**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 (small ubiquitin-like modifier protease 1) has been reported to target the IFNG receptor IFNGR1 for degradation (1,2). 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**

- Human tumor lines A-375 (melanoma), A549 (NSCLC) and NCI-H441 (NSCLC) were purchased from ATCC and maintained as recommended. STUB1 knockout (KO) cells were generated by nucleofection with CRISPR-Cas9 ribonucleoprotein complexes (RNPs). Successful modification and knockout (KO) were validated by Sanger sequencing and Western blotting.

- IFNGR1, PD-L1, and MHC-I (HLA-ABC) protein expression were measured by flow cytometry using the CellTrace Glo 2.0 Cell Viability Assay (Promega) and a colony formation assay.

- Early apoptosis and secondary necrosis were measured via the Annexin V Apoptosis and Necrosis Assay (Promega).

- Recombinant human IFNG for cell experiments was purchased from R&D Systems.

**Results**

**A549 STUB1 KO Cells Show Enhanced Sensitivity to IFNG Treatment**

- **A)**: Cell viability of A549 cells in response to treatment with IFNG. 5,000 cells were seeded in triplicate and treated for 4 days. Relative luminescence units (RLU) were normalized to untreated cells on a per-cell-line basis. (Left) Flow cytometry analysis of MHC-I expression in A549 CC cells following IFNG treatment. Baseline (no IFNG treatment) levels are indicated by solid or dashed lines. (**B)**: Flow cytometry analysis of PD-L1 expression in A549 CC cells following IFNG treatment. Baseline (no IFNG treatment) levels are indicated by solid or dashed lines. (Right) Flow cytometry analysis of PD-L1 expression in A549 CC cells following IFNG treatment. Baseline (no IFNG treatment) levels are indicated by solid or dashed lines.

**A549 STUB1 KO Cells Show Enhanced Levels of Growth Inhibition in Response to IFNG Treatment**

- **A)**: Flow cytometry analysis of MHC-I expression in A549 CC cells following IFNG treatment. Baseline (no IFNG treatment) levels are indicated by solid or dashed lines. (**B)**: Flow cytometry analysis of PD-L1 expression in A549 CC cells following IFNG treatment. Baseline (no IFNG treatment) levels are indicated by solid or dashed lines.

**H441 STUB1 KO Cells Show Enhanced Sensitivity to IFNG Treatment**

- **A)**: Flow cytometry analysis of MHC-I expression in H441 cells at baseline and with IFNG treatment. 20,000 cells were seeded in triplicate and treated for 24 hours. Data show quantification of all live cells. (**B)**: Flow cytometry analysis of PD-L1 expression in H441 cells at baseline and with IFNG treatment. 20,000 cells were seeded in triplicate and treated for 24 hours. Data show quantification of all live cells.

**Summary**

- 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.

**References**

1. Ng, S. et al. (2022). Scientific reports vol. 12, 1 14087.

Figures were prepared with BioRender.