Checkpoint Blockade Inhibitors in Combination with Radiation Therapy in Breast Cancer

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INTRODUCTION

Emerging data implicate stress-inducible heat shock protein 70 (HSP70) in the cytoprotection of malignant cells against chemotherapeutic agents and radiation as well as in generation of immunosuppressive tumor microenvironment. Recent clinical studies have established that increased levels of tumor-infiltrating lymphocytes (TILs) in TNBC predicted better clinical outcomes compared to other subtypes. These observations led to the hypothesis that women with TNBC may respond to checkpoint blockade therapy. However, early results from these trials using checkpoint inhibitors alone or with chemotherapy has shown very little promise in breast cancer patients. We recently demonstrated that TNFα distinctly induces A20 in TNBC subtype and protects these cells from TNFα-induced cytotoxic cell death by upregulating HSP70 protein and maintaining EMT/CSC phenotype. In contrast, luminal MCF7 or ZR75-1 cells display approximately 70% apoptosis when treated with TNFα. Furthermore, we show that A20/HSP70 pathway attracts tumor-infiltrating lymphocytes (TILs) while inducing the accumulation of immunosuppressive MDSCs in syngeneic mouse models. Interestingly, pulmonary DTCs as well as the immune infiltrates from 4T1 tumor-bearing mice exhibited significantly higher HSP70 expression. Therefore, we proposed that targeting HSP70 may potentiate the efficacy of immunotherapy when combined with radiation in preclinical models of breast cancer. To test the effectiveness of a combination of checkpoint blockade inhibitors and radiation therapy, this study utilized 6-week old female BALB/c 4T1 tumor-bearing mice as a model for TNBC. Combination treatment of HSP70 inhibitors and targeted irradiation was performed prior to tumor resection and data collection.

RESULTS

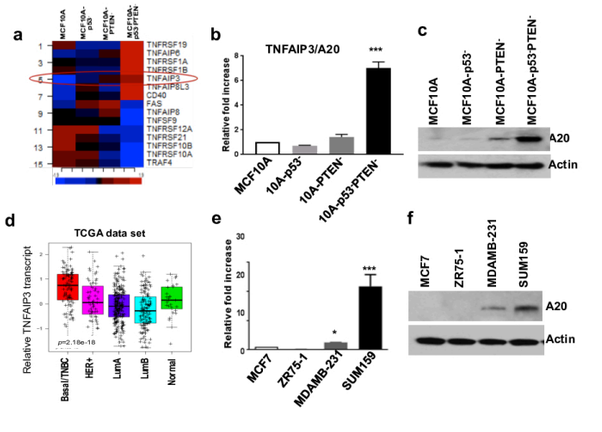

Figure 1. TNFAIP3/A20 is highly upregulated in transformed MCF10A-p53-PTEN- cells and patient basal/TNBC subtype. (a) TNFα-induced genes including TNFAIP3 (red circle) is highly expressed in MCF10A-p53-PTEN- cells. (b) Expression of TNFAIP3/A20 was verified by qPCR and (c) Western blot analyses. (d) TCGA data set indicates the upregulation of A20 in Basal/TNBC breast cancer subtype. (e, f) Higher expression of A20 in TNBC cell lines, compared with luminal subtype was confirmed by Western blot and qPCR assays. Results are presented as mean ±SD. *P<0.05, ***P<0.0001.

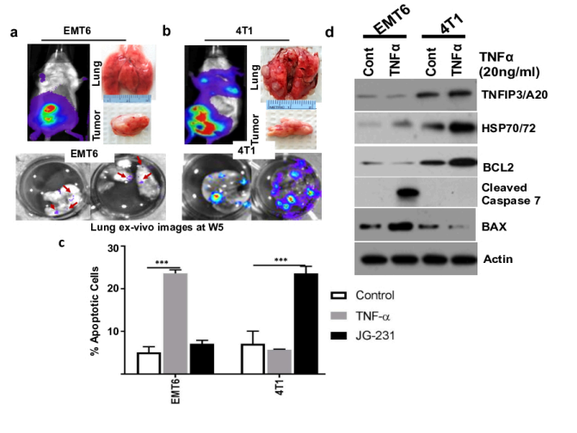

Figure 2. HSP70 protects metastatic 4T1 tumor cells from TNFα-induced apoptosis. (a, b) Both 4T1 and EMT6 murine breast cancer cells grow similar size primary tumor, while only 4T1 tumors generates spontaneous metastasis in syngeneic BALB/c mouse model. **(c)** TNFα induces apoptosis in non-invasive EMT6 tumor cells while it fails to do so in metastatic 4T1 tumor cells which are sensitive to HSP70 inhibitor, JG231. **(d)** TNFα stimulates the induction of pro-apoptotic proteins as well as the cleavage of Caspase 7 in EMT6 cells while it induces the expressions of anti-apoptotic proteins and HSP70 in metastatic 4T1 cells. . *P<0.05, **P<0.001, ***P<0.0001.

