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Reviewer Comments

This study raised an important diagnostic approach using differential expressed genes (DEGs) for restenosis. GO and KEGG have been applied to explore the related signal pathways. The subject is of interest yet several points need to be further elucidated as follows:

Comment 1: The authors have identified many genes related to ISR in this study. However, genes may play multiple roles depending on its microenvironment. For examples, STAT5 can regulate the expression of genes encoding proteins that are involved in cell proliferation, differentiation, angiogenesis, inflammation, apoptosis, extracellular matrix composition and cell signal transduction. Reduced expression levels of STAT5A may have different effects on cell proliferation and apoptosis, which can be inconclusive to ISR.

Reply 1: We thank the reviewer for bringing these points to our attention and we agree that different expression of genes may play different roles depending on its microenvironment. Considering the vital role of endothelial cells (ECs) in the occurrence of ISR, we mainly discuss the relationship between the expression of STAT5A and the proliferation of ECs. Yang et al. found that conditioned medium derived from constitutively active STAT5A ECs can induce EC invasion and tube formation but does not effect on EC proliferation (1). Li et al. showed that the proliferation of endothelium can be reduced by inhibiting STAT5A-related gene transcription or knocking down STAT5A (2). However, Bucher et al. demonstrated that knocking down STAT5 can eliminate cytokine-induced anti-angiogenesis effects, such as the proliferation, migration and tube formation of human lung microvascular endothelial cells (3). Considering the complexity of the entire gene regulation and the specificity of different cells and different diseases, the role of low STAT5A expression in ISR needs more experiments to confirm.

In general, our results suggested that 10 hub genes may have an effect on the ISR process and be useful in the clinical diagnosis of ISR, which still need confirming and supporting by more experiments and clinical practice. Thanks again for this comment. We have since added the following as a limitation (see Page 15, line 368-370).

Changes in the text: *"The role of STAT5A low expression in the occurrence and development of ISR is still controversial and there is no relevant literature to fully confirm it."*

Reference: 1. Yang X, Qiao D, Meyer K, et al. Angiogenesis induced bysignal transducer and activator of transcription 5A (STAT5A) is dependent on autocrine activity of proliferin. J Biol Chem. 2012 Feb 24;287(9):6490-502.
2. Li Y, Zhao Y, Peng H, et al. Histonedeacetylase inhibitor trichostatin a reduces endothelial cell proliferation by suppressing STAT5A-related gene transcription. Front Oncol. 2021 Sep 23;11:746266.
3. Bucher F, Lee J, Shin S, et al. Interleukin-5 suppresses Vascular Endothelial Growth Factor-induced angiogenesis through STAT5 signaling. Cytokine. 2018 Oct;110:397-403.

Comment 2: This study revealed that the expression profiles of 10 hub genes by AUC of ROC curves demonstrated the correlation order to ISR. However, the higher expression level of one gene may not be more related to the disease as compared to the one with lower expression level. It depends more important on the role of this gene. It would be more convincing to analyze if the defect of the specific gene is closely related to ISR.

Reply 2: We agree with the observation made by the reviewer that the expression of a single gene may play a vital role within the occurrence and development of disease. In this paper, we intended to detect the expression of related genes in ISR patients by bioinformatics analysis. It is necessary to establish cell and animal models with different gene expression and establish gene knockout models to confirm the role of hub genes in ISR fully. However, gene modification and gene expression are complicated processes, especially for polygenic diseases. Due to some compensatory mechanisms, the deletion or expression change of the single gene may have indeterminate effects of certain the disease. Moreover, except for the classic genetic laws Mendelian inheritance, epigenetics changes such as DNA methylation and histone modification also play an important role in the occurrence and development of diseases (PMID: 29540357). Under such circumstances, knocking out the target gene and changing the expression of the gene may lead to different disease phenotypes.
To verify the relationship between specific gene with certain disease, the defect of the specific gene might be more convincing to clarify of the gene. However, due to the limitation of clinical data sources, this study did not cover the validation experiment of gene defect. We have already elaborated on the limitations of this part in the

discussion section. We are grateful for this comment and have added these as further limitations to our study in the discussion section (see Page 18-19, line 450-465).

