Peer Review File

Article information: https://dx.doi.org/10.21037/tlcr-24-157

Reviewer A

The authors employ a dataset of TEP RNA profiles for marker selection, which results into tumor cell/tissue analyses. TRIM27 is selected as a potential oncogenic marker in NSCLC.

Comment 1: The work is interesting though the story is rather unlogical. If the authors can prove that TRIM27 expression in platelets is associated with tumor activity, the work would be more sound. Is there transfer of TRIM27 to platelets, or are miRNAs shuttling from platelets towards cells involved into TRIM27 expression in cells and/or platelets? Identifying such a link would provide biological rationale for the biomarker panels identified, and novelty to the paper.

Reply 1: Thank you for your valuable suggestion. Platelets, as circulating anucleated cells, originate from megakaryocytes in bone marrow and lung niches[1]. The majority of platelets remain quiescent during their 7 to 10-day lifespan[2], the relatively short lifespan makes stable platelets culture a difficult problem to perform. Although lacking nuclei, platelets retain cytoplasmatic RNA derived from megakaryocytes and may translate small amounts of mRNAs and process miRNAs[3]. By consulting numerous authoritative literatures, we tried to find the appropriate methods to prove the TRIM27 expression in platelets is associated with tumor activity. In vitro thrombogenicity testing, platelets, obtained from the whole blood, are used to detect specified parameter in short time, without the process of platelets culture[4]. In a cross-talk study of circulating tumor cells (CTCs) and platelets, transient co-culture of CTCs and platelets assays was performed for 24 h to observe the behavior/phenotype of both cell types[5]. Human pluripotent stem cells, including human embryonic stem cells and induced pluripotent stem cells, can induce platelets or their precursor megakaryocytes[6]. Megakaryocytes can be transfected miRNA, and the effect of miRNA on platelets also can be observed[7]. However, the process of obtaining human megakaryocytes requires a lot of preparation work. In the absence of relevant experience, the implementation of this complex technology requires sufficient time. Meanwhile, the revision need return back within 3 weeks. As for the shortcomings you raised, we have already made statements in the discussion section. Thanks your valuable advice again.

Reviewer B

The goal of this study was to identify a potential biomarker to aid the diagnosis of malignant GGO. The authors conducted TEP RNA-seq of 81 samples (59 malignant GGO and 22 benign GGO) and found 1,647 DEGs. They verified the differential expression of 17 out of 23 select DEGs. The authors then measured the expression of these 17 genes in 10 paired NSCLCs and matched adjacent tissues, and they found only TRIM27 was differentially expressed in the

cancer tissues. Using the A549 and PC-9 cell models with TRIM27 overexpression or knockdown, the authors found that TRIM27 regulates the expression of several genes involved in glycolysis in part through HMOX1. The study is interesting and utilized both human specimens and in vitro cell models.

There are two major issues I'd like to point out.

Comment 1: I found the transition to glycolysis in section 3.4 is abrupt. I'd recommend the authors perform pathway analysis (EnrichR or Gene Ontology) in their 1,647 DEGs and 17 verified ones to determine if the glycolysis-related pathway is one of the enriched pathways.

Reply 1: Thanks for your valuable advice, and we apologize for our omission. Based on TEP RNA-seq data, gene get enrichment analysis (GSEA) was performed and the results revealed that the malignant GGOs group was significantly enriched in glycolysis and gluconeogenesis and ranked first. The relative information has been already supplemented in result 3.2 section. Thanks very much.

Comment 2: The authors should measure PKM2, PKM1, LDHA, HK2, HK1, and HMOX1 in the 10 paired NSCLC tissues and paired normal to determine whether any of these genes are differentially expressed. The positive results could greatly strengthen the conclusion of this study.

Reply 2: Thanks for your valuable advice, and we supplemented experiment. Western blot assay was conducted to detect the protein expression. Due to limited revision time, we can only receive 7 pairs of clinical tissues that meet the requirements. So, the protein levels of PKM1/2, LDHA, HK1/2, GLUT1 and HMOX1 have been measured in seven paired NSCLC and adjacent tissues. The relative information has been respectively supplemented in result 3.6 section and result 3.7 section. Thanks very much.

Minor issues.

Comment 3: The sentence in lines 293-297 is difficult to understand and the sample numbers do not match with the numbers in the Figure 1.

Reply 3: We apologize for our negligence of confusing presentation. The sample numbers in the Figure 1 were the number of samples used for TEP RNA-seq, while the samples used for HT-qPCR included partially usable sequencing remaining samples and new collected samples. Thanks very much.

Comment 4: Line 334: NIC-H1975 should be NCI-H1975

Reply 4: We apologize for this mistake. We have modified NIC-H1975 to NCI-H1975 in the text (See page 3, line 101; page8, line 334; page 24, line 714). Thanks very much.

Comment 5: Figure 6D. Please label the samples with NC in red and TRIM27 OE samples in blue to be consistent with Figure 6C to avoid confusion.

Reply 5: Thank you for your valuable advice, and we apologize for our confused presentation. We have modified the label of NC and TRIM27 OE in Figure 6D.

Comment 6: Lines 389 and 390. The Figure number is missing in the parentheses.

Reply 6: We apologize for our negligence of perfunctory expression. We have added number 7 in the parentheses and modified to Figure 7A and Figure 7B (Page9, lines 389 and 390). Thanks very much.

Comment 7: Table 1. The breakdown numbers of the smoking status in the subjects with benign GGO do not appear to be correct. The sum is more than 22 patients.

Reply 7: Thanks for your suggestion, and we apologize for our negligence of incorrect data. When filtered the related options, the certain options were missed. We have revised the number of smoking status in table 1. Related data have been submitted with supplementary material. Thanks very much.

Reference

1. Lefrancais E, Ortiz-Munoz G, Caudrillier A, Mallavia B, Liu F, Sayah DM, Thornton EE, Headley MB, David T, Coughlin SR *et al*: **The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors**. *Nature* 2017, **544**(7648):105-109.
2. Roweth HG, Battinelli EM: **Lessons to learn from tumor-educated platelets**. *Blood* 2021, **137**(23):3174-3180.
3. Risitano A, Beaulieu LM, Vitseva O, Freedman JE: **Platelets and platelet-like particles mediate intercellular RNA transfer**. *Blood* 2012, **119**(26):6288-6295.
4. Lau S, Maier A, Braune S, Gossen M, Lendlein A: **Effect of Endothelial Culture Medium Composition on Platelet Responses to Polymeric Biomaterials**. *Int J Mol Sci* 2021, **22**(13).
5. Eslami SZ, Cortes-Hernandez LE, Glogovitis I, Antunes-Ferreira M, D'Ambrosi S, Kurma K, Garima F, Cayrefourcq L, Best MG, Koppers-Lalic D *et al*: **In vitro cross-talk between metastasis-competent circulating tumor cells and platelets in colon cancer: a malicious association during the harsh journey in the blood**. *Front Cell Dev Biol* 2023, **11**:1209846.
6. Wu X, Zhang B, Chen K, Zhao J, Li Y, Li J, Liu C, He L, Fan T, Wang C *et al*: **Baffled-flow culture system enables the mass production of megakaryocytes from human embryonic stem cells by enhancing mitochondrial function**. *Cell Prolif* 2023, **56**(12):e13484.
7. Garcia A, Dunoyer-Geindre S, Nolli S, Strassel C, Reny JL, Fontana P: **miR-204-5p and Platelet Function Regulation: Insight into a Mechanism Mediated by CDC42 and GPIIbIIIa**. *Thromb Haemost* 2021, **121**(9):1206-1219.