Enrichment of tumor-specific neoantigen reactive tumor infiltrating lymphocytes (TIL) in gastric cancer

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Abstract

Background

Tumor-specific neoantigens have been employed in cancer immunotherapies such as cancer vaccines and adoptive cell therapy (ACT) with tumor infiltrating lymphocytes (TIL). These protocols are used to enhanced immune response against solid tumors by enriching tumor specific immune cells. Gastric cancer is one of the most lethal cancers. The objective of this study is to determine the feasibility of using neoantigens to isolate tumor-specific TILs in upper gastric cancer (esophageal and stomach) and potentially increase anti-tumor response.

Methods

A total of 14 research samples gastric cancer patients were evaluated in this study. Tumors and PBMC samples were collected at Moffitt Cancer Center under an IRB approved study (Advarra Pro0043972). Tumor-specific neoantigens were identified using whole exome and RNA sequencing. Long peptides (25aa), designed to bind to autologous HLA molecules, were manufactured. TIL were expanded *ex vivo* from whole tumor digest in media containing high dose IL2 (pre-REP). Tumor neoantigens were pulsed onto antigen presenting cells (APC). APC were cocultured with bulk TIL and neoantigen-reactive TILs were isolated by FACS based on expression of OX40 and 4-1BB. Sorted TIL populations underwent rapid expansion (REP). Tumor-reactivity of the final product was validated by quantifying IFN-γ and Granzyme B in co-culture supernatants.

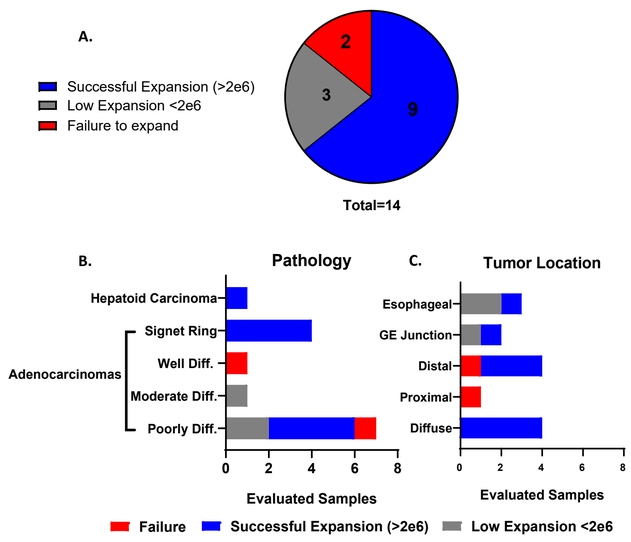
Results

Utilizing small tumor samples (~0.06g - 1.8g) a scale-down model of clinical TIL isolation prior to a rapid expansion protocol (preREP), TIL could be expanded from 12/14 (86%) of the available samples. Tumor-specific neoantigens were predicted (ranging from 130 to 321 peptides) for each tumor sample. Up to 186 individual peptides were synthesized to represent neoantigens for each sample, and in instances where >186 neoantigens were identified bioinformatics-based prioritization of peptide manufacture was performed. Neoantigen-reactive TILs, as measured by upregulation of OX40/4-1BB, were successfully enriched and expanded in a REP. Enriched TILs were tested and demonstrated significant reactivity against several individual neoantigens.

Conclusion

This study utilized a scaled-down research model of clinical TIL isolation and demonstrated successful TIL expansion from gastric tumors. Additionally, the use of tumor-specific neoantigens allowed the enrichment of TIL, which maintained neoantigen reactivity after TIL rapid expansion. This data support previous studies performed in melanoma patients and demonstrate that reactive TIL enrichment protocols represent a promising venue to enhance adoptive cell therapy with TIL for solid tumors.

