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Analysis of integrative clinical genomics of metastatic cancer

The use of tumor genome profiling to match patients to appropriate therapies and clinical trials is growing, particularly in the setting of advanced and often metastatic disease. Although metastatic tumors share key driver mutations with the primary site from which they arise, they frequently display additional mutations acquired during dissemination or treatment, or both. This suggests that it would be preferable to base therapeutic decisions on the genomic profile of a metastatic tumor sample rather than on a specimen from the primary tumor. Much of the data used to inform these personalized therapeutic decisions has been generated using tumor tissue from a primary site, with the genomic profiles of metastatic tissue samples being less well characterized. The authors conducted a large-scale clinical study using a combination of whole exome and transcriptome sequencing (referred to as integrative sequencing) to characterize 500 cancer patients with metastatic tumors. The cohort included 20 tumor types, the top three of which were metastatic prostate (18.6 percent), metastatic breast (18.2 percent), and soft tissue sarcomas (8.4 percent). The most common sites of metastases were liver (n=134), lymph node (n=114), lung (n=46), bone (n=42), and abdominal cavity/pleural fluid (n=32). The authors performed paired exome sequencing on tumor and germline tissue for each patient and identified an average of 119 somatic mutations per tumor. The most commonly mutated tumor-suppressor genes included *TP53* (53.2 percent), *CDKN2A* (16 percent), *PTEN* (15.8 percent), and *RB1* (13.6 percent), while the most commonly mutated oncogenes were *PIK3CA* (13.4 percent), *AR* (12.6 percent), and *KRAS* (10.2 percent). Through the use of germline sequencing, 12.2 percent of cases were found to harbor potentially pathogenic germline variants, the majority of which were related to DNA repair pathways. To identify known or potentially pathogenic gene fusions, the authors performed transcriptome sequencing on 496 metastatic tumor RNA samples. They detected at least one putative pathogenic fusion in 199 (39.8 percent) cases, with 138 activating fusions and 103 deleterious fusions resulting from translocations (38 percent), inversions (25 percent), deletions (15 percent), and duplications (22 percent) detected overall. The transcriptomes were also used to evaluate the expression profiles of the metastatic sites. The findings suggest that metastatic tumor tissue is dedifferentiated relative to primary tumor tissue and that metastatic tumors fall into one of two subtypes: an epithelial to mesenchymal transition group or proliferative group. Overall, this study is the first large-scale demonstration of combined whole exome and whole transcriptome sequencing in a clinical setting to scrutinize metastatic tumor samples. It serves to highlight the potential value of this approach in more completely categorizing these heterogeneous cancers functionally. Although the study did not directly compare matched primary and metastatic samples, it offers evidence that such a paired sequencing approach may provide additional clinically relevant information regarding genomic changes during tumor evolution and the therapeutic implications of these changes.

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Direct detection of early stage cancers using circulating tumor DNA

The use of cell-free DNA in screening or diagnostic applications is proving increasingly valuable. In patients with cancer, a small fraction of such DNA is derived from tumor cells and is referred to as circulating tumor DNA (ctDNA). Multiple studies have reported on the use of ctDNA as a liquid biopsy, with the expectation that mutational analysis of this material will reflect findings from a tissue biopsy of the tumor, thereby providing a less invasive means of tumor genotyping. Most studies of this type have been performed with late-stage disease. While some of these studies have been promising, others have demonstrated that the concordance may not be as expected and that the analytic sensitivity and specificity required to analyze ctDNA are difficult to achieve. The authors suggest that improvements to the technical aspects of analysis may extend the use of ctDNA to direct detection of early stage disease. They used a library preparation modified to minimize errors and deep sequencing (approximately 30,000×) followed by an optimized bioinformatic analysis to assess a panel of 55 driver genes frequently mutated in lung, colorectal, breast, and ovarian cancers. Furthermore, because mutations have been found in the blood of healthy people (presumably representing early stage clonal hematopoiesis), they analyzed three additional genes related to hematopoietic proliferation and used the results to eliminate false positives due to this cause. Using a series of mixed samples containing tumor cell line DNA diluted with varying amounts of normal DNA, the authors demonstrated a sensitivity of 100 percent for an allele fraction of 0.2 percent. No mutations were found in the plasma samples from 44 healthy subjects evaluated with this approach. An analysis of plasma samples from 194 untreated patients with localized or metastatic cancer (breast, 45; colorectal, 42; lung, 65; and ovarian, 42) demonstrated that 62 percent of stages I and II patients and 77 percent of stages III and IV patients had detectable driver gene mutations. Overall, the authors identified 313 candidate tumor-specific mutations in the plasma samples of 128 of the 194 patients. They further evaluated 216 of these mutations in 100 patients for whom matched tumor tissue and blood cells were available. The authors found that 155 of the 216 (72 percent) mutations were identical in both tissue and plasma. When examined by tumor stage, they found 77 percent concordance of variants in stages III and IV and 68 percent concordance for alterations in stages I and II. They also found that 70 of 75 (93 percent) alterations with a mutant allele fraction greater than one percent in the plasma were detected in the tumor tissue sample of the same individual. Finally, to assess the possibility that tumor heterogeneity may lead to a lack of plasma/tissue concordance, the authors examined 10 regions of the primary tumor as well as a metastatic site from a colorectal cancer patient found to have a *GNAS* mutation in the plasma but not in the tumor. Whereas a *BRAF* V600E mutation was found in all sites, the *GNAS* mutation was found in the plasma but only a portion of the primary tumor, suggesting that it represents a subclonal change developed later in tumorigenesis. Overall, the analytical performance characteristics of the approach used in this study suggest that continuing refinements to ctDNA sequencing assays may make them suitable for use in multiple applications, including early detection of disease.

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