Author's response to reviews

Title: Overexpression of CDC2/CyclinB1 in gliomas, and CDC2 depletion inhibits proliferation of human glioma cells in vitro and in vivo

Authors:

Chen Hua (cutv1220@yahoo.com.cn)
Huang Qiang (hq1936@yahoo.com.cn)
Dong Jun (didongjun@163.com)
Zhai De zhong (chuliang_1980@yahoo.com.cn)
Wang Ai dong (eagle.wangad@yahoo.com.cn)
Lan Qing (weizilong2007@163.com)

Version: 4 Date: 28 October 2007

Author's response to reviews: see over
Dear Scott Edmunds PhD:

I'm sorry to send the manuscript to you so late. The point-by-point response to the concerns was showed as follows. We look forward to receiving your decision.

With best wishes,
Dr. Huang Qiang

Reviewer: Jose Segovia

1. The Vector System for Expression of Short Interfering RNA used in our experiment is pSUPER RNAi System. The recombinant retroviral vector is a plasmid. The pSUPER.retro plasmid can be transfected directly into target mammalian cells, or utilize a packaging cell line to produce retroviral supernatants for a higher rate of stable cell integration. The vector uses the polymerase-III H1-RNA gene promoter, as it produces a small RNA transcript lacking a polyadenosine tail and has a well-defined start of transcription and a termination signal consisting of five thymidines in a row (T5). More information about this vector can be found in this website (www.oligoengine.com).


3. Injections were performed 100µl per 3 days, and the procedure repeated 3 times (make a total of 300 µl).

4. C4 was designed by oligoengine workstation (Figure 1). The software gives the effect target corresponds to positions 908(779+129) of the CDC2 open reading frame. But we designed target corresponds to positions 909. We want to know whether it was an “off target”. Our result indicated C4 had no effect on inhibition of CDC2. We think “off target” C4 have the same role that insures the specificity of the RNAi process with scrambled.
5. Characterizations of stem cells from gliomas and fetal tissue were not presented in this article, they were displayed in our published paper (Zhang QB, Ji XY, Huang Q, et al, Differentiation profile of brain tumor stem cells: a comparative study with neural stem cells[J]. Cell Research, 2006, 16(12): 909-915). This paper was cited in my manuscript.

6. Treatment was indicated in Figure7.

Reviewer: Michael Berens
All the minor essential revisions which professor Michael Berens suggested were corrected.

1. Abstract has been careful rewritten.
2. Background:
   a. 6th line down, “Increasing results demonstrates…” is not a complete sentence.
   b. 1st line last paragraph, “…progression associated with gene expression…”
   c. 1st sentence last paragraph, take out the word brain for it is understood with glioma.
   d. 2nd sentence last paragraph, last word of this sentence, “grades” should be plural.
   e. Last sentence last paragraph, “…overexpression of CDC2…”, overexpression of should be eliminated. This wording is confusing.
3. Materials and Methods:
   a. Section 4, “Construction of tissue…”. 3rd line down, “All this patients
didn’t…” should read “All of these patients did not…”.

4. Results:
   a. “Expression of CDC2…” title should all be in italics.
   b. First line of first section, “Expression of CDC2 in primer…”, primer should read primary.
   c. 2nd line of first section, “…first and second recurrence tumor respectively.” ‘respectively’ should be included.
   d. Second section, 3rd line down, “…showed appreciable strong staining...”. Should be appreciably.
   e. Second section, 9th line down, When describing figure 4, the manuscript should describe what panels A-R portray. The Figure legend is insufficient to convey the results from the panels in Figure 4. Also, there is no “Table 1” present that coincides with these results.
   f. Third section, there is no mention of results for U251 cells at 48-96 hrs. If this is due to death at the prior time point, should a different cell line have been chosen?
   g. Third section, figure 5 was eliminated.
   h. 4th section, last line of first paragraph, what figure is the real-time PCR results referring to? If not in the manuscript, please state “data not shown”.
   i. 4th section, last line, “Recombinant C3…” it is indistinguishable to see the differences between the effectiveness of C1 vs. C3.
   j. 6th section, “Therapeutic potential…” 3rd line down, “Virus titre (spelled titer)...RCFU...” Please indicate what the acronym RCFU represents.
   k. 6th section, 5th line down, Figure 10 results are said to be significant, therefore it would be good to possibly make a table indicating the size and weight of the tumors along with p-values.
   l. 6th section, 6th line down, “Tissue microarrays indicated that the percentage...” Please indicate what these percentages are.

