T Cell Recognition of Colon Adenocarcinoma and Pancreatic Cancer Cells

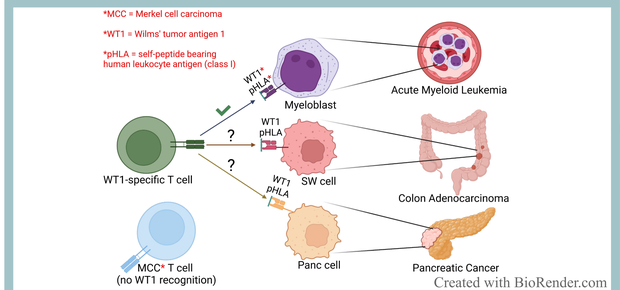
Carina Coalman[1,2], Elise Wilcox, Ph.D.[1], Aude Chapuis, M.D.[1]

[1] Fred Hutchinson Cancer Center, Clinical Research; [2] University of Washington - Tacoma, Sciences and Mathematics

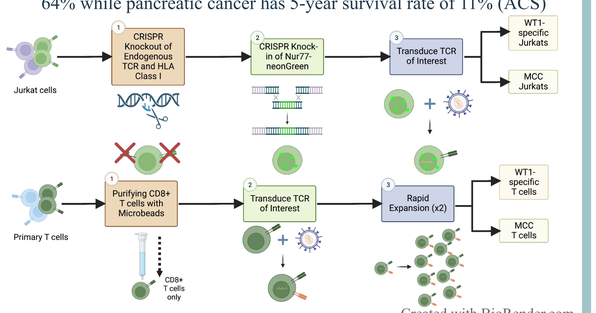
ABSTRACT

Adoptive T cell therapy, wherein a cancer patient receives T cells in an effort to fight cancerous tumors, relies on the identification of a protein whose antigen is expressed on tumor cells but not normal cells. Wilms' tumor antigen 1 (WT1) is overexpressed in a variety of solid tumor cancers, in addition to acute myeloid leukemia. WT1-specific T cells have previously been shown to be effective in recognizing and targeting myeloblasts in acute myeloid leukemia that express WT1, but efficacy of these T cells against colon adenocarcinoma and pancreatic cancer is being investigated. We determined the relative levels of WT1 expression in several colon adenocarcinoma and pancreatic cancer tumor lines via simple Western, in addition to previous work in the lab confirming HLA-A2 expression by these tumor lines using flow cytometry. WT1-specific and irrelevant Merkel cell carcinoma (MCC)-specific Jurkats were co-cultured against colon adenocarcinoma tumor lines to assess differences of Nur77 expression according to IFNg exposure. Then, WT1-specific and irrelevant MCC primary T cells were co-cultured against the same colon adenocarcinoma tumor lines along with pancreatic cancer tumor lines to assess cytokine and CD107a expression. While no significant difference was observed between Jurkat IFNg co-cultures, primary T cells appear to recognize tumor lines and demonstrate indication of cytotoxic activity through the expression of cytokines and CD107a, respectively. The SW480 and Panc10.05 tumor lines that instigated the highest T cell response to either Jurkats or primary T cells are promising targets for future T cell therapies.

BACKGROUND



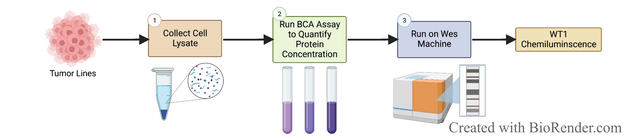
- Identifying antigens shared across various tumor lines but not normal cells is a challenge
- We are seeking out proteins that are expressed in tumor cells but not normal cells so that T cells can be transduced with receptors to recognize antigens of these proteins
- Wilms' tumor antigen 1 (WT1) is one such protein:
  - WT1 is overexpressed in acute leukemia and many solid tumor cancers, including lung, breast, colon, and ovarian cancers (Sugiyama 2010)
- Why compare colon adenocarcinoma and pancreatic cancer cells?
  - Overexpress WT1; colon adenocarcinoma has 5-year survival rate of 64% while pancreatic cancer has 5-year survival rate of 11% (ACS)

