T Cell Recognition of Colon Adenocarcinoma and Pancreatic Cancer Cells

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ABSTRACT

Adaptive 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 IFNγ 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 IFNγ 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, and ovarian cancers (Sugiyma 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)

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 IFNγ (diluted 1:1000 to 20 ng/mL) for 96 hours
  - IFNγ refreshed once at 48 hours since incubation start

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

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

- Impact of this work: WT1-specific T cells can be used in adoptive T cell therapy to target tumor cells other than AML, myeloblasts

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 and 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 IFNγ 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


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