Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2471-review.doc).

Title: Monitoring adenoviral based gene delivery in rat glioma by molecular imaging

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The manuscript has been improved according to the comments and suggestions of the reviwers. Please see the point-by-point answers.

Reviewer 1:

This is well prepared paper, only have some minor questions.

1. In introduction, “Viral vectors carrying oncolytic genes have been used to treat tumors in animals as well as in humans during early clinical trials in human [2, 3]. … Currently different viral vectors carrying oncolytic genes are being administered directly into the tumor sites by multiple injections [2, 4, 5].” The term of “oncolytic gene” is not accurate. Virus-induced oncolysis is mostly caused by virus replication, not by transgene carried in virus vectors.

   Answer: This paragraph is corrected from oncolytic genes to oncolytic vectors

2. In Material and methods, “After 3 days of incubation supernatant containing adenovirus was collected and the concentration of purified viral particles was determined UV spectrometer.” Here misses the purification of adenovirus or reference for purification.

   Answer: Method for concentration of adenovirus was added, the modified method is as follows:

   After 3 days of incubation supernatant containing adenovirus was collected and concentrated using PEG solution. Viral supernatants were mixed with PEG solution (final concentration 7.3% PEG, 0.92 M NaCl) and incubated overnight at 4^{o}C. After incubation, virus was collected by centrifugation at 1500g for 30 min. Concentration of viral particles were measured UV spectrometer.

3. In result, “(Figure 3). These results indicate that the EPCs are migrating from the injection sites to the periphery as well as center of the tumors.” The injection sites should be indicated in the figure.

   Answer: Injection site was indicated in the Figure 3.

4. In Discussion, “In our previous report, we showed systemic injection of lentivector-transduced EPCs to carry transgene to glioma, while in this present study we have used

replication competent adenovirus to transfect EPCs and deliver transgenes directly into tumor via intratumor injections." Comparing the two delivery systems would be appreciated.

Answer: we added sentences to compare our previous studies with present studies. Following paragraph was added to the discussion section:

In our previous report, we showed systemic injection of lentivector transduced EPCs to carry transgene to glioma. Where we used a replication deficit lentivirus to integrate the reporter gene and showed their deliver capacity of transgenes into tumors neovessels [14]. In this present study, we have used replication competent adenovirus to transfect EPCs and deliver transgenes directly into tumors via intratumor injections. The advantage with this delivery system is one way it can deliver transgenes to tumors and secondly it can destroy the tumor cells by its self-replication properties [7,23].

5. Proofreading is needed. For example, in Abstract, "The results indicate the EPCs' ability to deliver adenoviral vectors carrying therapeutic genes directly into the **glioma.."** Two periods. "Images were obtained with **Three dimensional (3D)** isotropic FIESTA with parameters". "Our recent studies suggest that cord blood derived EPCs can be used as *therapeutic gene delivery vehicles to deliver therapeutic genes* to brain tumors [15]." Repeats of words.

   Answer: Correction was made and proofreading was performed.

Reviewer 2

1. This manuscript needs to be thoroughly revised. The following points require special attention: The English of the manuscript needs to be improved. A native speaker should read the article. There are numerous and often quite basic grammatical and stylistic errors.

   Answer: Proof was read by English native speaker and manuscript was revised.

2. The quality of the photographs in Figure 3 is not sufficient to decide whether there is largely necrotic or viable tumor tissue. A standard haematoxylin & eosin section should be included. The images need to be properly focused including at low magnification.

   Answer: Figure 3 is replaced with new figure and 2X and 40X images were taken with good focus. H & E staining was included as figure 4. We have done the HE staining on frozen sections. We do not have paraffin sections, because of the transgene expression (hNIS) To test the gene expression it is always better to process sections in frozen conditions than paraffin sections. We know frozen section would not give good quality of H&E staining.

3. The adenovirus construct (AD5/carrying hNIS gene) is only mentioned in the abstract. No convincing evidence is presented to support the authors' claim that "Histological analysis further confirmed EPC migration to periphery of the tumor" (abstract).

