THERAPY-INDUCED CANCER CELL SENESCENCE PROMOTES TUMOR IMMUNOSUPPRESSION IN CHOLANGIOCARCINOMA

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BACKGROUD

Cholangiocarcinoma (CCA)

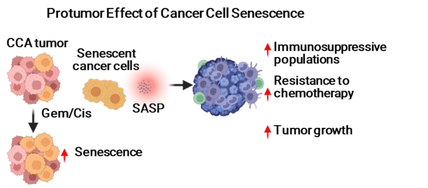
- 5-year overall survival (OS) less than 10%.
- First-line therapy (gemcitabine/cisplatin [Gem/Cis] plus durvalumab or pembrolizumab) yields a median overall survival of ~12.8 months.
- The majority of CCA patients have disease progression on first-line systemic therapy. Rapid progression suggests a treatment induced adaptation and is an unmet.
- **Unmet clinical need: identification of treatment-induced resistance mechanisms we can target.**

Senescence

- **Definition**: Characterized by an irreversible cell cycle arrest and a proinflammatory phenotype.
  - Senescent cells express p16^{INK4A}, p$^{21WAF1/Cip1}$, SA-β-galactosidase, DNA damage markers and are Ki67 low.
  - Senescence-associated secretory phenotype (**SASP**) includes a variety of growth factors, cytokines, chemokines, etc.
- **Role in cancer biology**: context dependent, both protumor and antitumor roles have been described.

HYPOTHESIS

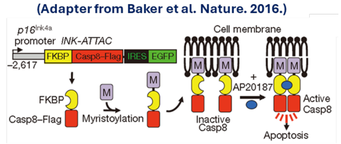
Gem/Cis Induces Tumor Senescence that Drives Tumor Immunosuppression and Progression



MATERIAL AND METHOD

- **Murine CCA cell lines**
  - *YAP^{S127A}/myr-Akt* [SB] (*Illyas et al., et al*, *Oncotarget* 2017).
  - *Kras^{G120D}p53$^{L/L}$* [KPPC] (*O'Dell et al., Cancer Res* 2012).
  - *Fbxw7ΔF/myr-Akt* [FAC] (JL Tomlinson & B Li, *et al., JHEP* 2024).
- **Syngeneic orthotopic murine models of CCA**
  - CCA cells were orthotopically implanted into livers of the mice. Mice are sacrificed 2-4 weeks after implantatio.
- **SB-*INK/p21-ATTAC* Cells (targeted senescence depletion)**

Senescence-based suicide gene cassette (Adapter from Baker et al. Nature. 2016.)



ATTAC (apoptosis through targeted activation of caspase 8)

FIGURE 1

The Preponderance of Senescent Cells in Human CCA Are Cancer Cells

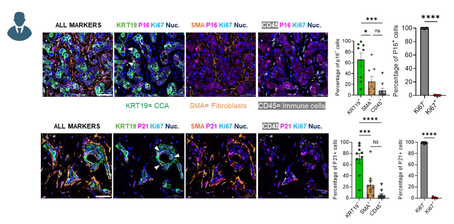


Fig. 1. The preponderance of senescent cells in human CCA are cancer cells. Sequential IF was conducted in human CCA specimens. Representative images and quantification of Senescent (P16+ or P21+) cancer cells (CK19+), CAFs (SMA+), and immune cells (CD45+) in human CCA patients. (C-D) Quantification of p16 and p21 expression in Ki67+ and Ki67- cells. Scale bar 50 μM. ***, P < 0.001; ****, P<0.0001.

FIGURE 2

Senescent cancer cells accumulate in human CCA with standard-of-care chemotherapy (Gem/Cis)

TAF: Telomere-associated DNA damage foci (≥3 Foci)

Fig. 2. Senescent cancer cells are significantly increased in chemo-therapy treated patient tumors. (**A**) Representative images and quantification of p16+CK19+ tumor cells in treatment-naive (n=18) and Gem/Cis treated patients (n=23). (**B**) Representative images and quantification of p21+CK19+ tumor cells in treatment-naïve (n=13) and Gem/Cis treated patients (n=10). (**C**) Representative images of telomere-associated foci (TAF) staining for DNA damage in treatment-naïve (n=9) and Gem/Cis treated patients (n=9). Scale bar 100 μM for A &B, 20 μM for C . *, *p*<.05; ****, *p*<0.0001.

