Assessing the contribution of activated STAT1 and STAT3 on survival and resistance to platinum-based chemotherapy and radiation in head and neck squamous cell carcinoma cells

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Methods

- CRISPR
- IC50 of inhibition of constitutive pY-STAT1 and pY-STAT3 in 12 HNSCC cell lines by TT1-101 the dual STAT3/STAT1 inhibitor was estimated using bead-based Luminex assay

Platinum-based chemotherapy and radiation

- Sensitivity of HNSCC lines to fixed ionized radiation dosage was estimated using clonogenic assays
- TTI-101 sensitization of HN31 to cisplatin, oxaliplatin, carboplatin platinum-based chemotherapy drugs by MT

CRISPR STAT1, STAT3, HNSCC KO

- gRNA CRISPR transfection of mouse HNSCC cells ROC1, ROC2 and human UM-SCC-17B was done and single clones with validated KOs (protein, Luminex) were selected
- Clones selected from pools, ISTAT3 and STAT3 KO’s validated by Luminex. Measurements of beta-normalized values were plotted for various cell lines

MTT proliferation assay

- Effect of TTI-101 on cell growth of human HNSCC cell lines (IC50) and STAT3 KO on ROC2 cells (Fold change over day zero after 24, 48, 96, and 144 hours, in 2% FBS media) were measured by MTT

Background

- Signal Transducers and Activators of Transcription 3 (STAT3) is an oncogenic transcription factor involved in cell growth and survival.
- Activated STAT3 has been correlated with poor prognosis and resistance to chemotherapy and radiation therapy in HNSCC.
- STAT5 is a downstream target of STAT3 and is involved in radiation resistance.

Hypothesis

- STAT3 supports growth and chemoresistance while activates STAT1 leads to radiosensitization in HNSCC tumor cells

Results

- pYSTAT1 and pYSTAT3 inhibition in HNSCC
- IC50 of inhibition of constitutive pY-STAT1 and pY-STAT3 in 12 HNSCC cell lines by TT1-101 the dual STAT3/STAT1 inhibitor was estimated using bead-based Luminex assay

Platinum-based chemotherapy and radiation

- Sensitivity of HNSCC lines to fixed ionized radiation dosage was estimated using clonogenic assays
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Conclusion

The data indicates a role of activated STAT3 in growth and chemoresistance in HNSCC mice and human cell lines, while indicating the role of activated STAT1 in radiosensitization. Ongoing work to assess the effects of STAT1, STAT3 double KO in HNSCC cell growth and survival will extend the finding to the chemoresistance and radiotherapy. A more detailed analysis of the mechanisms of these effects is under way.

Discussion

- Activation of IFN/STAT1 pathway correlated with resistance to ionizing radiation (Pearson R: 0.9150, p = 0.0294) of HNSCC cell lines.
- Ability of TT1-101, the dual STAT3/1 inhibitor to inhibit cell-growth correlated with its ability to inhibit pY-STAT3 in these cells (Pearson R: 0.8354, p = 0.0014).
- Co-treatment of 3 μM of TT1-101 sensitized the chemoresistant line HN31 to platinum-based chemotherapy drugs cisplatin (IC50 shift from 14 μM to 0.01 μM) and carboplatin (IC50 shift from 163.4 μM to 0.04 μM) and oxaliplatin (IC50 shift from 15.7 μM to 2.9 μM) through successful reduction of pY-STAT3 (IC50; p=7μM), indicating the role of activated STAT3 in HNSCC cell chemoresistance.
- Beta-subunit normalized ISTAT3 protein levels via Luminex showed successful STAT3-CRISPR-KO ROC2 clones (p=0.05).
- STAT3-KO ROC2 cells proliferated in 2% serum at lower rates than control cells validating that STAT3 augments cell-growth of HNSCC tumor cells

References


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