STUB1 REGULATES IFNGR1 EXPRESSION AND IFNG SENSITIVITY IN HUMAN TUMOR CELL LINES

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Background Interferon gamma (IFNG) is a cytokine that is essential for anti-tumor immune activity in a variety of tumor models and settings. IFNG activity induces responses important for anti-tumor immunity, such as antigen processing and presentation molecules, but can also induce immunosuppressive molecules such as PD-L1. IFNG additionally exhibits direct growth inhibition or apoptosis-inducing potential in some cell settings. Regulation of IFNG sensing in tumor cells therefore represents a potential mechanism to enhance anti-tumor immunity. STUB1 is a ubiquitin ligase that has been reported to target the IFNG receptor IFNGR1 for degradation. Inactivation of STUB1 may therefore increase IFNGR1 expression and cellular sensitivity to IFNG. Here, we aimed to study the effects of genetic inactivation of STUB1 on IFNG responses in human tumor cell lines.

Methods CRISPR-Cas9 was used to genetically inactivate STUB1 in the human tumor lines A-375 (melanoma), A549 (NSCLC) and H441 (NSCLC). Successful modification and knockout were validated by Sanger sequencing and Western blotting. IFNGR1 expression was measured by flow cytometry. IFNG response was measured through multiple assays, including upregulation of canonical IFNG-inducible surface markers (PD-L1 and MHC-I), secretion of IFNG-inducible chemokines (CXCL9 and CXCL10), and inhibition of cell growth.

Results STUB1 knockout resulted in higher protein expression levels of IFNGR1 in all tested cell lines. This effect was specific to IFNGR1, as other measured surface receptors did not change on untreated STUB1 knockout cells. STUB1 knockout cell lines all showed elevated responses to IFNG, with enhanced sensitivity (lower concentration of IFNG necessary to induce a response in knockout cells) and/or enhanced efficacy (higher maximal detected response to IFNG in knockout cells) in a cell line and assay-dependent manner.

Conclusions STUB1 inactivation increases IFNGR1 expression and increases cellular responsiveness to IFNG treatment. This increase is detected in pathways that are important for anti-tumor efficacy (e.g., growth inhibition, MHC-I expression) as well as pathways that contribute to immune evasion (e.g., PD-L1 expression). Further investigation of the role of STUB1 in promoting immune sensitivity is warranted and STUB1 inhibition may be attractive to enhance anti-tumor immune responses in appropriate settings.

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