FIGURE 3

Targeted deletion of *P16/P21+* senescent cancer cells reduces murine CCA tumor burden

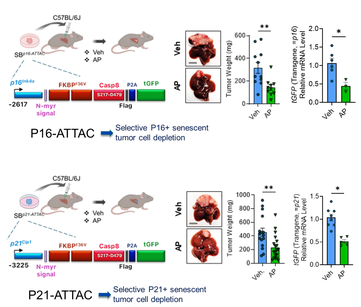


Fig. 5. Selective senescent cancer cells depletion results in decreased CCA tumor burden in mice. (**A**) Experimental strategy and schematic of ATTAC suicide cassette driven by *p16* or *p21* promoter fragment. This allows for the selective elimination of p16+ or p21+ senescent cancer cells upon administration of AP20187 (AP), which dimerizes caspase-8 causing apoptosis. Stromal cells are not affected since they don't express ATTAC. (**B**) Representative images and quantification of SB1-*p16-ATTAC* tumors treatment with vehicle or AP20187 in WT mice. (**C**) Representative images and quantification of SB1-*p21-ATTAC* tumors treatment with vehicle or AP20187 in WT mice.* , *p*<.05; **, *p*<0.001.

FIGURE 4

Cancer cell senescence is associated with low survival in both human and mouse

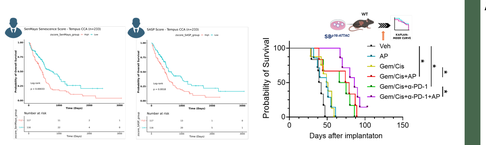


Fig. 4. Cancer cell senescence results in low survival rate in both CCA patients and tumor-bearing mice. *Kaplan-Meier* analysis of SenMayo senescence score High vs Low (left panel, *Log-rank p*=0.00033) or SASP score High vs Low (middle panel, *Log-rank p*=0.0018) in Mayo tempus CCA patient cohort (n=233). Right panel: Genetic depletion of senescent cancer cells overcomes standard-of-care cytotoxic chemotherapy(Gem/cis) ± ICI (α-PD-1) in SB$^{HI-ATTAC}$ tumor-bearing mice (n≥7). *, *P*<0.05.

FIGURE 5

Chemotherapy-induced senescence augments tumor-associated macrophages in murine CCA

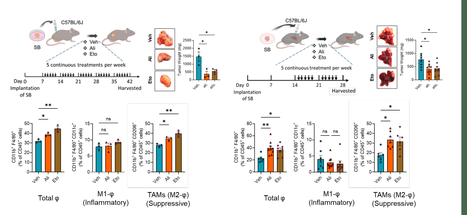


Fig. 5. Chemotherapy increase immunosuppresive TAMs in murine CCA tumors. alisertib (Aurora kinase A inhibitor) and etoposide (Topoisomerase II inhibitor) were used in subcutaneous (**Left**, n=3) or orthotopic (**Right**, n≥6) murine CCA tumor models as indicated. Percentage of Tumor-infiltrated total macrophages, M1 and M2-like TAMs of CD45+ cells in the tumors was analyzed by flow cytometry. *, *P* < 0.05; **, *P* < 0.01.

FIGURE 6

Senescent CCA cells foster Macrophage abundance and suppressive function in vitro

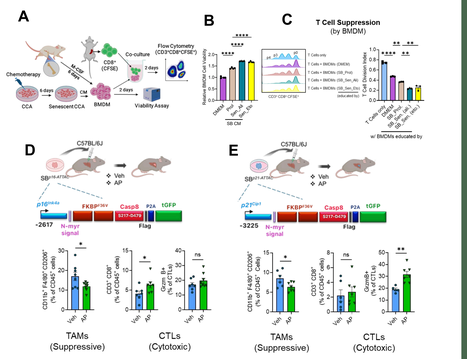


Fig. 6. Cancer senescence augments macrophage proliferation and function. (**A**) Schematic of in vitro myeloid cell studies. (**B**) Murine CCA cells (SB1) were treated with vehicle, alisertib, or etoposide. Bone marrow-derived macrophages (BMDMs) of non-tumor bearing mice were treated with conditioned medium from CCA cells and viability was assessed using CellTiter-Glo® 2.0. (**C**) BMDM were treated with proliferating (vehicle) or senescent (Ali. Eto. Palbo) CCA cell CM and subsequently co-cultured with CFSE-labeled CD8+ T cells as described in A. T cell proliferation was assessed by flow cytometry. Representative flow plots (left) and quantification (right) shown. (**D-E**) Selective elimination of P16+ (**D**) or P21+ (**E**) senescent cancer cells reduces TAM abundance and increases cytotoxic T lymphocytes (CTLs) *, *p*<0.05; **, *p*< 0.01; ****, *p*<0.0001.

