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Mutations causing acquired resistance to PD-1 blockade in melanoma

Immunotherapy in metastatic cancer has achieved durable responses in a wide variety of cancer types. Antibodies that block programmed cell death protein-1 (PD-1) are particularly effective in metastatic melanoma, but a recent study showed that approximately 25 percent of patients that achieved a durable response ultimately had disease progression at a median follow-up of 21 months. Although the mechanism for tumor progression is not well understood, it has been proposed that alterations in the interferon signaling pathway can cause resistance to immunotherapy. The authors of this study examined 78 patients who had metastatic melanoma and had been treated with the anti-PD-1 antibody pembrolizumab. Of these 78 patients, four met all three selection criteria: an objective initial tumor response to pembrolizumab therapy, late resistance to therapy after more than six months of tumor response, and adequate biopsy material before and after therapy. The paired tumor samples for these four patients were analyzed by whole exome sequencing. It was found that the relapsed tumors were closely genetically related to their original tumors prior to therapy. However, in two of the patients, the relapsed tumors showed new homozygous deleterious mutations in pathways associated with interferon receptors. The first patient developed a Q503* nonsense mutation in the gene encoding Janus kinase 1 (*JAK1*), while the second developed a F547 splice-site mutation in the gene encoding Janus kinase 2 (*JAK2*). To further examine the functional effects of these mutations, the authors established cell lines from the second patient, one from the tumor prior to therapy with wild type JAK2 and a second from the relapsed tumor after acquiring the *JAK2* F547 splice-site mutation. Western blot analysis showed that the initial tumor responded to interferon alpha, beta, and gamma signaling with appropriate activation of downstream targets. However, the cell line that acquired the *JAK2* mutation showed a complete lack of response to interferon gamma and was ineffective in upregulating pathways involved in antigen presentation and T-cell chemotaxis. Finally, the authors demonstrated causality by engineering *JAK1* or *JAK2* mutations into a separate melanoma cell line and showing that the mutations rendered the cell line resistant to interferon-induced growth arrest. In a third patient, exome sequencing revealed a homozygous frame-shift deletion in exon 1 of the β-2-microglobulin component of major histocompatibility complex (MHC) class I. Immunohistochemical studies showed a loss of membrane localization of MHC class I consistent with β-2-microglobulin being required for proper MHC folding and localization to the membrane. The authors suggested that this mutation could be an alternative mechanism for acquired resistance to immunotherapy. No mutations were identified in the fourth patient that could potentially explain the acquired resistance to immunotherapy. With the increased use of immunotherapy, it will be important to understand the molecular causes of acquired resistance.

Zaretsky JM, Garcia-Diaz A, Shin DS, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma [published online ahead of print July 13, 2016]. *N Engl J Med.* doi:10.1056/NEJMoa1604958.

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Human SRMAtlas: mass spectrometric assays to quantify the human proteome

The ability to reliably detect and reproducibly quantify any protein in the human proteome would revolutionize the

study of human disease in a fashion similar to the advances seen since the discovery of the human genome and the development of next-generation sequencing. However, the proteome is not well-characterized, as tools to investigate it are cumbersome and have low throughput, and complicating factors, such as alternative splicing and post-translational modifications, abound. Mass spectrometric and affinity-based techniques are used to detect and quantify proteins. Mass spectrometric methods can be broadly characterized as shotgun or targeted. In both techniques, proteins initially are enzymatically digested into peptides. In shotgun techniques, these peptides are separated by chromatography, ionized, and then subjected to tandem mass spectrometry in which collision-induced dissociation breaks the peptide ions into fragment ions that are detected via a survey scan. These fragment ion spectra are interpreted, and the proteins from which the peptides originated can be inferred. This method allows the identification of thousands of proteins in a given sample. However, it is not well-suited for accurate quantification. Alternatively, spectrometric method selected reaction monitoring (SRM) is a targeted quantitative technique with a lower limit of detection, wider dynamic range, and increased reproducibility. In this method, a triple quadrupole mass spectrometer filters the peptide ions, then subjects only selected ions to collision-induced dissociation, and finally isolates predetermined fragment ions for quantification. While extremely precise, it has a drawback: the optimal peptides for each protein must be predefined and the assay parameters experimentally optimized. The authors of this study generated a database of SRM assays for targeted identification and quantification of any human protein. They identified all 20,277 proteins and 14,677 isoforms in the human proteome and then selected unique peptides to unambiguously identify each human protein. These peptides were selected based on predicted and empirical biophysical properties that make them suitable for SRM assays. Additional peptides were then added to the library to account for sequence variants and post-translational modifications. The final library consisted of 166,174 peptides that provided coverage of 99.9 percent of the predicted human proteome. To develop the SRM assays, all 166,174 peptides were individually chemically synthesized and analyzed via quadrupole time-of-flight mass spectrometry (MS) to create spectral libraries at multiple collision energies. These data were then used to obtain chromatographic traces and full MS/MS spectra using triple quadrupole and quadrupole-linear ion trap mass spectrometers in the SRM assays. The authors were able to recover 95.1 percent of the synthesized peptides in the SRM assays, for 99.7 percent coverage of the predicted human proteome. All of the data are available in a free, searchable database (www.srmatlas.org), allowing users to search for SRM assays for any human protein. The data in the database include precursor and fragment ion type, charge state, elution times, chromatograms, and MS/MS spectra, and are integrated with bioinformatics knowledge. Finally, the authors performed two pilot studies to demonstrate the utility of the SRMAtlas. The first examined the effect of statins on cellular cholesterol regulation by quantifying enzymes in cholesterol synthesis pathways. The second study examined docetaxel treatment in differentially responsive prostate cancer cell lines and correlated mRNA transcriptional data with corresponding protein products. The authors concluded that, overall, the human SRMAtlas allows investigators to reliably identify and reproducibly quantify any protein in the human genome. There will be ample opportunity to use these assays in systems biology and biomedical research projects to revolutionize understanding of the human proteome.

Kusebauch U, Campbell DS, Deutsch EW, et al. Human SRMAtlas: a resource of targeted assays to quantify the complete human proteome.*Cell.* 2016;166:766–778.

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