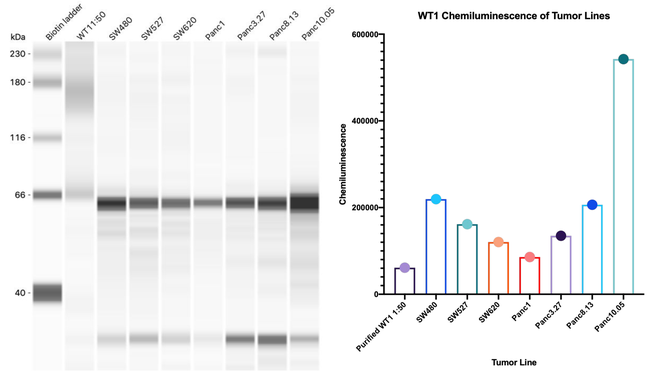


- Nur77+ % of cells correlates to T cell activation as Nur77 indicates T cell receptor (TCR) stimulation (Ashouri and Weiss 2017)
- Impact of this work: WT1-specific T cells can be used in adoptive T cell therapy to target tumor cells other than AML myeloblasts

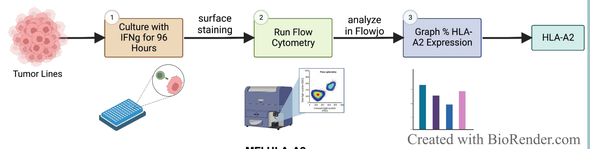
RESULTS

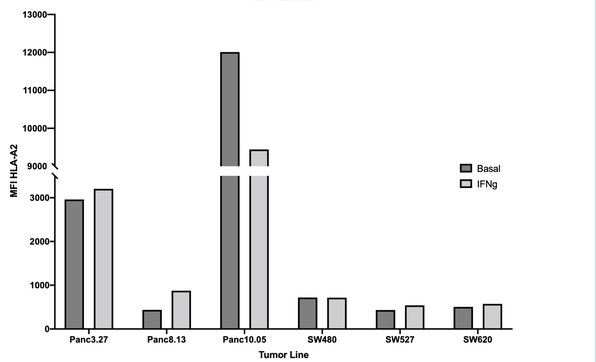
All tumor lines tested express WT1 and HLA-A2, Panc10.05 expressed highest levels of both





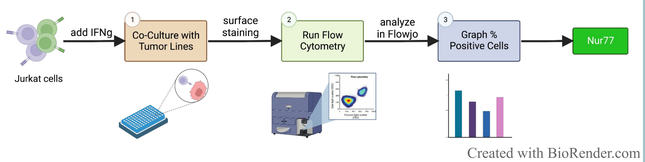
- Simple Western measures WT1 (~55 kDa) via chemiluminescence
  - 0.4 mg/mL per lane determined by BCA assay
  - Positive control: purified WT1 protein diluted 1:50 from 0.5 mg/mL to 10 µg/mL
  - Confirms and compares expression of WT1 in tumor lines being used

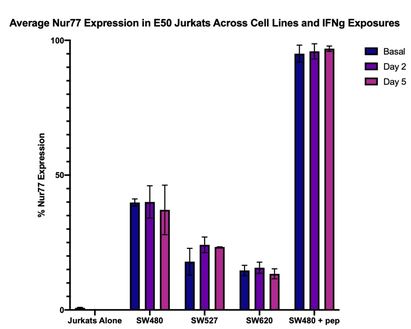




- Previous lab data: HLA-A2 measured by FITC on flow cytometer
  - Tumor lines were cultured with IFNg (diluted 1:1000 to 20 ng/mL) for 96 hours
  - IFNg refreshed once at 48 hours since incubation start

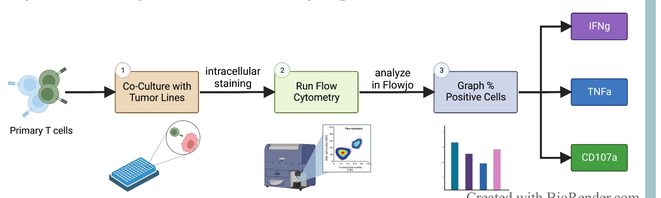
Co-culture with SW480 results in highest % of Nur77-expressing Jurkats regardless of IFNg exposure