FIGURE 8

Growth/differentiation factor-15 (GDF-15) is abundant in senescent human CCA cell secretome

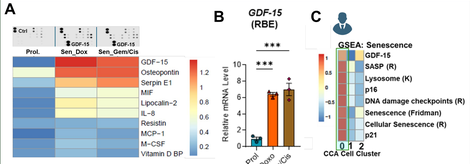


Fig. 7. GDF-15 is abundant in the secretome of senescent human CCA cells. (**A**) Conditioned medium (CM) was collected from human CCA cells (RBE) treated with either vehicle (Prol.), doxorubicin (Sen_Dox) or Gem/Cis (Sen_Gem/Cis) for 6 days to induce senescence. Proteome Profiler Human XL Cytokine Array was conducted using the CM. Representative dot blot (top) and heatmap (bottom) depicting the top ten proteins with the greatest differential increase in the senescent CCA cell secretome. (**B**) Relative mRNA level of *GDF-15* in Prol. or Sen. RBE cells was confirmed by qPCR. n=3 ***, *p*<0.001. (**C**). snRNA-seq using 9 human CCA tumors. The cluster 0 with the highest senescence signatures also shows the highest *GDF-15* expression.

FIGURE 8

GDF-15 knockdown in CCA significantly reduces TAM markers and suppressive function

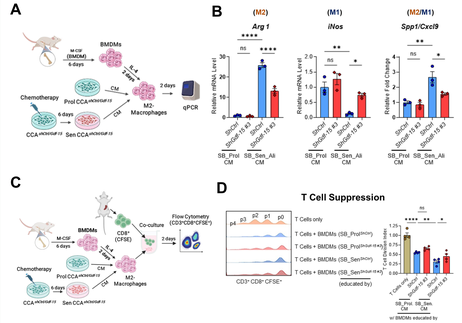


Fig. 8. GDF-15 knockdown abolishes TAM feature and suppressive function. (**A, C**) Schematic of in vitro myeloid cell studies. (**B**) M2-polarized BMDMs were treated with CM from Prol. or Sen. SB cells carrying shRNA targeting *Gdf-15*, and representative markers of M1 (*iNos, Cxcl9*) or M2 (*Arginase 1, Spp1*) were determined by qPCR. (**D**) CM treated M2-BMDMs were co-cultured with CFSE-labeled CD8+ T cells as described in C. T cell proliferation was assessed by flow cytometry. Representative flow plots (left) and quantification (right) shown. *, *p*<0.05; **, *p*<0.01; ****, *p*<0.0001.

FIGURE 9

Senescent Cancer Cell Restricted GDF-15 Knockdown Significantly Reduces CCA Tumor Burden and Immunosuppressive TIME

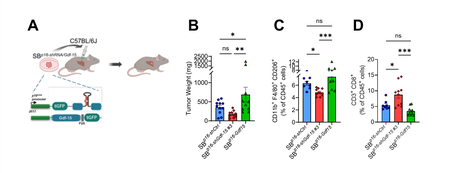
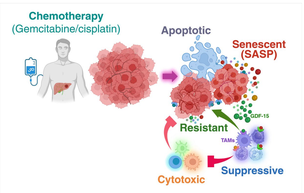


Fig. 9. Senescent Cancer Cell Restricted GDF-15 Knockdown Significantly Reduces CCA Tumor Burden and overcomes Immunosuppressive TIME. (**A**) Schematic of *in vivo* studies. (**B**) 4 weeks post implantation, mice were sacrificed and tumor weight were measured. (**C, D**) Percentage of Tumor-infiltrated TAMs (**C**) or CD8+ T cells (**D**) of total CD45+ immune cells were determined by flow cytometry. *, *p*<0.05; **, *p*<0.01; ***, *p*<0.001.

LAY SUMMARY

Senescent CCA Cells Drive Tumor Immunosuppression through TAMs via GDF-15



Background/Objective Cholangiocarcinoma (a type of bile duct cancer) is difficult to treat, and many patients stop responding to standard chemotherapy and immunotherapy within a few months. One possible reason is that cancer cells can enter a state called "senescence," where they stop growing but release substances that may help the tumor evade the immune system. This study aimed to understand whether chemotherapy causes this effect and to identify key factors involved.

Methods We examined tumor samples from patients with cholangiocarcinoma before and after chemotherapy. They also used mouse models to study how senescent cancer cells affect tumor growth and immune response. Laboratory experiments were performed to see how substances released by these cells influence immune cells, especially those responsible for attacking cancer. A specific protein, GDF-15, was identified and further tested.

Results Our study found that chemotherapy increases the number of senescent cancer cells in tumors. These cells release signals that weaken the immune system's ability to fight cancer, particularly by changing immune cells called macrophages into a suppressive form. Removing senescent cells in mice reduced tumor growth, improved survival, and enhanced response to treatment. The protein GDF-15 was identified as a key factor driving this immune suppression. Blocking GDF-15 reduced tumor growth and improved immune activity.

Conclusion Chemotherapy can unintentionally create conditions that help tumors resist treatment by promoting senescent cells and immune suppression. Targeting these senescent cells or blocking the effects of GDF-15 may improve treatment outcomes for patients with cholangiocarcinoma.

ACKNOLEDGEMENTS

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