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Molecular characterization of pancreatic cancers as seen in the *SLUG* gene revealing cancer progression

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Introduction

- Epithelial mesenchymal transition (EMT) is a crucial process during invasion or metastasis of cancer. The *SLUG* gene plays an important role in EMT by repressing E-cadherin together with Zinc-finger family, i.e., *SNAIL* and *ZEB1/2*, and is accelerated in the oxygenic or nutritional deficient environment [1].
- The *SLUG* gene is reported to be frequently overexpressed in pancreatic cancer (PC) [2,3] which is characterized by hypovascularization and poor prognosis due to the high frequency of invasion and metastasis even in the early stage; however, its contribution to characteristics or metastatic features in PC remains elusive.

Methods

- A total of 2928 pancreatic tumors collected from March 2016 through August 2020 were analyzed at Caris Life Sciences (Phoenix, AZ) using whole transcriptome sequencing (WTS), next generation sequencing (NGS) with a 592 gene panel (NextSeq), and/or whole exome sequencing (WES) (NovaSeq).
- Microsatellite instability (MSI)/mismatch repair (MMR) status was tested by fragment analysis, immunohistochemistry (IHC) and NGS.
- PD-L1 was tested by IHC. Tumor mutational burden (TMB) was measured by counting all nonsynonymous missense/nonsense/indel/fs mutations found per tumor that had not been previously described as germline alterations according to dbSNP (single-nucleotide polymorphism) and 1KG databases. A universal cutoff point of ≥10 mutations per MB was used.
- Immune cell fraction was calculated by QuanTIseq using transcriptomic data (Finotello 2019, Genome Medicine).

References

1. Recouvreux MV, et al. *J Exp Med* 2020; 217(9): e20200388.
2. Fujiwara S, et al. *Cancer Med* 2019; 8(4): 1671–8.
3. Hotz B, et al. *Clin Cancer Res* 2007; 13: 4769–76.

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Results

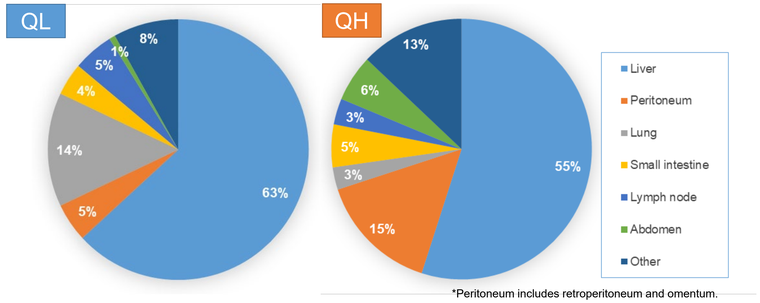
Patient characteristics

Pancreatic *SLUG* expressed tumors (N=2958)						
				Avarage age		
Quartiles	Primary/Local	Metastatic	Male, N (%)	Male	Female	Total
Q1: QL	344	396	391 (52.8)	64.2	66.3	740
Q2	319	420	368 (49.8)	65.4	66.6	739
Q3	304	435	432 (58.5)	65.2	66.5	739
Q4: QH	307	433	406 (54.9)	65.2	65.3	740
Total	1274	1684	1597 (54.0)			2958

Samples were divided equally into 4 classes in each group, according to their *SLUG* expression levels. Q1 is the quartile with the lowest expression levels: QL. Q4 is the quartile with the highest expression levels: QH.

Student's *t*-Test showed that average age for females in QL was significantly higher than in males (p=0.021).

Metastatic distributions

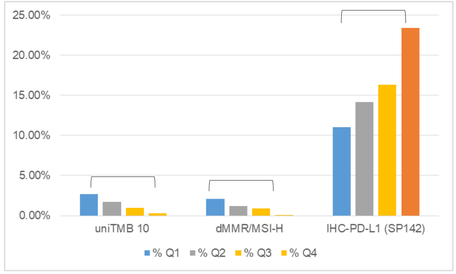


*Peritoneum includes retroperitoneum and omentum.

