P08.07 IDENTIFICATION OF STRESS GRANULE FORMATION AS A THERAPEUTIC TARGET IN CHEMOTHERAPY TREATED COLORECTAL CANCER

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Background Under certain stress conditions, such as oxidative stress or nutrient deprivation, specific RNA-binding proteins aggregate with actively translated mRNAs to facilitate translational reprogramming and cell survival.[1] High levels or deregulated activity of these RNA-binding proteins, which include Ras GTPase-activating protein-binding protein 1 (G3BP1) or Y-box-binding protein 1 (YB-1) contribute to tumour progression and metastasis.[2] Inhibition of stress granule (SG) formation may therefore exert a synergistic effect with cytotoxic chemotherapy.

Materials and Methods Formalin-fixed paraffin-embedded sections from neoadjuvant-treated colorectal cancer (CRC) liver metastasis patients (n=33) were immunohistochemically (IHC) stained for YB-1. CRC cell-lines as well as organoids and tissue slice cultures from surgical specimen were treated with oxaliplatin/5-fluorouracil alone or in combination with the histone deacetylase inhibitor (HDACi) MS-275. Incubation with arsenic acid served as positive control for SG aggregation. Immunofluorescence co-staining of YB-1 and G3BP1 was used to detect SG formation. Cell viability and apoptosis induction were analysed using viability (cellular adenosine triphosphate) and cytotoxicity (lactate-dehydrogenase release) assays, flow-cytometry (active caspase 3, viability dye) and IHC (haematoxylin & eosin, active caspase 3, Ki-67).

Results In the cohort of CRC liver metastasis patients, YB-1 protein expression was a negative predictor for overall survival. Oxaliplatin-based chemotherapy induced SG formation in CRC cell-lines and primary tumour tissue culture. Pre-treatment with the HDACi MS-275 prevented stress-granule aggregation and increased the sensitivity of CRC cell lines to oxaliplatin.

Conclusions Clinical data and CRC cell-line or primary tissue cultures identify SG formation as a resistance factor for chemotherapy and as a therapeutic target in CRC.

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P08.08 IMMUNOMODULATION BY LACTATE DEHYDROGENASE C INDICATES A POTENTIAL NEW OPPORTUNITY FOR COMBINATION THERAPY

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Background Despite the success ofcancer immunotherapy in the treatment of advanced cancer, the clinical benefit is limited to a subgroup of patients. One of the major challenges remains the lack of a durable anti-tumor immune response within an immunosuppressive tumor microenvironment. Cancer testis antigens (CTAs) are lucrative anti-cancer targets with restricted expression patterns and defined roles in multiple cancer hallmarks. Lactate dehydrogenase C (LDHC) is a promising target with a highly tumor-specific expression that correlates with poor prognosis in breast cancer. We previously reported that silencing *LDHC* improves treatment response to DNA damage response drugs through dysregulation of the DNA damage response pathway. Here, we investigated the effect of *LDHC* silencing on the immune response to gain insight into the potential benefit of combining LDHC targeting with immunotherapy.

Materials and Methods Breast cancer transcriptomic data from TCGA and METABRIC were used to assess *LDHC* expression and association with cytotoxic T lymphocyte (CTL) infiltration. LDHC silencing of breast cancer cell lines was followed by analysis of immune-related gene expression (RT2 Profiler Cancer Inflammation & Immunity Crosstalk array), cytokine protein secretion (ProteomeProfiler cytokine antibody array) and immune checkpoint expression (flow cytometry). Finally, T cell activation within a co-culture model with LDHC-silenced cells was determined by IFN-γ ELISpot.

Results Transcriptomic analysis demonstrated a higher LDHC expression in basal-like and HER2-enriched breast tumors than in luminal tumors, and a significantly poorer overall and disease-specific survival for *LDHC* expressing tumors. Tumor Immune Dysfunction and Exclusion (TIDE) analysis showed that high *LDHC* expression in Her2 (TIDE score=1.97, p=0.049) and triple negative breast tumors (TIDE score=0.466, p=0.642) dampens the beneficial effect of CTLs on overall survival. Concurrently, *LDHC* silencing of breast cancer cells induced substantial dysregulation of immunosuppressive cytokines. Furthermore, gene ontology analysis of differentially expressed immune-related genes and secreted cytokines predicted that *LDHC* silencing upregulates the granzyme-mediated cell death pathway; T cell proliferation, activation and differentiation; cytolysis and interferon gamma production while downregulating TLR signaling pathway, macrophage activation, natural killer cell activation, and monocyte and lymphocyte chemotaxis. In addition, *LDHC* silencing reduced the expression of the PD-L1 and Gal-9 immune checkpoint ligands, suggesting additional levels of immunomodulation. In line with these observations, functional analysis confirmed that *LDHC* silencing affects T cell activation in a co-culture setting.

Conclusions Our current findings suggest that targeting *LDHC* may have a dual anti-cancer benefit, impairing tumor cell survival while supporting a favorable tumor immune microenvironment. As such, LDHC-based therapy could potentially improve clinical outcome when used in combination with DNA damage response drugs or immunotherapy.

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