next-generation sequencing can provide important diagnostic and prognostic information that can be used to inform treatment strategies. There is an abundance of information in the literature about molecular and genomic characteristics of solid tumors, but less is known about how these characteristics could influence clinical care for individual patients. In two prospective studies—the Integrated Molecular Profiling in Advanced Centers Trial (IMPACT) and Community Molecular Profiling in Advanced Cancers Trial (COMPACT)—conducted at the Princess Margaret Cancer Centre, in Toronto, Canada, oncologists use molecular profiling information on solid tumors to enroll patients in clinical trials with targeted therapies. In studies conducted from March 2012 to July 2014, formalin-fixed, paraffin-embedded archival tumor and matched peripheral blood samples from 1,893 patients were analyzed by three molecular profiling assays: a MALDI-TOF mass-spectrometry platform to genotype 279 mutations in 23 genes, MiSeq sequencer covering regions of 48 genes, and Ion Proton sequencer covering regions of 50 genes. Variants were then classified using a five-class scheme. Tumor profiling was successful in 1,640 of the patients, and these patients' molecular information was entered into their electronic medical records. Treating oncologists, often with the aid of a multidisciplinary molecular tumor board, used this information to plan treatment strategies. A total of 245 patients were subsequently enrolled in therapeutic clinical trials, including 84 in genotype-matched trials. The latter were defined as trials restricted to specific somatic mutations, trials in which a targeted drug inhibited a biological pathway linked to the specific somatic mutation, or trials in which targeted drugs had been shown to have increased activity in patients with the specific somatic mutations. Most of the patients enrolled in genotype-matched trials had somatic mutations in *PIK3CA, KRAS, BRAF,* and *EGFR*. When comparing patients enrolled in genotype-matched trials with those enrolled in genotype-unmatched trials, no significant difference was found in age or gender distribution or number of previous treatments. However, genotype-matched trials were significantly more likely to be phase one studies and significantly more likely to involve treatment with targeted drug combinations without chemotherapy or immunotherapy. The overall response rate, as measured by tumor shrinkage, was significantly higher in genotype-matched trials—19 percent versus nine percent in genotype-unmatched trials—although no significant difference in overall survival was seen. While the difference in response rate was significant, a rate of only 19 percent in the genotype-matched trials suggests a potential for improvement, possibly by refining models or using synergistic therapies or alternative treatment strategies. In addition, a multivariate analysis showed that the only significant predictors of tumor response were enrollment in a genotype-matched trial and female gender. One disappointing outcome was that only five percent of patients enrolled in the IMPACT or COMPACT trials were ultimately enrolled in a genotype-matched clinical trial, a finding seen in many similar studies at academic cancer centers. Overall, however, although the study is a nonrandomized comparison, it demonstrated that patients are more likely to achieve tumor response if selected for a clinical trial based on molecular tumor profiling.

Stockley TL, Oza AM, Berman HK, et al. Molecular profiling of advanced solid tumors and patient outcomes with genotype-matched clinical trials: the Princess Margaret
IMPACT/COMPACT trial. *Genome Med.* 2016;109. doi:10.1186/s13073-016-0364-2.

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Comprehensive molecular profiling of mucinous gastric carcinoma

Mucinous gastric carcinoma composes 2.6 percent to 8.7 percent of all gastric cancers and is characterized by abundant extracellular mucin. It has an aggressive clinical course and is associated with advanced stage and poor survival. It is often refractory to intraperitoneal chemotherapy. The authors conducted a study in which they evaluated 68 patients who had mucinous gastric carcinoma at various clinical stages. They classified the tumors into differentiated or undifferentiated mucinous gastric carcinoma. The authors extracted DNA from formalin-fixed, paraffin-embedded tissue and performed whole exome sequencing on a series of 16 carcinomas. A median of 62.5 mutations per case was identified, corresponding to a somatic mutation density of 1.9 mutations per megabase. Three tumors, all of which lacked MLH1 expression by immunohistochemistry, were found to be hypermutated, with greater than 1,000 nonsilent mutations. Based on the whole exome sequencing results and findings from previous studies, a targeted sequencing panel of 114 genes was developed and performed on a subsequent set of 52 mucinous gastric carcinomas. The most frequently mutated gene was *TP53*, which was mutated in 56 percent of carcinomas, followed by *ARID1A* (21 percent), *CDH1* (21 percent), *MLL2* (19 percent), *RBMXL3* (19 percent), and *MLL3* (15 percent). The authors found that mutations affecting chromatin structure and histone methylation appear to play a key role in mucinous gastric carcinoma, as 32 of the 68 tumors were found to have somatic mutations in any of nine genes related to chromatin remodeling. For example, the mutations affecting the *MLL2* and *MLL3* genes were shown to be nonsense or frameshift mutations that resulted in the production of truncated proteins lacking histone methyltransferase activity. Another recurrently mutated gene was *MYH9*, seen in nine of the 68 tumors, all of which were undifferentiated mucinous gastric carcinomas. The *MYH9* gene encodes the heavy chain of nonmuscle myosin IIA, a component of the cytoskeleton, although its biological significance in gastric carcinoma was unclear. Using siRNA knockdown in two gastric carcinoma cell lines, the authors demonstrated that loss of *MYH9* increased cell migration, reduced cell adhesion, and, in one of the cell lines, caused a signet ring phenotype. Therefore, mutations in *MYH9* could represent a key event in the development of undifferentiated mucinous gastric carcinoma. Finally, the two- tiered histological subclassification of mucinous gastric carcinoma into differentiated and undifferentiated subtypes revealed clinical and molecular differences. The patients with undifferentiated carcinoma were shown to have increased nodal involvement, more advanced stage, and an overall poorer prognosis than those with differentiated mucinous gastric carcinoma. Furthermore, when comparing molecular aberrations, differentiated mucinous gastric carcinoma was found to be similar to intestinal-type gastric carcinoma, whereas undifferentiated mucinous gastric carcinoma appeared to be biologically distinct.

Rokutan H, Hosoda F, Hama N, et al. Comprehensive mutation profiling of mucinous gastric carcinoma. *J Pathol.* 2016;240:137–148.

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