Bi-Specific Killer Engager (BiKE) targeting anti-B7H3 enhances NK cell-mediated cytotoxicity toward pancreatic cancer cells

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Introduction

Pancreatic ductal adenocarcinoma (PDAC), the most common type of pancreatic cancer, is predicted to be the second leading cause of cancer death in the US by 2030. The poor 5-year survival rate (10%) of PDAC patients is due to a lack of early detection and that most tumors are detected at a very late stage. Thus, new innovative therapeutic therapies are needed.

Natural killer (NK) cells are an innate lymphocyte that is an essential part of tumor immunosurveillance. In fact, patients lacking NK cells or devoid of NK cell lytic activity have a higher risk of cancer. Also, patients with cancer who have poorer outcomes show low NK cell numbers or reduced cytotoxicity. Therefore, NK cell-based immunotherapies are being developed for anti-tumor therapies.

However, while the targeting and persistence of NK cells to tumors has remained a significant challenge, recent work developing bispecific killer engagers (BiKE) and trispecific killer engagers (TriKE) can redirect NK cells to various tumors expressing cell surface tumor associated antigens such as B7-H3 (see Schematic Diagram).

Study Aims

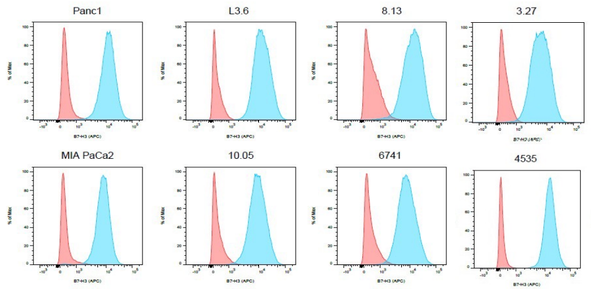
- Test the expression ofB7-H3 in PDAC cell lines.
- Knockout B7-H3 in PDAC cell lines using CRISPR/Cas9 technology.
- Derive B7-H3 null PDAC cell lines using flow sorting
- Evaluate the contribution of B7-H3 on BiKE-mediated NK cell killing of PDAC cell lines.

Methods

Flow cytometry Pancreatic was used to measure the expression on PDAC cell lines. CRISPR/Cas9-mediated gene editing was conducted using nucleofection of CRISPR/Cas9 guide complexes into PDAC cells. NK cell killing was performed in the presence or absence of anti-B7-H3 BiKE.

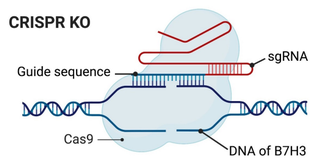
Results

1. B7-H3 is expressed in PDAC cells



Anti-B7H3 antibody (blue) and isotype control (red) were used to detect cell surface expression of B7H3 in PDAC cell lines.

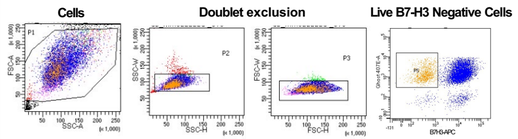
2. Generating of B7H3 knockout pancreatic cancer cells.



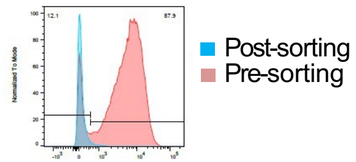
B7-H3 was knocked out using CRISPR/Cas9 technology.

3. Isolation of B7H3 knockout PDAC cell lines using FACS

(A) Sorting strategy

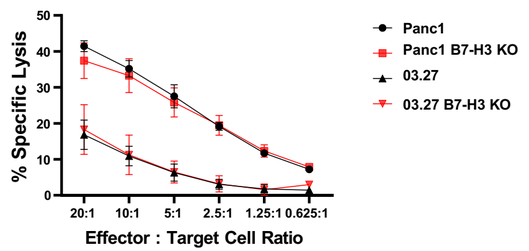


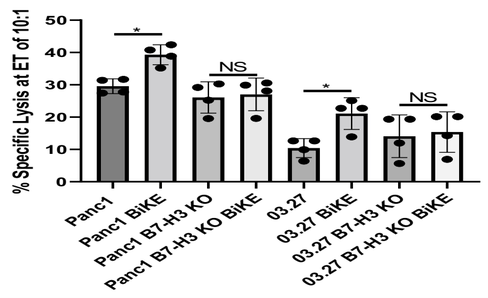
(B) Validation



B7H3 knockout cells were enriched through flow sorting (A) and expression of B7H3 was examined by flow before and after sorting (B).

4. B7-H3 is required for BiKE-mediated enhancement of NK cell killing.

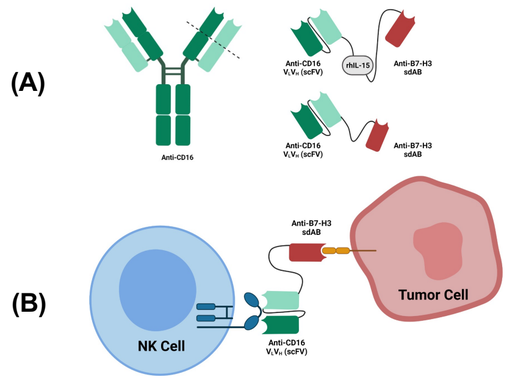




Conclusion

- B7H3 is expressed on PDAC cell lines.
- Using CRISPR/Cas9 and flow sorting we have obtained PDAC cells lacking.
- The anti-B7-H3 BiKE enhances killing of PDAC cells expressing B7-H3 but not B7-H3 null cells.

Schematic Diagram



Schematic diagram was created with Biorender showing the structure of TriKE and BiKE (A) and mechanism by which it tethers the NK cell to the B7-H3 expressing tumor

Reference

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