Molecular pathology selected abstracts

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VEXAS: an autoinflammatory disease associated with the *UBA1* gene

May 2021—Autoinflammatory and rheumatologic disorders in adults often present with overlapping clinical features and are challenging to treat. A genotype-first approach has been helpful to guide proper clinical management in other analogous disease settings. The authors conducted a study in which they analyzed the exome/genome sequencing data of peripheral blood cells from two large cohorts: 1,477 people who had undiagnosed recurrent fevers or systemic inflammation, or both, and 1,083 people affected by atypical, unclassified disorders who were identified through the National Institutes of Health Undiagnosed Diseases Program. Recurrent novel missense mutations affecting the methionine-41 codon of the X-linked gene *UBA1*, which codes for a major E1 enzyme that initiates ubiquitylation, were identified in three men. These *UBA1* mutations were confirmed as somatic because they were not found in the matched fibroblasts of the affected individuals and their family members. *UBA1* M41 missense mutations were also identified in 22 additional study participants who presented with symptoms overlapping those of the aforementioned three men. These *UBA1* mutations were absent in a control population comprising 77,162 unaffected adults and 64,438 adults referred for diagnostic testing for neurodevelopmental disorders. Furthermore, the *UBA1* mutations appeared to be restricted to the myeloid cells in peripheral blood and immature progenitor cells in bone marrow, suggesting impaired hematopoietic proliferation in the mutant cells. Common clinical features observed in the 25 study participants with *UBA1* mutations included male gender, adult-onset inflammatory manifestations (including chondritis and inflammatory skin lesions such as dermatosis and vasculitis), cytopenia, thromboembolic disease, and highly elevated levels of serum acute-phase reactants (erythrocyte sedimentation rate and C-reactive protein). Almost half the patients met the diagnostic criteria for a hematological neoplasm, such as myelodysplastic syndrome or multiple myeloma/monoclonal gammopathy of undetermined significance. All patients had vacuoles observed in their myeloid and erythroid precursor cells, which led to the name VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome. All 25 patients were refractory to treatment with multiple disease-modifying antirheumatic drugs, except high-dose glucocorticoids. The clinical outcome was dismal, with 10 (40 percent) patients succumbing to disease-related manifestations. Consistent with the elevated serum cytokines (interleukin-8, interferon-inducible protein 10, and interferon-γ) observed in these VEXAS patients, gene-expression profiling of the mutated cells revealed a picture of cell-intrinsic severe inflammation characterized by upregulation of genes involved in inflammatory response pathways, including tumor necrosis factor, interleukin-6, and interferon-γ. In addition, functional studies of neutrophils isolated from the VEXAS patients revealed dysregulated proinflammatory activation. In the cultured cells and mutant monocytes isolated from VEXAS patients, the authors demonstrated that missense mutations affecting codon Met41, which is the initiation codon that produces the cytoplasmic isoform UBA1b, resulted in a switch of UBA1b to the catalytically impaired UBA1c isoform that was initiated at the downstream start codon Met67. This isoform switch resulted in decreased ubiquitin activation and activated innate immune pathways. It was observed in the mutant monocytes isolated from the VEXAS patients and in a zebrafish model with CRISPR-engineered knockout of the cytoplasmic UBA1b isoform. The authors concluded that testing for *UBA1* gene mutations in adult patients with an autoinflammatory condition, and in some adult patients with cytopenias and myeloid cell vacuolization, could help guide clinical management of these patients.

Beck DB, Ferrada MA, Sikora KA, et al. Somatic mutations in *UBA1* and severe adult-onset autoinflammatory disease. *N Engl J Med*. 2020;383(27):2628–2638.

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Clinical-grade WGS and 3′ transcriptome analysis of colorectal cancer patients

Disease-focused next-generation sequencing gene panels and, to a less extent, whole exome sequencing are the mainstream clinical assays for identifying therapeutic targets in colorectal cancer patients. However, the PCR-free whole genome sequencing (WGS) method has a number of advantages over the aforementioned methods, including increased coverage of the difficult-to-sequence regions intrinsic to the amplicon- or hybridization-based approaches used by the targeted panels or whole exome sequencing (WES). WGS also offers increased accuracy in calling structural variants (gene fusions and copy number alterations) and tumor mutational burden (TMB). To evaluate the potential clinical utility of WGS, the authors analyzed clinical-grade WGS coupled with 3' RNA-seq on the fresh-frozen tumor and paired normal sample in 54 patients diagnosed with sporadic primary colorectal cancer (CRC). The read depth was at least 60× for the tumor samples. Germline and somatic mutations were called using the bioinformatics pipeline. The coding-region mutations most frequently identified were in known CRC-associated cancer genes (*APC, TP53, KRAS, FBXW7, PIK3CA,* and *BRAF*) and in novel drivers not previously reported in colorectal cancer, including those that would potentially be targetable by molecular agents, such as *KIT, ATM,* and *SMARCA4*. Similarly, the differentially expressed pathways in this cohort, as identified by transcriptome analysis, included the p53 signaling pathway, NF-kappa-B signaling pathway, and "colorectal cancer pathway," each of which were known to be overexpressed in CRC. Among other novel pathways that were overexpressed was one associated with platinum drug resistance. This was clinically relevant since oxaliplatin is commonly given as a part of the adjuvant regimen in CRC. In structural variant analysis, recurrent and unique gene fusions that may be targetable were also found in this cohort, including the *FGFR2/3* fusion (a target for pemagatinib in cholangiocarcinoma), *BRAF/DLG1* (potentially targetable by BRAF kinase inhibitors), and *ERBB2/HAP1* (potentially targetable by laparinib). In addition, the high TMB phenotype that is indicative of anti-PD1 immunotherapy responses was observed in a higher proportion in this patient cohort than in a previous cohort featured in The Cancer Genome Atlas (30 percent versus 16 percent). This may be due to WGS' ability to comprehensively detect mutations when compared to other methodologies. Furthermore, many recurrently mutated noncoding regions were identified for the first time in this study's patient cohort and may warrant further investigation. Overall, this study demonstrated that WGS can reveal additional "druggable" alterations that the targeted panel or WES approach would not be able to capture in colorectal cancer. There are practical limitations to this comprehensive methodology, namely cost, long turnaround time, and poor performance with formalin-fixed, paraffin-embedded material (the common sample type for CRC). However, the combination of WGS and RNA-seq has potential to become the future standard of clinical care for colorectal cancer molecular pathology.

Stodolna A, He M, Vasipalli M, et al. Clinical-grade whole-genome sequencing and 3′ transcriptome analysis of colorectal cancer patients. *Genome Med*. 2021. doi:10.1186/s13073-021-00852-8

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