1376 REGULATION OF NON-CANONICAL PROTEINS ENCODED BY SMALL OPEN READING FRAMES VIA NONSENSE-MEDIATED DECAY PATHWAY

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Background Immunotherapy has shown great promise in the treatment of cancer, but its effectiveness is limited by the availability of neoantigens on cell surfaces.[1] While PD-L1 inhibitors have demonstrated encouraging outcomes in clinical trials for advanced melanoma and non-small cell lung cancer, their applicability is restricted to a select group of patients.[2] In a recent study by Chong et al.,[3] it was found that non-canonical proteins encoded by the human genome may possess peptides that can act as neoantigens, providing a promising avenue for immunotherapeutic targeting. However, these proteins tend to be expressed at low abundance thereby reducing their abundance and restricting their utility.

Methods In the present study, we adopted a proteogenomics methodology to identify multiple non-canonical proteins synthesized by breast cancer cell lines. To investigate whether the production of intracellular non-canonical proteins is enhanced and whether non-canonical protein-derived neoantigens are upregulated, we perturbed a conserved quality control mechanism within cells.

Results These non-canonical proteins exhibited low abundance and inconsistent expression patterns, which could be attributed to the degradation of these proteins by conserved quality control mechanisms in cells, such as the nonsense-mediated decay (NMD) pathway. By targeting this pathway through the knockdown of UPF1, a key regulator of NMD,[4] we were able to increase the levels of non-coding transcripts and non-canonical proteins. We also observed increased expression of unannotated transcripts and human leukocyte antigen transcripts associated with antigen presentation. Our in vivo analysis of UPF1 protein expression in triple-negative breast cancer (TNBC) patient tumor samples (n=48) demonstrated that UPF1 is highly expressed at the protein level compared to adjacent stroma. Moreover, our expanded analysis of UPF1 transcript expression across multiple cancer types revealed that UPF1 is highly expressed in cancer tissues compared to adjacent normal tissue.

Conclusions Our observations suggest that TNBC and other cancer types upregulate the NMD pathway to suppress the production of mutant transcripts and non-canonical proteins, which could potentially mask neoantigens presentation on cancer cell surfaces. By modulating the expression level of UPF1, we could potentially increase the reservoir of neoantigens and enhance neoantigen presentation, ultimately augmenting immunotherapeutic responses in cancer patients.

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