Peer Review File

Article Information: http://dx.doi.org/10.21037/cdt-20-773

Reviewer A:

Comment #1: how many experiments were performed?

Reply：three times. In this revision, we added in methods section where necessary in each method.

Reviewer B:

Comment 1: I do have some suggestion to improve the article concept....**the presentation is lacking in introduction to justify the use of this work**...there for its should be revised most importantly the **introduction** part which looks lacking the soul...

Reply1： We revised the introduction with the intention to better lead in the topic of this study. The revised part is as pasted below. We removed the less relevant cites and added the most relevant introductions relatwd to our study.

Cardiac muscle tissues are highly sensitive to hypoxic environment, which can not only lead to heart failure after traumas, but also induce injuries of organs and multi organs dysfunction [1]. Previously, there have been mounting studies on the molecular regulators in myocardial cells[2]. Hypoxia models are adopted in myocardial infarction research and often used to evaluate the effects of active substances on the cellular morphology and functions [3].

Long non-coding RNAs (lncRNAs) emerged as influential modulators in various diseases including cancers these years, mediating the disease phenotypes via interplay with other molecules in cells, like proteins, DNA and other RNAs, potentiating these molecules as therapeutic targets[4]. Also, lncRNAs were investigated in cardiovascular diseases. lncRNA MALAT1 was recognized as a cancer biomarker with abnormally high expression in many cancers and thereby regarded as a potential target for cancer treatment[5]. LncRNA GAS5, has been identified as a tumor suppressor in gastric cancer, prostate cancer, bladder cancer, etc[6].

In recent years, LncRNAs have also been increasingly investigated in hypoxia-induced myocardial cells[7]. In myocardial ischemia-reperfusion in-vitro model, GAS5 upregulation contributed to the elevated apoptosis rate of H9C2 cells by enhancing LAS1 via P38/MAPK signaling pathway [8]. Recently, it was reported that GAS5 can be downregulated by a traditional Chinese medicine Astragaloside IV, resulting in the reduced cell injury via Pi3k/mTOR signaling in H9C2 cells[9]. Previously, the apoptosis of hypoxia-induced myocardial cells has been disclosed to be adjusted by GAS5. However, whether GAS5 can modulate inflammation and cell viability in hypoxia-induced H9C2 cells remains to be known. Hypoxia-inducible factor-1α (HIF-1α) has been frequently researched in both in-vitro and in-vivo models of myocardial hypoxia and is stimulated by hypoxia treatment[10]. Furthermore, HIF-1α inhibition was uncovered to alleviate apoptosis and inflammatory cytokine in glucose-induced cardiomyocyte injury model [11]. In hypoxia-induced H9C2 cells, one study revealed that Genistein could decrease H9C2 cell apoptosis from hypoxia injury through inhibition of HIF-1α[12]. Although it has been widely identified that GAS5 or HIF-1α could function in myocardial hypoxia in vitro, no further research has correlated them in detail so far. This study utilized physical methods to establish hypoxia myocardial in-vitro model so as to facilitate an in-depth exploration into the functions of lncRNA GAS5/ HIF-1α in hypoxic myocardial cellular model. JAK1/STAT3 signaling has been proved to be closely correlated with cell viability and apoptosis in various diseases, yet few findings have been connected to hypoxia myocardial in-vitro model [13, 14]. Therefore, in this study, we evaluated the interplay of lncRNA GAS5/ HIF-1α and JAK1/STAT3 signaling in the functions of lncRNA GAS5/ HIF-1α in hypoxic myocardial cellular injuries.

Comment 2: However you have used a cancer model ? cell line is it so? but i really do not see use of any cancer line cell line and also you can remove "Cancer" from title.There are various reason behind a hypoxia status. Please clarify is you have used a cancer cell line and then you need to elaborate that particular caner causing hypoxia?

Reply2：No， we used the hypoxia-induced H9C2 model in this study. Please check in the revision.(esp the method section).

Comment3: So it important that you give background of "H9C2 cell line" in method section.

Reply3: In this revision, we added the background for H9C2 in the cell culture method pasted as below.

“Rat H9C2 cell line is originally from the embryonic rat cardiac tissues, sharing a lot in common with primary cardiocytes [5]. Compared to primary cardiocytes, derived from neonatal rats, H9C2 is easier to acquire and there has been increasing research using the H9C2 to establish hypoxia-stimulated cellular model of myocardial infarction [6]. Therefore, in this study, we used H9C2 cells to simulate the primary cardiocytes in hypoxia conditions.”

Comment 4: How hypoxia was created and how it was created please elborate and how you confirmed the status of hypoxia?

（Discussion 2nd paragraph）In this study, we exposed the H9C2 cells to hypoxic conditions to induce hypoxia injury in cells so as for in-vitro analysis. To confirm the establishment of hypoxia H9C2 model, we observed the changes in cell morphology under microscope at 0h, 12h and 24h after hypoxia exposure and the increasingly notable vacuolation signified that hypoxia induced H9C2 death. Furthermore, functional assays also witnessed the occurrence of hypoxia injuries in hypoxia groups, presenting the reduced viabilities, activated inflammatory cytokines, and increased apoptosis rates in hypoxia groups in comparison with the normal ones. Such characteristics of hypoxia injury in myocardial hypoxia cells have been reported before

Reply 4: We elborated the hypoxia-induced model in method (cells were cultured in incubator for 12 and 24 hours with 95% N2 and 5% CO2 in 37℃ to induce anoxic injury and form hypoxia group of H9C2 cells.) . First, when we created thehypoxia model , we can control the air content in incubator. In addition, as shown in the cell morphology uner microscope after hypxia exposure . This is mentioned in results and discusson.