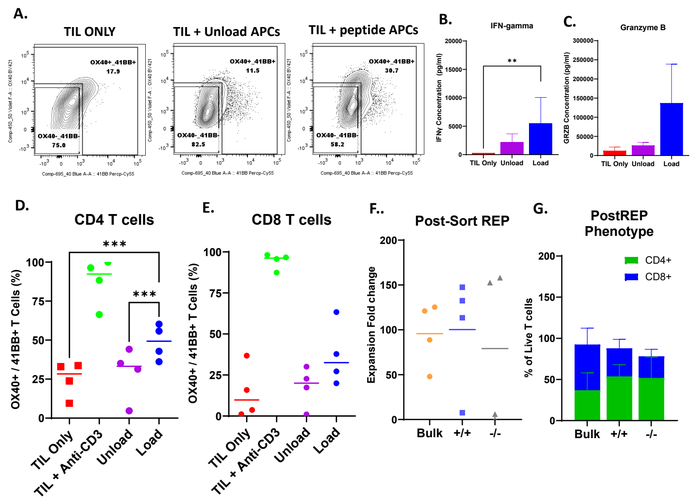
Tumor Infiltrating Lymphocytes Expansion from Gastric Cancer



Successful TIL expansion from upper gastric tumors

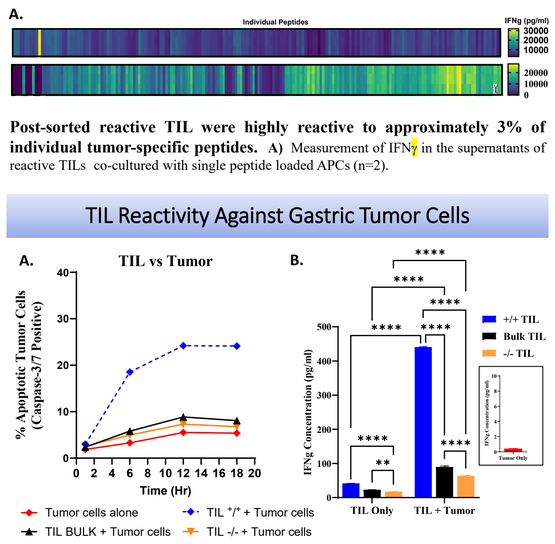
A) Pie-chart demonstrating the TIL expansion success. TIL expansion was categorized as successful expansion (>2e6), Low expansion (<2e6) and failure expansion (no expansion) according to **B)** pathology and **C)** tumor location. **D)** Expanded TIL was phenotypical characterized in CD56+, CD3+, CD4+ and CD8+ cells. Statistical significance was determined by means of Two-way ANOVA (n=4); * = p<0.05; ** p<0.01

Selection and Expansion of Neoantigen Reactive TILs



Gastric TILs were co-cultured with neoantigen-presenting APCs resulting in the identification and isolation of reactive TIL. A) Representation of flow cytometry analysis of reactive TIL enrichment after TIL co-cultured with neoantigen loaded/unloaded APC (n=1). Analysis of **B)** Interferon gamma and **C)** granzyme B in the co-culture supernatant. Expression of OX40 and 41BB determined in **D)** CD4+ and **E)** CD8+ TILs co-cultured in presence or absence of neoantigens; anti-CD3/CD28 dyna beads treatment served as positive control and TIL only was set as baseline control. **F)** Rapid expansion (REP) fold change of peptide loaded TIL sorted for: OX40+/41BB+ and OX40-/41BB- TILs, and bulk TIL (not sorted). **G)** Post-REP TIL (Bulk, OX40+/41BB+, OX40-/41BB-) were phenotypically characterized into CD4+ and CD8+ TILs. Statistical significance was determined by means of Two-way ANOVA; * = p<0.05; ** = p<0.01, *** = p<0.001; n =4

TIL Reactivity to Single Neoantigen Peptides



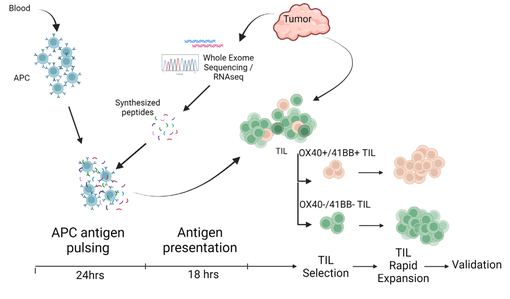
Post-sorted reactive TIL were highly reactive to approximately 3% of individual tumor-specific peptides. A) Measurement of IFNγ in the supernatants of reactive TILs co-cultured with single peptide loaded APCs (n=2).

