Checkpoint coexpression landscape in gastroesophageal adenocarcinoma

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Objectives

Obesity, measured by an increased body mass index (BMI), creates chronic inflammation, which leads to immune dysfunction in various cancers. We hypothesized that obesity-driven immune dysfunction manifests as changes in the checkpoint expression landscape. Our primary objective was to look at the coexpression of different known immune checkpoints in gastroesophageal adenocarcinoma (GEAC) and correlate them with BMI.

Methods

- Targeted RNA-seq (OmniSeq INSIGHT by LabCorp) was performed on 46 metastatic GEAC tumors [1].
- Gene expression was measured for 394 immune transcripts.
- Coexpression analyses were conducted by calculating Pearson correlations for every possible pair of 15 checkpoint genes and clustering groups of similarly expressed genes.
- The immunogenic and microenvironmental effects of each checkpoint were also interrogated by calculating correlations with tumor immunogenic (TIGS) [2] and cell proliferation (CP) [3,4] signatures.

Table 1: Cohort BMI composition.

BMI Group	n	Percent
Normal (BMI < 25)	13	28.3%
Overweight (BMI ≥ 25)	33	71.7%

	Step	Description
Ranked	4 Gene Expression (GEX) Rank	For each gene, GEX rank is calculated as nRPM percentile against a reference population of 735 tumors. Rank for transcript (t): $\text{Rank(t)} = 100 \times \frac{\#\ of\ samples\ in\ reference\ pop. < nRPM(t)}{735}$
Normalized	3 Normalized Reads Per Million	$\text{nRPM}_{(t)} = \frac{Background\ subtracted\ absolute\ read\ count_{(t)}}{Normalization\ ratio}$, Where Normalization ratio = $\frac{Background\ subtracted\ abs\ read\ count\ of\ house\ keeping\ genes}{Pre-defined\ reads\ per\ million\ profile\ of\ house\ keeping\ genes}$
Raw	2 Background subtracted Absolute read Count	For each transcript (t), Background subtracted Absolute read Count = $(absolute\ read\ counts(t) - absolute\ read\ counts\ from\ NTC\ (t))$
Raw	1 Absolute Read Count	RNA-seq absolute reads for each transcript (t) were generated with Torrent Suite's plugin immuneResponseRNA.

Gene Expression Rank

Figure 1: Flow chart showing calculation of gene expression normalized reads per million (nRPM) from raw absolute read count values.

Results

The overweight (BMI≥25) and normal (BMI<25) groups demonstrated distinct checkpoint coexpression patterns. Overweight patients had a larger amount of coexpression between almost all checkpoints, and normal BMI patients had fewer groups of coexpressing checkpoints.

In normal BMI patients, a total of seven small groups of coexpressing checkpoints were observed.

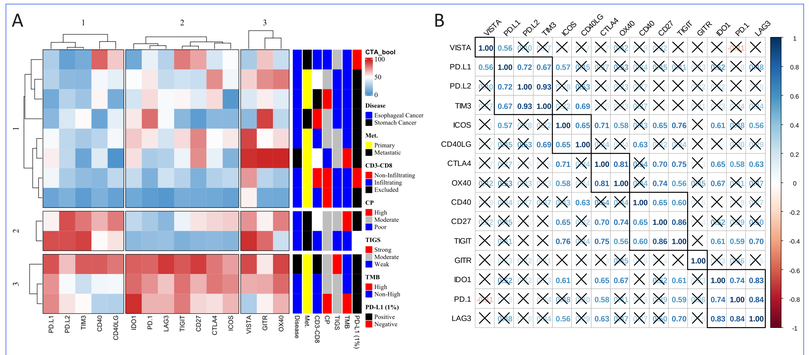

Figure 2: Normal BMI cohort (BMI < 25) checkpoint expression: A) checkpoint expression heatmap. Rows represent patients and columns represent checkpoint genes. Rows and columns are clustered using unsupervised k-means analysis into three clusters each. Rows are annotated with disease type, metastatic status, CD3-CD8 status, cell proliferation (CP) group, TIGS group, TMB status, and PD-L1 IHC. B) checkpoint expression correlation plot showing all pairwise Pearson correlation coefficients between checkpoints. Nonsignificant ($p > 0.05$) correlations indicated by an "X" over a box. Clustered groups of coexpressing checkpoints indicated by black rectangles about main diagonal of plot.