Comment5: what was rationale behind "**AG-490 treatment"**

Reply5: We added one sentence in method

The cells in hypoxia group (24h) were selected and treated with 10nM of JAK1/STAT3 inhibitor AG-490 agent for an hour (HY-12000, MCE, Shanghai, China) to inactivate the JAK1/STAT3 signaling pathway. **Therefore, two sub-groups were formed with different JAK1/STAT3 activity.** The cells were prepared for further use.

Comment6: CCK-8 and cell apoptosis has same goals and the later one is confirmatory so why CCK-8 is used? can it be removed?

Reply6: We believe that it will be more complete if we include both cell apoptosis and cell viability. Therefore, if there is no hurt ,we insist that we keep that .

Comment7:The introduction doesn´t lead to the topic of the paper, lacking a clear hypothesis.

Reply7：we added "Therefore, we hypothesized that GAS5 might co-regulate the cell functions with HIF-1α in hypoxia-induced H9C2 cells." nearly at the end of the intoduction.

Comment8:The lncRNAs were identified to possess the ability to enhance or prevent the progression? of what? sentence looks incomplete...

Reply 8: We really feel sorry to make such sentence mistakes.

In this revision, we first proof-read by ourselves and correct such similar mistakes and others that we can recogrnize and then we resort to outside help from a foreign expert in this field. Please kindly check the changes in writing traced in the revision.

Comment9 :Please justify how you think **LNCRNA Gas5 Could be related to Myocardium**? any previous literature?

Reply 9: In introdction, we added some citations on GAS5 and H9C2 cells.

“In recent years, LncRNAs have also been increasingly investigated in hypoxia-induced myocardial cells[4]. In myocardial ischemia-reperfusion in-vitro model, GAS5 upregulation contributed to the elevated apoptosis rate of H9C2 cells by enhancing LAS1 via P38/MAPK signaling pathway [5]. Recently, it was reported that GAS5 can be downregulated by a traditional Chinese medicine Astragaloside IV, resulting in the reduced cell injury via Pi3k/mTOR signaling in H9C2 cells[6].”

Comment 10: Its not very clear what **Statistical analysis** was used to determine the significant of data?Please mention each **tools used for particular figure or table**?

Reply10: we added the statistial software used in thispaper. Graphpad is the one that we used to generate figures.

“One-way ANOVA was applied for groups more than two. Post-hoc analysis was conducted using Bonferroni’s correction. Figures were formed and pieced together by GraphPad Prism8 (Graphpad Software, USA).”

Reviewer C:

Comemnt1 :In my opinion, the articel is well written and well designed what I would sugesst is add for intorduction section some details about miRNA or lncRNA in different branches of science

Reply1: Thank you for your opinion, in the revised version, we added the introduction of lncRNA and miRNA in different diseases including cancers. Related section is pasted below.

“Long non-coding RNAs (lncRNAs) emerged as influential modulators in various diseases including cancers these years, mediating the disease phenotypes via interplay with other molecules in cells, like proteins, DNA and other RNAs, potentiating these molecules as therapeutic targets[4]. Also, lncRNAs were investigated in cardiovascular diseases. lncRNA MALAT1 was recognized as a cancer biomarker with abnormally high expression in many cancers and thereby regarded as a potential target for cancer treatment[5]. LncRNA GAS5, has been identified as a tumor suppressor in gastric cancer, prostate cancer, bladder cancer, etc[6]. In recent years, LncRNAs have also been increasingly investigated in hypoxia-induced myocardial cells[7].”

Commment2: What is earlier known about Long noncoding RN**A GAS5 and HIF-1α** role in hypoxia? In normal how do **lncRNA and HIF interpla**y? Please make a note in introduction with 2-3 lines. While LnRNA GAS5 is an important LnRNA in myocardial cell prognosis, it is only LnRNA with a central role in promoting myocardial cell prognosis

Reply2：

Comment3：. Although methods are adequately described. Authors should write in each of the method sections the number of performed experiments, concentrations of antibodies, number of cells, incubation times, ….

Reply 3： We added the number of performed experiments and concentrations of antibodies, number of cells, incubation times in the methods where proper.

Comment 4: AT end of discussion it would be good if author may add something about clinical perspective of interplay between lncRNA and HIF

Reply4: We added one cite in the last paragraph of discussion.

“In practice, there have been some clinical trials in HIF inhibitors mostly in cancers[34]. However, there hasn’t been clinical trial related to lncRNA and HIF-1α as targets in cardiovascular diseases yet.”

Comment5. Fig 4D in the Results section does not describe any result. Check whether Figure 14A is wrong in line 51 on page 8.

Reply 5: We colored the Fig4D in results, please check. Also, We modified the figure legends ,figure and also results mainly focusing on the comparison highlighting the si-GAS5 VS siGAS5+siHIF-1α with the differential significance marker && rather than **. Please note that.

Comment6: Please note the unity of the manuscript. For example, JAK-STAT, JAK/STAT3 and JAK-STAT3.

Reply 6: Dear reviewer, we carefully proof-read the manuscript after settling all the rest issues mentioned by you and also other people .In this version, we are sure that there is no spelling mistake or typo related to JAK-STAT, JAK/STAT3. We unify them as JAK1/STAT3 specifically for the results description.