TIL Reactivity Against Gastric Tumor Cells

Enrichment of OX40+/41BB+ population demonstrates enhanced anti-tumor response compared to OX40-/41BB- and Bulk (not sorted).

A) Immunofluorescence intensity analysis of Caspase 3/7 analysis in tumor cells co-cultured with enriched OX40+/41BB+ TILs (blue line), bulk TIL (black line) and OX40-/41BB- TILs (orange line); tumor cells only culture served as negative control (red line). **B)** Measurement of IFNγ concentration in the co-culture supernatant of enriched OX40+/41BB+ TIL (blue bars), Bulk TIL (black bars) and OX40-/41BB- TIL (orange bars) with autologous tumor cells. Concentration of IFNγ in tumor cells only (red bar) supernatant was used as negative control. Statistical significance was determined by means of Two-way ANOVA; * = p<0.05; ** = p<0.01, *** = p<0.001, **** = p≤0.0001; n =1

Methods



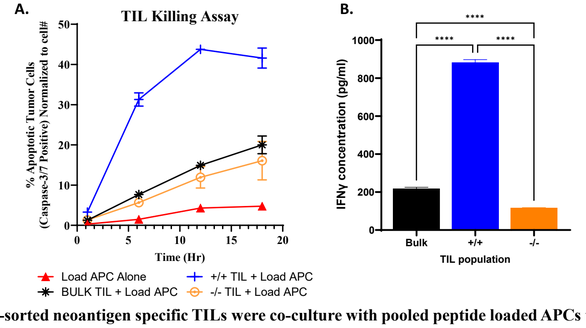
Schematic summary of protocol to identify, select and expand neoantigen reactive TILs.

Identification of Tumor-Specific Neoantigens

Sample ID	Neoantigens Predicted					Synthesized Peptides
	Total	MHC I binding		MHC II binding		
		Strong (IC50<50nM)	Weak (50nM<IC50<500nM)	Strong (IC50<50nM)	Weak (50nM<IC50<500nM)	
P.9	273	28	134	86	172	185
P.12	130	40	57	19	91	124
P.23	321	72	168	70	223	186
P.38	242	60	123	34	178	178

Tumor-specific neoantigens of each evaluated patient were identified and classified as strong or weak MHC I/II binders.

Reactivity Validation of Neoantigen specific TILs



Post-sorted neoantigen specific TILs were co-cultured with pooled peptide loaded APCs to validate reactivity. A) Measurement of Caspase 3/7 performed in loaded APCs in presence/absence of enriched OX40+/41BB+ TIL (blue line), OX40-/41BB- TIL (orange line) and Bulk TIL (black line) to determine TIL reactivity towards pooled peptides. **B)** Soluble concentration of IFNγ in the supernatant of Bulk TIL (black bar), OX40+/41BB+ TIL (blue bar) and OX40-/41BB- TIL (orange bar) co-cultured with neoantigen loaded APC. IFNγ measurement in loaded APC alone was below detectable concentration. Statistical significance was determined by means of Two-way ANOVA; * = p<0.05; ** = p<0.01, *** = p<0.001, **** = p<0.0001. Data represents n=1.

Conclusions

- Tumor infiltrating lymphocytes were expanded from 12 out of 14 upper gastric tumors, demonstrating TIL expansion protocol feasibility.
- Over 120 neoantigens were identified and synthesized from gastric tumors
- Co-culture of TIL with neoantigens led to increased expression of OX40/41BB and increased reactivity to neoantigen peptides.
- Enriched TIL demonstrated reactivity against approximately 3% of tumor-specific peptides.
- Enrichment of neoantigen-reactive TIL led to enhanced cytotoxicity of TIL against autologous tumor.

Acknowledgements

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