Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-3665

Reviewer

In this study, the authors show that a high positive rate of LSECtin existed in the NSCLC patients who were non-smokers, at advanced stages, or had lung adenocarcinoma, and could be related to the poor prognosis. The experiments are well controlled for most of the figures. Suggestions follow.

Comment 1: *The authors said, 'Univariate results showed that smoking history (P = 0.050) and pathological pattern (P < 0.001) were related to tumor PVR expression. (l.211~212). I thought these were related to LSECtin expression.*

Reply 1:

Thank you for your careful checking. I apologize for this typographical error. Your comment is reasonable and correct. Herein, I want to express, " Univariate results showed that smoking history (P = 0.050) and pathological pattern (P <0.001) were related to tumor LSECtin expression." I have corrected it and rechecked the manuscript.

Changes in the text: Page 8, line 211-213

Comment 2: *From the TCGA database, LSECtin and PVR mRNA expressions were found to have a positive correlation trend in only lung squamous cell carcinoma. Table 5 showed that in NSCLC patients, the expression of LSECtin was positively correlated with PVR. How about in adenocarcinoma in authors patients.*

Reply 2:

We deeply appreciate your comments. According to your suggestion, we performed a correlation analysis between LSECtin and PVR in patient samples of different pathological pattern. The result was shown in Supplementary Table 1. We found that the expression of LSECtin and PVR was positively correlated in patients with lung squamous cell carcinoma (LUSC), which was consistent with the results from the TCGA database. Although the results in lung adenocarcinoma(LUAD) patients showed that there is no significant statistical correlation, it could be seen from the data that there was a certain positive correlation trend. Meanwhile, the probability of double-positive LSECtin and PVR in LUAD patients was greater than that in LUSC patients. Therefore, if we want to verify the correlation between the expression of these two proteins in different pathological pattern, we will need a larger sample size for support. In the future, we will expand the sample size for subsequent analysis and verification. Finally, we considered to put this supplementary table 1 in the supplementary data if necessary. Meanwhile, this result has been described in the manuscript.

Changes in the text: Page 9, line 258-269

Supplementary Table 1 added in Supplementary data

Supplementary Table 1. Association of LSECtin and PVR expression in NSCLC

Patients in different pathological pattern

	Pathological pattern			
	LUAD		LUSC	
	LSECtin positive	LSECtin negative	LSECtin positive	LSECtin negative
PVR positive	30	7	8	7
PVR negative	8	5	6	27
$\chi 2$	2.014		6.168	
P-value	0.156		0.013	

Comment 3: *The authors said, 'Myeloid-derived cells had a wide range of immunosuppressive effects, and both PVR and LSECtin could be found in myeloid-derived cells.'(l.311~313). Please check these expression in MDSCs in tumor tissues of 98 patients.*

Reply 3:

Thank you for your valuable comments. MDSCs are derived from myeloid progenitor cells and immature myeloid cells, and they are known for their ability to suppress T cell immunity[1]. MDSCs are characterized by uncertain phenotypes, and their molecular expression levels often change with environmental conversion[2]. MDSCs are mainly divided into two categories, which are MDSCs in peripheral lymphoid organs and tumors. They have different functional expertise in tumor development[1]. The current research method on MDSCs is usually mouse tumor-bearing models, the clinical research about MDSCs in tumor patients is more complicated. The complete research needs to collect the patient's peripheral blood and tumor samples for detection and analysis at the same time[3,4].

The sample collection time of the cases in this study was long, and some cases lacked peripheral blood samples. In addition, multispectral IHC is required to check the LSECtin expression of MDSCs in tumor pathological sections[5]. However, the tumor samples used in this study were collected and embedded as early as 2014. Paraffin sections can generate autofluorescence due to preparation reasons, and the storage time of samples is relatively long. These two reasons may lead to false positives and results that make the results unreliable. Therefore, we think that the samples in this experiment are not suitable for detecting the expression of LSECtin in MDSCs.

Finally, your suggestion is very enlightening to us. In the future, we plan to improve the collection of samples and consider performing in vitro and in vivo experiments for verification.

Changes in the text: N/A

Reference:

[1] Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The Nature of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. Trends Immunol. 2016;37(3):208-220.

[2] Wang Y, Ding Y, Guo N, Wang S. MDSCs: Key Criminals of Tumor Pre-metastatic
[3] Niche Formation. Front Immunol. 2019;10:172. Published 2019 Feb 7.
Huber V, Vallacchi V, Fleming V, et al. Tumor-derived microRNAs induce myeloid suppressor cells and predict immunotherapy resistance in melanoma. J Clin Invest. 2018;128(12):5505-5516.
[4] Yamauchi Y, Safi S, Blattner C, et al. Circulating and Tumor Myeloid-derived Suppressor Cells in Resectable Non-Small Cell Lung Cancer. Am J Respir Crit Care Med. 2018;198(6):777-787.
[5] Li XY, Das I, Lepletier A, et al. CD155 loss enhances tumor suppression via combined host and tumor-intrinsic mechanisms. J Clin Invest. 2018;128(6):2613-2625.

Comment 4: *The author said that LSECtin was associated with cell motility and metastasis. Is it possible to add analysis of metastatic tissues if there are resected specimens of metastases?*

Reply 4:

We sincerely thank you for your review. The purpose of this study is to explore the clinical value of LSECtin and the correlation of LSECtin with PVR in NSCLC. Therefore, the samples collected in this study did not include resected specimens of metastases. I apologize for not being able to analyze the metastatic tissues as this kind of tissue are rarely available as surgery will not be done usually in late stage metastatic patients. In previous reports, the research on LSECtin about tumor migration is mainly based on mouse models.

Changes in the text: N/A