

CHARACTERIZING CANCER'S DARK MATTER, SHORT-LIVED PROTEINS, AND DEFECTIVE RIBOSOMAL PRODUCTS, PRESENTED BY CANCER AND CONTAINED IN THE DPV-001 CANCER VACCINE

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Background Short-lived proteins (SLiPs), defective ribosomal products (DRiPs), and Cancer's dark matter/non-canonical peptides (NCP) are unstable and rapidly degraded, loaded onto MHC and represent a large proportion of the epitopes presented by cancer cells. Recent data suggests that NCP may play a role in molecular control of some malignant processes, further strengthening the rationale for characterizing their presence and developing pharmacologic and immunologic approaches to impact their effect.¹ Immunologic approaches seem particularly promising as NCP, that are not expressed in the thymus, represent potential shared alternative cancer neoantigens.² Our group developed a vaccine strategy that concentrates SLiPs, DRiPs, and NCP in dendritic cell-targeted microvesicles.³ Preclinical combination immunotherapy studies documented efficacy in difficult to treat animal models^{4 5} and an off-the-shelf human vaccine was developed and has entered clinical trials. The current studies were undertaken to characterize the breadth of peptides presented by head and neck squamous cell cancer (HNSCC) and non-small cell lung cancer (NSCLC), as well as the proteins contained in the DPV-001 vaccine.

Methods Cancer cells were lysed and recovered lysates were treated with detergent in the presence of protease inhibitor. HLA peptides were collected from HLA complexes purified by anti-HLA-I antibody (w6/32). Recovered HLA peptides were analyzed by the microLC-QTOF MS (LCMS-9030. Shimadzu Corporation). The two components of DPV-001, UbiLT3 and UbiLT6, were assessed via deep proteomic profiling on an Orbitrap mass spectrometer (ORBITRAP FUSION LUMOS with FAIMS-Pro interface (ThermoFisher Scientific)). Spectra from whole proteome tryptic digests were acquired in data-dependent acquisition (DDA) mode, and Peptide-Prism was used to identify canonical and noncanonical peptides/NCPs.

Results The approaches outlined above provide evidence of canonical and NCP, derived from noncoding or out-of-frame translations, presented by HLA of HNSCC cell lines and contained within DPV-001. These peptides provide a starting point for immunological studies.

Conclusions These results support our efforts to identify shared canonical and NCP that are targets of therapeutic immune responses. The discovery of NCP derived from supposed non-coding regions that are not expressed in the thymus, represent a novel class of potentially shared alternative cancer neoantigens. Their association with malignant processes may provide them properties similar to that of driver mutations, and increase their relevance as cancer vaccine targets.

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