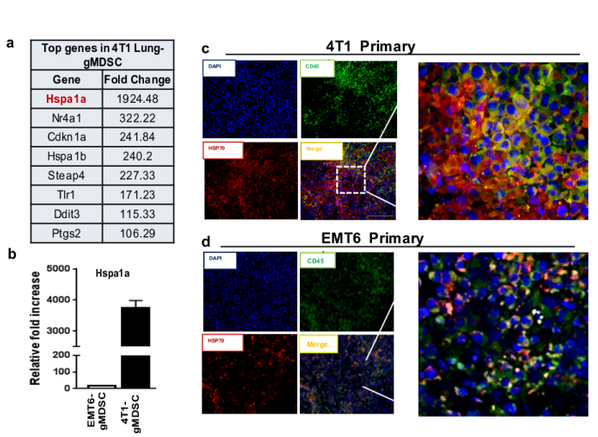

Figure 3. Elevated HSP70 (*Hspa1a*) expressions in tumor microenvironment. (a) Mouse transcriptome analyses revealed a significantly higher Hspa1a expression in lung-gMDCs derived from 4T1 tumor-bearing mice compared to the EMT6. **(b)** Elevated Hspa1a expression in 4T1 lung-derived gMDSCs is validated by qPCR. **(c)** Murine 4T1 tumors exhibited significantly higher HSP70 expression (red) and immune infiltrated as shown by CD45 staining (green). **(d)** EMT6 tumors expressed significantly lower HSP70 and showed CD45+ immune infiltrates.

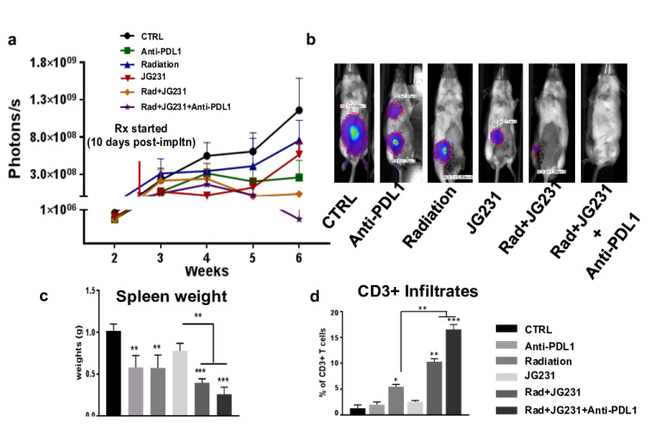

Figure 4. Blockade of HSP70 using JG-231 sensitizes tumor cells to Radiation and potentiate the efficacy of check point blockade inhibitors. (A, B) 4T1 tumor-bearing mice were treated with HSP70 inhibitor, anti-PDL-1 antibody or radiation alone or in combination as indicated. Tumor growth over the course of 6 weeks was monitored by luciferase intensity and tumors at the end point were shown. **(C)** Spleen weight of animals treated with indicated drugs. **(D)** CD3+ immune infiltrates were analyzed by flow cytometry and shown that mice treated with triple combination showed a highest level of CD3 infiltrates. Increased immune infiltrates may have exhibited anti-tumor immune response eliminating tumors.

CONCLUSIONS

- The studies outlined in this presentation test a novel hypothesis that HSP70 plays a dual role; protects tumor cells from cytotoxicity and radiation by inducing EMT/CSC phenotype, while regulating immunosuppression by promoting the MDSC accumulation.
- Our studies in most relevant syngeneic mouse model provided evidence that HSP70 blockade significantly enhances the efficacy of immunotherapy/radiation herapy by reducing the primary tumor growth more than 20-fold and eliminating metastasis.
- Furthermore, there was more than 10-fold increase in tumor-infiltrating lymphocytes when animals treated with triple combination. Therefore, proven that our preclinical is supported in other models, our approach may potentiate the effectiveness of check point blockades in clinical settings leading to improved therapeutic options for breast cancer.