TUMOUR IMMUNOLOGY

Helping tumour antigens to the surface

Omics-based analyses, mainly involving transcriptomics, have amplified our understanding of the mutational profile of melanoma and the make-up of tumour-infiltrating immune cell populations. Now, using high-resolution proteomic analysis of clinical samples from patients with advanced melanoma, Harel et al. show that the metabolic state of melanoma is associated with response to immunotherapies.

Recently published in *Cell*, the study presented analyses of samples from 116 patients with stage IV melanoma who were treated with tumour-infiltrating lymphocyte (TIL; $n=42$)-based or anti-programmed cell death protein 1 (PD1; $n=74$)-based immunotherapy; in total, 61 patients showed partial or complete responses. Stable-isotope labelling with amino acids in cell culture-based high-resolution LC-MS/MS analysis of dissected melanoma regions with >80% tumour cells revealed 10,376 non-redundant protein groups, with further data filtration producing a final list of 4,400–4,600 proteins, thus covering a range of intracellular processes. When comparing non-responders with responders, 414 proteins or 636 proteins were differentially expressed in the TIL cohort or the anti-PD1 cohort, respectively.

Next, the researchers performed functional cluster analysis based on Kyoto Encyclopaedia Genes and Genomes pathway annotations. In both cohorts, high levels of metabolic proteins (specifically, proteins of the tricarboxylic acid cycle, fatty acid oxidation and ketone body metabolism) as well as antigen presentation-related

proteins (including type I and type II interferon (IFN) signalling proteins) dominated the responder group. By contrast, spliceosome and RNA metabolism-related proteins dominated the non-responder group. The dominance of mitochondrial metabolism and IFN signalling-related pathways in responders was also apparent when both cohorts were pooled and subjected to weighted gene correlation network analysis, and on the RNA level based on published RNA sequencing datasets of similar cohorts (although, here, the enrichment was lower than at the proteome level).

Exploring more specifically which proteins were of importance in determining treatment responses, a support vector machine-based classification helped the researchers to produce an 8-protein signature that identified responders in the TIL cohort, and a 15-protein signature that identified responders in the anti-PD1 cohort. However, these protein signatures did not overlap — the TIL response signature contained proteins of fatty acid and ketone body metabolism, whereas the anti-PD1 response signature contained antigen presentation-related proteins. Still, a protein interaction network created on the basis of significantly altered protein expression in responders versus non-responders in both cohorts showed that the mitochondria–IFN network was associated with response in both patient cohorts. In line with this, proteins of the TIL signature were upregulated in anti-PD1 responders compared with non-responders; and vice versa, antigen presentation-related proteins present in the anti-PD1 signature

were higher in TIL responders than in non-responders.

Encouragingly, expression levels of three proteins of the TIL signature (ACAT1, SUPV3L and HTATIP2) were associated with longer progression-free survival (PFS) in the TIL cohort, and most of the anti-PD1 signature proteins were associated with longer PFS in the anti-PD1 cohort (associations that were not found when analysing RNA expression levels).

Next, the authors turned to cell culture experiments, testing whether increasing mitochondrial respiration in melanoma cell lines would lead to changes in antigen presentation-related proteins. Proteomic analysis of cells treated with dichloroacetate to induce respiration confirmed their hypothesis, showing increased expression of immunoproteasome components and MHC class I proteins (resulting in increased HLA surface presentation), amongst others. Likewise, when either of the TIL signature genes (*ACAT1* and *HADHA*) or the key fatty acid oxidation gene *CPT1A* were knocked out using the CRISPR–Cas9 system, expression levels of antigen presentation-related proteins and IFN-signalling proteins were reduced compared with control cells. Overall, the reduced functionality of the antigen presentation machinery affected specific T cell-mediated killing in co-culture experiments. *Acat1*-knockout tumours derived from YUMMER1.7 mouse melanoma cells grew more quickly in mice and showed reduced MHC class I expression and lower CD8⁺ T cell infiltration compared with control tumours. These results showcase the significance of mitochondrial metabolism in melanoma cells for antigen presentation and T cell-mediated killing in vivo.

This study provides an important proteomics resource for further studies into the mechanisms and biomarkers that determine immunotherapy responses, and contributes to our understanding of the immune–metabolic network.

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