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 **BMI Group** Normal (BMI < Overweight (BN

			Ste	p	Description
Gene Expression Rank	\wedge	paya	4	Gene Expression (GEX)	For each gene, GEX rank is calculated as nRPM per against a reference population of 735 tumors. Ran transcript (t):
		- (1) 2		Rank	Rank(t) = 100 x ^{# of samples in reference pop. < n} 735
		izad	2		nRPM _(t) = <i>Background subtracted absolute read co</i> <i>Normalization ratio</i>
	ı Rank	lemon	3	Normalized Reads Per Million	Where Normalization ratio = Background subtracted abs read count of house k
	ssior		-		Pre–defined reads per million profile of house ke
	zpre				For each transcript (t),
	Gene I	Raw	2	Background subtracted Absolute read Count	Background subtracted Absolute read Count = (absolute read counts(t) — absolute re counts from NTC (t))
		Mea		Absolute Read Count	RNA-seq absolute reads for each transcript (t) wer with Torrent Suite's plugin immuneResponseRNA.

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

composition.											
	n	Percent									
25)	13	28.3%									
MI ≥ 25)	33	71.7%									

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nce pop. < nRPM(t)

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Count = absolute read

cript (t) were generated

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.



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VISTA	1.00	0.56	Xo	×7	X	X	X	2	X	×2		X	X	-)×1	-×6		-
PD.L1	0.56	1.00	0.72	0.67	0.57	× 5	×7	3	₩4	× 5	×1	×3	2	X	<mark>) </mark>	-	- (
PD.L2	$\mathbf{\times}$	0.72	1.00	0.93	X	X 3	\times	⋈3	∕∕7	X	Х	×2	×2	Х	X		
TIM3	\times	0.67	0.93	1.00	×2	0.69	\times 5	╳3	X 7	X	X	\times	×3	×4	★4		-
ICOS	\times	0.57	X	×2	1.00	0.65	0.71	0.58	X 3	0.65	0.76	×	0.61	X 8	0.56	-	-
CD40LG	\times	5	3	0.69	0.65	1.00	₩4	×4	0.63	×2	₩4	\times	Х	X	×9		
CTLA4	\times	×7	X_5	\times 5	0.71	₩4	1.00	0.81	0×4	0.70	0.75	\mathbf{X}_{3}	0.65	0.58	0.63		-
OX40	2	3	⋈3	×3	0.58	\mathbf{X}_{4}	0.81	1.00	0∕√4	0.74	0.56	5	0.67	×1	X 7	-	-
CD40	\times	⋈4	×7	×7	X 3	0.63	× 4	₩4	1.00	0.65	0.60	$\mathbf{\times}$	X	X	✓7		
CD27	×2	× 5	\times	\times	0.65	×2	0.70	0.74	0.65	1.00	0.86	\times	2	×9	× 0		
TIGIT	\mathbf{X}	×1	Х	\times	0.76	₩4	0.75	0.56	0.60	0.86	1.00	Х	0.61	0.59	0.70		- <u>-</u>
GITR	\times	×3	X2	\times	X	X	X	× 5	X	\times	Х	1.00	\times	×6	\times 5		
IDO1	\times	2	X2	×3	0.61	Х	0.65	0.67	Х	2	0.61	X	1.00	0.74	0.83		
PD.1	-0×1	X	Х	\times	X 8	Xo	0.58	X 1	X	×9	0.59	× 6	0.74	1.00	0.84		
LAG3	X	X 8	X	₩4	0.56	×9	0.63	X 7	×7) (0	0.70	\times	0.83	0.84	1.00		L

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	GITR	1.00	0.47	×5	0.52	0.57	0.39	0.36	×	\times	×6	X	X	X	X	X
	PD.L1	0.47	1.00	0.69	0.57	0.58	0.51	0.71	0.62	0.52	0.47	0.49	0.61	0.53	0.54	0.42
	IDO1	×5	0.69	1.00	×4	0.50	0.56	0.65	0.64	0.57	X	×9	0.48	0.39	0.53	0.47
ncer	OX40	0.52	0.57	₩4	1.00	0.69	0.56	0.55	0.56	0.52	0.49	0.49	0.60	0.42	0.42	0.37
	CTLA4	0.57	0.58	0.50	0.69	1.00	0.79	0.79	0.74	0.76	0.38	0.35	0.59	0.43	0.59	0.46
	CD27	0.39	0.51	0.56	0.56	0.79	1.00	0.65	0.66	0.72	0.40	⋈3	0.54	0.42	0.65	0.50
	LAG3	0.36	0.71	0.65	0.55	0.79	0.65	1.00	0.77	0.80	0.40	0.62	0.79	0.59	0.60	0.52
	PD.1	\times	0.62	0.64	0.56	0.74	0.66	0.77	1.00	0.80	×9	0.58	0.79	0.72	0.70	0.66
	TIGIT	\times	0.52	0.57	0.52	0.76	0.72	0.80	0.80	1.00	0.38	0.54	0.78	0.67	0.79	0.66
	VISTA	\mathbf{X}_{6}	0.47	×6	0.49	0.38	0.40	0.40	×9	0.38	1.00	0.58	0.49	0.52	×1	0.46
	CD40	\times	0.49	×9	0.49	0.35	⋈3	0.62	0.58	0.54	0.58	1.00	0.82	0.83	0.55	0.58
	PD.L2	X	0.61	0.48	0.60	0.59	0.54	0.79	0.79	0.78	0.49	0.82	1.00	0.84	0.68	0.70
	ТІМЗ	X	0.53	0.39	0.42	0.43	0.42	0.59	0.72	0.67	0.52	0.83	0.84	1.00	0.61	0.65
	ICOS	X	0.54	0.53	0.42	0.59	0.65	0.60	0.70	0.79	X	0.55	0.68	0.61	1.00	0.81
С	D40LG	X	0.42	0.47	0.37	0.46	0.50	0.52	0.66	0.66	0.46	0.58	0.70	0.65	0.81	1.00

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: Pearso (TIGS), and cel sh 6heck.	on correlations Il proliferation CE	of the expres score (CP) in)8	sion of 15 che normal (BMI · TM	eckpoint genes < 25) and ove 1B	with CD8 exp rweight (BMI TIC	ression, TMB, ≥ 25) groups. SS	tumor immun Only significa	ogenic score nt (p < 0.05) 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
ID01		0.492	0.632			0.626		

Conclusions

- immune escape mechanisms.

References

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• The increased checkpoint coexpression in overweight patients suggests that they have more

• 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.

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