For overweight patients, checkpoint coexpression was divided into two groups: the single checkpoint GITR and all other checkpoints.

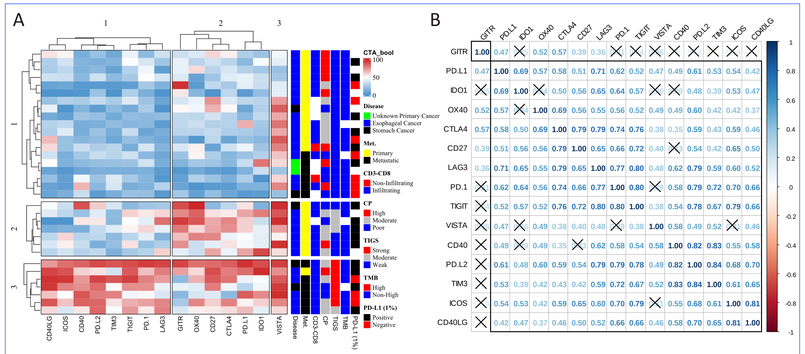

Figure 3: Overweight BMI cohort (BMI ≥ 25) checkpoint expression: A) checkpoint expression heatmap. Rows represent patients and columns represent checkpoint genes. Rows and columns are clustered using unsupervised k-means analysis into three clusters each. Rows are annotated with disease type, metastatic status, CD3-CD8 status, cell proliferation (CP) group, TIGS group, TMB status, and PD-L1 IHC. B) checkpoint expression correlation plot showing all pairwise Pearson correlation coefficients between checkpoints. Nonsignificant ($p > 0.05$) correlations indicated by an "X" over a box. Clustered groups of coexpressing checkpoints indicated by black rectangles about main diagonal of plot.

In the normal BMI group, CD8 was significantly correlated with 6, TMB was significantly correlated with 2, and TIGS was significantly correlated with 8 of the 15 checkpoints analyzed. CP did not correlate with any checkpoints analyzed.

In the overweight BMI group, CD8 was significantly correlated with 14, TMB was significantly correlated with 1, TIGS was significantly correlated with 14, and CP was correlated with 2 of the 15 checkpoints analyzed.

Table 2: Pearson correlations of the expression of 15 checkpoint genes with CD8 expression, TMB, tumor immunogenic score (TIGS), and cell proliferation score (CP) in normal (BMI < 25) and overweight (BMI ≥ 25) groups. Only significant ($p < 0.05$) shown.

Check.	CD8		TMB		TIGS		CP	
Gene	BMI<25	BMI≥25	BMI<25	BMI≥25	BMI<25	BMI≥25	BMI<25	BMI≥25
CTLA4	**0.74**	**0.714**			**0.675**	**0.772**		
PD.1		**0.813**				**0.867**		
PD.L1		**0.443**			**0.722**	**0.671**		
PD.L2		**0.763**				**0.858**		
OX40	**0.58**	**0.413**	**0.751**		**0.75**	**0.65**		
TIM3		**0.694**				**0.785**		
LAG3	**0.681**	**0.808**				**0.8**		
VISTA		**0.448**				**0.59**		**-0.347**
ICOS		**0.646**			**0.727**	**0.778**		
CD40LG		**0.636**			**0.656**	**0.744**		
CD27	**0.768**	**0.62**			**0.845**	**0.771**		
GITR				**0.417**				
TIGIT	**0.781**	**0.865**			**0.753**	**0.884**		
CD40	**0.626**	**0.66**			**0.763**	**0.718**		**-0.418**
IDO1		**0.492**	**0.632**			**0.626**		

Conclusions

- The increased checkpoint coexpression in overweight patients suggests that they have more immune escape mechanisms.
- The increased association of TIGS with checkpoints suggests that in the presence of immune activity, immunosuppression is more common in overweight patients.
- Further studies are necessary to elucidate the exact mechanisms underlying checkpoint coexpression patterns and their relationship with BMI.

References

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