   Answer: The full details about the adenovirus construct were added to the methods and also included full reference to original work to represent. We rewrote sentences in the abstract to reflect the right meaning. The changes as follows: We further showed EPC distribution around the tumor from the injection site using histological staining. We also showed transgene expression in the tumor using immune-staining. The results indicate EPCs' ability to deliver adenoviral vectors into the glioma upon intratumor injection

4. “Malignant glioma” is a rather broad category. Ref. 1 should be replaced by a reference to the WHO classification for CNS tumors.

   Answer: Glioblastoma is a primary brain tumor and tumor model we used in this study is orthotopic glioma. WHO classification for CNS tumors was added to the line

5. What is novel in this manuscript when compared with Janic B, Jafari-Khouzani K, Babajani-Feremi A, Iskander AS, Varma NR, Ali MM, Knight RA, Arbab AS. MRI tracking of FePro labeled fresh and cryopreserved long term in vitro expanded human cord blood AC133+ endothelial progenitor cells in rat glioma. PloS one 2012, 7(5):e37577. [PMID:22662174 DOI 10.1371/journal.pone.0037577]?

   Answer: Janic et al., 2012 works are on to test develop a method to preserve (freezing) cord blood stem cells. Further we tested the cryopreserved EPCs can keep their migration properties, where we tested the long time stored EPCs can be used for cell based therapies. For these we injected cryopreserved cells into the tail vain of the rats carrying glioma, we observed migration of EPCs into tumor area using MRI imaging.

   In this present study, we showed EPCs can be used as adenoviral gene delivery vehicles and at same time can be tracked using molecular imaging. For our knowledge the concept using EPCs as adenoviral gene delivery vehicle in brain tumors has not been reported previously.

Reviewer 3:

1. In this study, authors have shown application of SPECT in monitoring EPCs transfected by recombinant adenoviral vectors harboring hNIS-reporter for the final aim of gene therapy in glioma cells (although such strategies has been widely used in seperate studies but the jioned combination of them has not been addressed before. All together, results are clear and article is relatively well-written but the following comments might be considered for a revised version (as following): -

   There was no page # in the pdf file of the article which made it difficult to exactly address the comments. -In the Introduction part, there is a large section including a few (informative) paragraphs which are left out of any references. In addition, this section should be summarized and revised with more improved writing skills.

   Answer: New references are given at large section (informative paragraph) in introduction, few lines were included and some parts were rewritten.

2. This section starts with …. “[12-15]. Based on the characteristics of…..” and ends with ” ……to be more effective”. The same goes for some sections in discussion (for example the part starting with…. Cord blood derived EPCs……” and ending with “….gene delivery vehicles.” However, there are a number of (informative) paragraphs in discussion section which are left without an obvious citation.

   Answer: Several references included in these Paragraphs

3. -In the materials and methods, sections dealing with; CD133+ cell collection and Production of replication competent viral vectors, transfection procedures and intra tumor injection would absolutely require an accessible and well described references (rather than the institutional Protocol #).

Answer: We have rewritten the methods to include the brief method with appropriate references.

4. Production of adenoviral vector carrying hNIS genes (which has been used in this study) should also be described or addressed to a previous study.

   Answer: We have included reference to the published method and modifications

5. -In the results section, please indicate in the legend of figure 1, the SD and significance level.

   Answer: SD and significance level indicated in the figure 1 legend.

6. Discussion section suffers from lack of a real comparative explanation on the obtained data by this study and other prior adenoviral based gene delivery studies (for example in human glioma; Szentirmai et al, J Neurosurg. 2008) or even other prior studies utilizing Adenoviral vector carrying hNIS genes in other Image-guided therapies (e.g. Spitzweg et al, Hum Gene Ther. 2007). Authors are encouraged to provide a revision by addressing the comments.

   Answer: Discussion section was further improved by adding the previous works and compared with our studies.

Thank you again for publishing our manuscript in the *World Journal of Clinical Oncology.*

Sincerely,

Nadimpalli RS Varma