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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Background

- Signal Transducers and Activators of Transcription 3 (STAT3) has been validated as a target in head and neck squamous cell carcinoma (HNSCC)[1,2]
- 37-75% of HNSCC tumor samples and correlated with more advanced disease stage, tumor size, and lower survival[1]
- Activated STAT1 has also been correlated with radio-resistance[2,3]
- TTI-101, the dual STAT3 and STAT1 inhibitor, inhibited growth of xenografts of pY-STAT3-high, pY-STAT1-high chemo and radioresistant cell line UM-SCC-17B by successfully reducing both pY-STAT3 and pY-STAT1 levels and downregulating oncogenic gene[1]
- Role of STAT1 and STAT3 in cell-growth and radioresistance by suing knockouts (KOs) has not been clearly delineated

Hypothesis

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

Methods

pYSTAT1 and pYSTAT3 inhibition in HNSCC

- IC50 of inhibition of constitutive pY-STAT1 and pY-STAT3 in 12 HNSCC cell lines by TTI-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 MTT

CRISPR STAT1, STAT3, STAT1/3 KO

- gRNA CRISPR transfection of mice 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, tSTAT3 and STAT1 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

Results

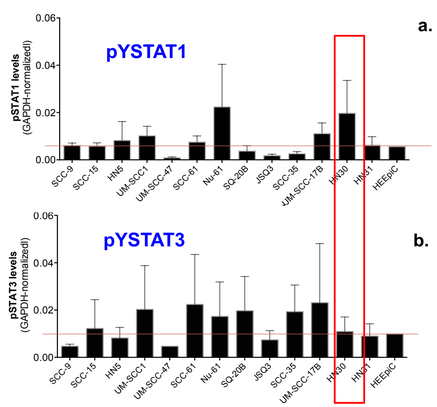


Figure 1. pY-STAT1/3 levels in 12 HNSCC lines: UM-SCC-17B selected for STAT3/1 CRISPR KO.

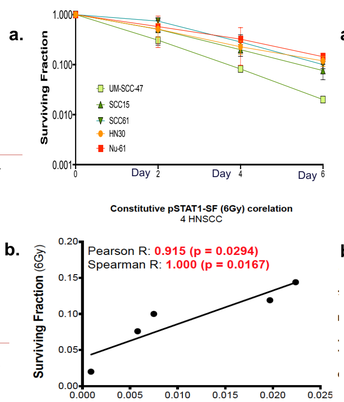


Figure 2. (a) pYSTAT1 high cells are more resistant to ionizing radiation. **(b)** pYSTAT1 activation is correlated with resistance to ionizing radiation.

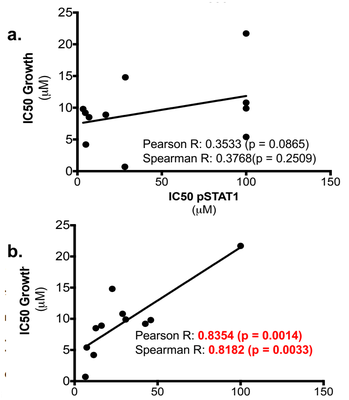
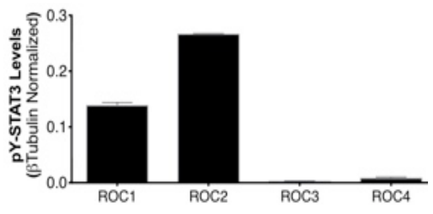


Figure 3. (a) pYSTAT1 levels are not significantly correlated with growth inhibition. **(b)** Growth inhibition correlates to pYSTAT3 inhibition in UM-SCC-17B cells.



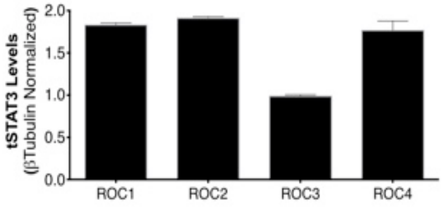


Figure 4. Roc2 beta-normalized pYSTAT3 and tSTAT3 levels. High constitutive pYSTAT3 expression was seen in Roc1, Roc2 cells was exhibited; selection of Roc1 and Roc2 as HNSCC lines for tSTAT3 CRISPR KO's. Roc2 exhibited highest constitutive pYSTAT3 activation.

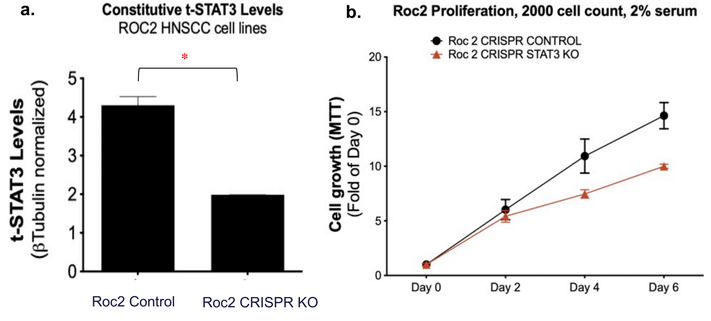


Figure 5. **(a)** Luminex validation of tSTAT3 levels in CRISPR controls and CRISPR KO Roc2 HNSCC lines ($p < 0.05$) **(b)** STAT3 CRISPR KO reduces anchorage dependent growth of ROC2 cells under low serum conditions.

Treatment	Growth Inhibition (IC50, μM)	
	HN30 (sensitive)	HN31 (resistant)
C188-9	4.2 ± 0.07	4.0 ± 0.14
CDDP	11.1 ± 0.07	14.8 ± 0.64
CDDP + C9 (3.0 μM)	1.1 ± 1.55	0.01 ± 0.00
CDDP + C9 (10.0 μM)	13.4 ± 18.9	0.01 ± 0.00
CBP	89.4 ± 3.11	163.4 ± 4.95
CBP + C9 (3.0 μM)	NA	0.04 ± 0.01
CBP + C9 (10.0 μM)	0.30 ± 0.14	0.06 ± 0.01
OXP	4.85 ± 29.56	15.70 ± 1.84
OXP + C9 (3.0 μM)	0.08 ± 0.08	2.95 ±1.06
OXP + C9 (10.0 μM)	0.42 ± 0.54	0.002 ±0.00

Figure 6. TTI-101 treatment sensitized chemo-resistant line HN31 to platinum-based chemotherapy drugs cisplatin (CDDP), carboplatin (CBP), oxaliplatin (OXP).

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 TTI-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 with 3 μM of TTI-101 sensitized the chemo-resistant line HN31 to platinum-based chemotherapy drugs cisplatin (IC_{50} shift from 14 μM to 0.01 μM), carboplatin (IC_{50} shift from 163.4 μM to 0.04 μM) and oxaliplatin (IC_{50} shift from 15.7 μM to 2.9 μM) through successful reduction of pY-STAT3 (IC_{50} ~7μM), indicating the role of activated STAT3 in HNSCC cell chemoresistance
- Beta-tubulin-normalized tSTAT3 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

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 radioresistance. Ongoing work to assess the effects of STAT1, STAT3 double KO in HNSCC cell growth and susceptibility to chemo and radiotherapy is needed to delineate the independent or synergistic effects of their activation.

References

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