1020 PROTEOMIC ANALYSIS OF T CELL EXHAUSTION UNVEILS DIFFERENTIAL MODULATION OF THE DNA DAMAGE RESPONSE

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Background T cells are subjected to a variety of genotoxic events in the tumor microenvironment including replication stress, off-target toxicity of chemotherapy and radiation therapy, and mitochondrial-derived reactive oxygen species.[1] Our preliminary data suggests that accumulation of DNA damage accelerates T cell exhaustion. Using an antibody-stimulation model of T cell exhaustion, we performed proteomic analyses to compare regulation of the DNA damage response in acutely stimulated T cells versus chronically stimulated T cells.[2]

Methods Human CD8+ T cells were isolated from three healthy donors and stimulated using CD2/3/28 beads either once (acute stimulation), or every 2 days for eight days (chronic stimulation). Samples were taken at days 4, 6, and 8 for proteomic analysis and at day 8 for phospho-proteomic analysis through the IDEA National Resource for Quantitative Proteomics. To validate exhaustion, flow cytometry for exhaustion markers was performed at days 2, 4, 6, and 8, and qPCR for T cell phenotype markers was performed at day 8.

Results Flow cytometry, qPCR, and proteomic analysis confirmed an increase in exhaustion markers after chronic stimulation. In our proteomic analysis, we observed downregulation of pro-apoptosis factors including PYCARD, Bax, and several Caspases. Mismatch repair proteins including ERCC4, MLH1, and PMS2 were upregulated in exhausted T cells. Interestingly, we observed a downregulation of ATM, a kinase upstream in the DNA damage response to double-stranded breaks, while it's downstream repair factor 53BP1 is upregulated. Phospho-TMT analysis revealed that 53BP1 is inactivated through phosphorylation in chronically stimulated T cells.[3] Overall, we identified 654 proteins and 1176 phospho-sites which were differentially regulated, log2FC >1 or <-1 with an adjusted p-value < 0.05.

Conclusions T cells must be able to overcome DNA damage to continue to proliferate and perform its cytotoxic functions. We found that DNA damage contributes to T cell exhaustion. To combat this, exhausted T cells alter their proteome to promote DNA repair and downregulate apoptotic mechanisms, but ultimately the T cell still becomes dysfunctional. These results highlight repair pathways which could be exploited in future T cell therapies.

REFERENCES

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