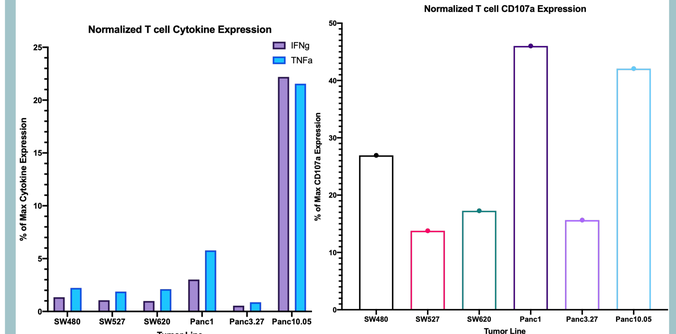




Jurkat cells were co-cultured with each tumor line, exposed to either no IFNg, IFNg for two days, or IFNg for five days. Positive control wells with added peptide only used SW480 for the target tumor line. Jurkat cells were gated on CD8-BV421+ and expression of Nur77 was measured via the FITC channel through flow cytometry. SW480 instigates the highest level of Nur77 expression in Jurkat cells. Bars show standard deviation around the mean. Experiment needs to be repeated to verify whether there is no significant difference between basal, Day 2, or Day 5 conditions within a tumor line.

WT1-specific primary CD8+ T cells can recognize and perform cytotoxic activity against WT1+ tumor lines





Primary T cells were co-cultured with each tumor line, stimulated either with or without peptide. T cells were gated on CD8-FITC+ and expression of IFNg, TNFa, and CD107a was measured via the APC, PeCy7, and Pacific Blue channels, respectively, through flow cytometry. Panc10.05 instigates the highest level of cytokine expression in T cells, while Panc1 instigates the highest level of CD107a on T cells. Experiment needs to be repeated with more replicates in order to have statistical significance.

CONCLUSIONS AND FUTURE DIRECTIONS

- WT1 expressed in both colon adenocarcinoma and pancreatic cancer tumor lines
- In co-culture:
  - WT1-specific CD8+ Jurkat cells indicated activation through Nur77 expression
  - WT1-specific CD8+ primary T cells indicated both:
    - activation through cytokine expression
    - cytotoxic function through CD107a expression
- Most promising targets for WT1-specific T cell therapy SW480 and Panc10.05:
  - high WT1 expression
  - high T cell response
- Future directions include:
  - Re-assessing effects of IFNg exposure in WT1-specific Jurkats co-cultured against colon adenocarcinoma tumor lines
  - Repeating co-culture of primary T cells against colon adenocarcinoma and pancreatic cancer tumor lines
  - Using more physiologic tumor model (i.e. in a mouse)
  - Determining other indicators of T cell expression and cytotoxic function

REFERENCES

[ACS] American Cancer Society. 2022. Survival rates for pancreatic cancer. Atlanta (GA): American Cancer Society, Inc. [accessed 2022 Aug 8]. https://www.cancer.org/cancer/pancreatic-cancer/detection-diagnosis-staging/survival-rates.html

[ACS] American Cancer Society. 2022. Survival rates for colorectal cancer. Atlanta (GA): American Cancer Society, Inc. [accessed 2022 Aug 8]. https://www.cancer.org/cancer/colon-rectal-cancer/detection-diagnosis-staging/survival-rates.html

Ashouri JF, Weiss A. 2017. Endogenous Nur77 is a specific indicator of antigen receptor signaling in human T and B cells. J Immunol. 198(2):657-668. doi: 10.4049/jimmunol.1601301

Sugiyama H. 2010. WT1 (Wilms' tumor gene 1): biology and cancer immunotherapy. Jpn J Clin Oncol. 40(5):377-387. doi: 10.1093/jjco/hyp194

ACKNOWLEDGEMENTS

This work is funded by the MPN Research Foundation grant "A JAK2 V617F-DIRECTED T CELL RECEPTOR TRANSGENIC T CELL IMMUNOTHERAPY FOR THE TREATMENT OF MYELOPROLIFERATIVE NEOPLASMS". The Summer Undergraduate Research Program is supported in parts by the Fred Hutch Internship Program and individual labs/research groups.