Background

Treatment with rapid expanded Tumor Infiltrating Lymphocytes (TILs) has shown remarkable results, especially in non-resectable melanoma. The primary mediators of effect are thought to be the CD8+ T cells, which secrete cytotoxic molecules such as granzymes A, B, H, K, and M upon tumor cell recognition. Despite their importance, the different granzyme functions and profiles are poorly understood.

Using an in vivo model of matched rapid expanded CD8+ TILs and melanoma tumor cell lines we simulated the TIL-TIC interaction in the tumor microenvironment and measured the secreted granzyme levels. Data from bulk and single cell mRNAseq were used to further validate and characterize the TIL populations secreting the molecules.

Materials and Methods

**Results I: The secretion profile is dominated by granzyme B**

Fig. 3: Expanded CD8+ tumor infiltrating lymphocyte total secretion of granzyme is dominated by granzyme B

- Granzyme B dominated the anti-tumor granzyme response from CD8+ TILs both in terms of total secreted granzyme and inducibility upon tumor cell recognition in melanoma.
- Granzyme B and perforin were upregulated on mRNA level in CD8+ TILs upon tumor cell recognition in melanoma.
- The upregulation of granzyme B and perforin could almost exclusively be attributed to reacting CD8+ Tumor infiltrating lymphocytes, whereas the signal disappeared in non-reacting cells.
- The findings on mRNA level were reproduced in colorectal cancer.
- Granzyme A, H, K, and M did not seem important for the CD8+ TILs anti-tumor response

**Results II: Granzyme B is upregulated upon tumor recognition**

Fig. 4: Bulk mRNAseq analysis of n=10 melanoma showed an upregulation of Perforin and Granzyme B upon TIL recognition compared to allogeneic control.

- Granzyme B is rapidly induced in melanoma in comparison to wildtype tumor.
- The expanded CD8+ tumor infiltrating lymphocyte tumor-specific granzyme response is dominated by Granzyme B.

**Results III: Single cell mRNAseq analysis of n=1 melanoma patients showed A) an upregulation of perforin and Granzyme B after tumor cell recognition in CD8+ TILs. B) When categorizing the the CD8+ TILs as either IFN resonance, non-tumor reacting or tumor reacting, the effect can be almost entirely attributed to tumor reacting TILs.

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**Disclosures**

The first (ACKR and CAC) and presenting (ACKR) authors declare no conflict of interest.