Changes in the text: *"Under the limitation of clinical data sources, we have not yet done the validation experiment of hub genes, including the detection of hub gene expression in peripheral blood samples of ISR patients and construction of ISR-related cell and animal models. It would be more convincing to analyze the knockout of specific genes. Changes in epigenetics also needed to be considered."*

Comment 3: The authors concluded that CA1, STAT5A and HBQ1 can be the relevant biomarkers for ISR. The rationales need to be further justified why only these three genes were regarded as the important biomarkers for ISR out of the 10 hub genes obtained through Cytoscape.

Reply 3: We appreciate the comment of the reviewer. After the identification of 10 hub gene through Cytoscape, we performed receiver operating characteristic (ROC) curve analysis, which can be used to assess the predictive ability of two or more biomarkers for the same disease. The area under the ROC curve (AUC) is an effective way to summarize the test's overall diagnostic accuracy, and AUC > 0.85 is considered to have excellent predictive value (PMID: 20736804). In fact, the AUC values of all these 10 genes are > 0.7, which means that their accuracy can be considered acceptable. But we prefer a higher predictive value. Between the 10 hub genes, CA1, STAT5A and HBQ1 have excellent diagnostic value in distinguishing ISR samples from the negative ones with AUC > 0.85. We elaborated this in the method part (see Page 10, line 241-247) and the result part (see Page 14, line 331-333).

Changes in the text: **"***Given that ROC curve analysis> 0.85 is considered to have excellent predictive value, CA1, STAT5A and HBQ1 appear to be the most important biomarkers for ISR among these ten genes."*

Comment 4: On line 175, it lacks of Kyoto for the abbreviation of KEGG.

Reply 4: We appreciate this correction from the reviewer and have amended this section accordingly. We have added Kyoto to the abbreviation of KEGG in the subheading on line 210 (see Page 9, line 210).

Changes in the text: *"Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs."*

Comment 5: Does this study using data set from human require IRB approval?

Reply 5: We are grateful for this comment and we agree that it would be better to get IRB approval. Considering that the GEO database is a public database, the patients involved have obtained ethical approval. Numerous of high level bioinformation articles published using public databases we saw before had not provided IRB approval. And we consulted the ethics committee of Xiangya Hospital and they think that ethics approval is not necessary for pure bioinformatics articles, so we didn't prepare IRB approval. If you think it is necessary to get IRB approval, we can try to communicate with the ethics committee of Xiangya hospital to see if the ethics approval can be obtained. For ethical issues, we have made a brief explanation in the ethics part (see Page 20, line 503-508).

Changes in the text: *"The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The GEO database we used is a public database, in which the patients involved have obtained ethical approval. The data that users downloaded for research and publish is based on public resources, so there is no ethical issues and other conflicts of interest."*

Comment 6: Gender difference needs to be considered between restenosis and control groups.

Reply 6: We agree with the reiviewer that differences in sex should be taken into account in any research. However, we don't think the differences in sex will affect the results of this article. Firstly, we calculated the sex difference between the experimental group and the control group. There was no statistical difference in gender between the two groups (see Table 1 in the original text for details). Secondly, we compared the gene expression between men and women respectively in all samples, experimental groups, and control groups. There was no significant sex difference in the expression of 10 hub genes in three comparisons (see in table 1 below). Finally, in the follow-up of large clinical trials, no gender differences in the occurrence of major adverse cardiovascular events after stent placement have been observed, whether it is a drug-eluting stent (1) or the current bioresorbable vascular scaffolds (2). In the follow-up after coronary revascularization with drug-eluting stents, angiography confirmed that there were no significant sex differences in terms of in-stent late loss and in-segment binary restenosis (3).