%QL > %QH	%QL	%QH	p-value
Liver	63.1%	55.0%	0.0197
Lung	14.1%	2.8%	0.0001
Lymph node	5.1%	3.2%	0.2208
Biliary tree	2.3%	1.6%	0.6154
Large intestine	1.5%	0.9%	0.5319
Ovary/Uterus	1.0%	0.7%	0.7149
Pelvis	0.5%	0.2%	0.6085

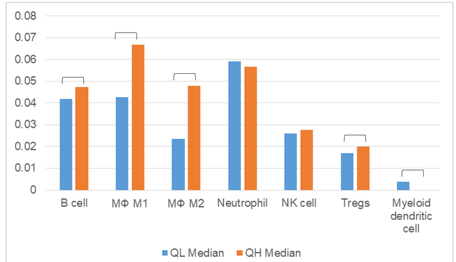
%QL < %QH	%QL	%QH	p-value
Peritoneum	4.8%	15.0%	0.0001
Small intestine	4.0%	5.3%	0.4157
Abdomen	0.8%	5.8%	0.0001
Bone	0.0%	2.8%	0.0005
Connective tissue	0.8%	1.4%	0.5094
Gastroesphageal	0.3%	0.5%	1
Skin	0.3%	1.6%	0.0712
Adrenal gland	0.3%	0.7%	0.6256
Other	1.3%	2.5%	0.213

Immune oncological markers



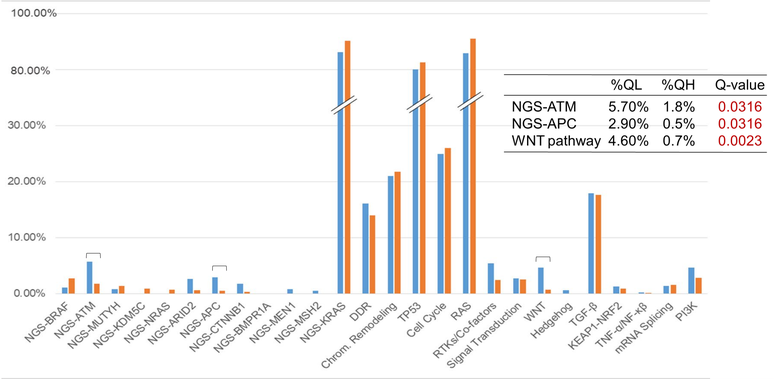
Test	%Q1	%Q2	%Q3	%Q4	Q value (Q1vsQ4)
uniTMB 10	2.7%	1.7%	1.0%	0.3%	0.0315
dMMR/MSI-H	2.1%	1.2%	0.9%	0.1%	0.0315
IHC-PD-L1	11.0%	14.2%	16.3%	23.4%	<0.001

Tumor microenvironment



Cell type	QL median	QH median	p-value
B cell	0.0419	0.0474	<0.0001
MΦ M1	0.0426	0.0667	<0.0001
MΦ M2	0.0234	0.0478	<0.0001
Neutrophil	0.0592	0.0567	0.0938
NK cell	0.0261	0.0277	0.0592
Tregs	0.0170	0.0201	<0.0001
Myeloid dendritic cell	0.0039	0.0000	<0.0001

Molecular characteristics & pathway expressions



	%QL	%QH	Q-value
NGS-ATM	5.70%	1.8%	0.0316
NGS-APC	2.90%	0.5%	0.0316
WNT pathway	4.60%	0.7%	0.0023

Summary

- Tumors in QH showed significantly higher frequency compared to QL in peritoneal-retroperitoneal-omentum metastasis (15.0% vs 4.8%), abdomen (5.8% vs 0.8%), and bone (2.8% vs 0.0%).
- Contrastingly, the metastases are occurred frequently the most in QL and the least in QH in liver (55.0% in QH vs 63.1% in QL) and lung (2.8% vs 14.1%), and a similar trend can be seen in lymph node (3.2% vs 5.1%, not significant).
- **This data indicated that tumors with high *SLUG* gene expression levels tend to lead to disseminated metastasis and, with low expression levels, tend to spread intravascularly.**
- Binary TMB-H and MSI-H tumors had higher frequencies in QL compared to QH (2.7% vs 0.3% and 2.1% vs 0.1%) and PD-L1 expression levels were higher in QH compared to QL (23.4% vs 11.0%).
- They had a linear relationship with the expression levels among Q1–Q4.
- The median values of the population of B cells, M1 and M2 macrophages were significantly higher in QH compared to those in QL, but those of myeloid dendritic and CD8⁺T cells conversely decrease as the *SLUG* expression increases.
- Significant differences were detected among genetic mutations in *ATM* (5.7% in QL vs 1.8% in QH) and *APC* (2.9% vs 0.5%), and in the expression level of Wnt signaling pathway (4.6% vs 0.7%).

Conclusions

Our data indicate the *SLUG* gene expression level could determine the tumor characteristics in progression, especially the pattern of metastasis in PC, which can possibly predict the prognosis and/or therapeutic effects. Immune oncologic markers have some relationships with the *SLUG* gene expressions. These results will lead to better understanding of invasive behavior and proper selection of therapies.