We are grateful for this comment and we have amended the limitations in the discussion to address this (see Page 18-19, line 450-465).

Table 1

	Total		Experimental group		Control group	
Gene	P.Value	logFC	P.Value	logFC	P.Value	logFC
CLTA	0.196	-0.620	0.244	0.936	0.102	-1.520
CAT	0.227	-0.277	0.729	-0.178	0.134	-0.565
STAT5A	0.006	0.631	0.095	0.722	0.052	-1.260
CD300A	0.340	-0.923	0.555	0.493	0.070	-2.120
CA1	0.327	0.152	0.629	-0.215	0.268	-1.270
NCF2	0.219	-1.157	0.170	-0.346	0.166	-1.760
HBQ1	0.255	-1.455	0.760	-0.233	0.172	-2.410
AHSP	0.162	-1.797	0.197	-0.834	0.164	-2.490
SLC4A1	0.775	-0.074	0.519	0.150	0.273	-0.343
EPB42	0.163	-2.389	0.477	-0.471	0.089	-3.920

Changes in the text: *"Under this limitation, the sex ratio between the control group and the experimental group may not be the same, although there was no statistical difference. Despite in the follow-up of large clinical trials, no sex differences in the occurrence of major adverse cardiovascular events after stent placement have been observed, whether it is a drug-eluting stent or the current bioresorbable vascular scaffolds."*

Reference：1. Hong SJ, Ahn CM, Kim BK, et al. Prospective randomized comparison of clinical and angiographic outcomes between everolimus-eluting vs. zotarolimus-eluting stents for treatment of coronary restenosis in drug-eluting stents: intravascular ultrasound volumetric analysis (RESTENT-ISR trial). Eur Heart J. 2016 Dec 1;37(45):3409-3418.

2. Włodarczak A, Rola P, Szudrowicz M, et al. Sex Differences in the Clinical Features and Outcomes of Patients with Acute Coronary Syndrome Treated with Two Generations (Absorb and Magmaris) of Bioresorbable Vascular Scaffolds. J Clin Med. 2021 Aug 24;10(17):3768.

3. Stefanini GG, Kalesan B, Pilgrim T, et al. Impact of sex on clinical and angiographic outcomes among patients undergoing revascularization with drug-eluting stents. JACC Cardiovasc Interv. 2012 Mar;5(3):301-10.

Comment 7: Since the sample size is rather small, it would be better to have same

sample number between groups to have a more precise comparison. (1 female and 4 males for ISR vs 1 female and 5 males for control).

Reply 7: We appreciate the suggestion from the reviewer. This concern had been encountered before we started this study and we consulted a statistician on this problem. As a small sample size study, we included as many patients as possible in order to improve the reliability of statistics. In the case of a relatively small number of experimental groups, the ratio of the experimental group to the control group can increase to 1:2 or 1:3. Although we have not reached this ratio, increasing a case to the control group will not reduce the accuracy of statistics in principle. Due to the limitation of data volume, we included these samples and we have added this limitation in the discussion part (see Page 18-19, line 449-465).

Changes in the text: *"As a small sample size study, we have included as many patients as possible in order to improve the reliability of statistics. Under this limitation, the sex ratio between the control group and the experimental group may not be the same, although there was no statistical difference. Despite the follow-up of large clinical trials, no gender differences in the occurrence of major adverse cardiovascular events after stent placement have been observed, whether it is a drug-eluting stent or the current bioresorbable vascular scaffolds."*

Comment 8: Check the grammar on line 292 "there have been many studies have been exploring the role of these genes in ISR".

Reply 8: We thank the reviewer for their observations. The grammar has been checked according to this advice, and has been corrected (see Page 14-15, line 348-350).

Changes in the text: *"CA1, STAT5A, NCF2, CAT and CD300A are closely related to ISR and recent studies have made an effort to explore the role of these genes in ISR."*