5. Discussion:
   a. 3rd paragraph, for in vivo experiments, were mice ever treated with current CDC2 inhibitors to run along side with C3 to make a true comparison of the treatments?
      1. Current CDC2 inhibitors also inhibited other CDKs and lack specificity. We haven’t treated mice with current CDC2 inhibitors to run along side with C3. So we didn’t know their inhibition effect compared with C3.

   b. It was stated that this viral treatment was unable to infect all tumor cells when injected directly. What effects would this CDC2 inhibitor (C3) have on patients if it cannot be injected directly into the tumor?
      2. We can replace the biopsy needle with a silastic ventricular catheter using stereotactic techniques and inject the C3 into the tumor as a single bolus via the catheter. This is the most efficient method.
6. Figures:
   a. There was one figure per page.
   b. Figure 2 was cited in the Materials and Methods.
   c. Figure 3 was flipped horizontally.
   d. Figure 5 was eliminated.
   e. Figure 6 was labeled A-C; panels A and B was flipped horizontally to read left to right; panel C was labeled the lanes and also formatted to grayscale.
   f. Figure 6 legend, panel C mentioned the “same membrane”. What is this referring to?
      “same membrane” was eliminated. It means that the membrane was stained with antibodies against CDC2 and GAPDH at the same time.
   g. Figure 7 figure legend needs to be more specific about each point shown, not just the overall figure in general.
   h. The % apoptosis within the quadrant of interest was label in Figure 8.
   i. Figure 9, Are these images the same size or is one expanded? Why are there two separate images?
      The two separate images show the two separate experiments in vivo under the same condition. The first experiment in vivo was eliminated.

1. The manuscript was revised by a native English scientist who is doing something similar to me to quality of written English. The corrections were red in the article.

2. Informed consent (and consent to publish) documents were attached. The original documents were written in Chinese. So the patients could well understand the document. Tumor specimens derived from Song Da-Mei (宋大妹) were used for gene microarray. Tumor specimens derived from Zhao Yun-Xia (赵云霞) were used for isolation of brain tumor stem cells. One-month-old abortive dead embryo derived from Pan Lin-Lin (潘淋淋) was used for isolation of neural stem cells.
苏州大学附属第二医院神经外科
知情同意书

患者 宋大妹，□男 ■女 性，36 岁，住院号 48955。

根据目前的病史、体格检查、辅助检查（影像学资料等），初步拟诊
为 颅内占位性病变：胶质瘤
，并拟行手术切除肿瘤。

手术日期确定于 1999 年 02 月 05 日。

鉴于手术中所切除的脑肿瘤组织具有进一步科学研究的价值，为
此，我们在取得肿瘤组织标本后，拟对其进行一系列的相关研究，包
括 基因芯片、相关分子病理检测 等
，并就所取得的成果发表相应的文章。为此，希望得到患方的同意，取
得对这些组织标本的处置权。

【患方意见】医生已就上述内容向我们进行了详细、明确的解释：我
们也就所关心的问题与医生进行了讨论，并得到了满意的答复。为此，
我们在完全知情并在自愿的情况下签署本同意书，同意将手术所取得
的组织标本进行上述研究并发表相应的文章和申请成果奖。

患者姓名： 宋大妹 身份证号： 320523631020146
被委托人： 沈桂通 关 系： 丈夫
医生签字： 周炳炎 患者或被委托人签字： 宋大妹
签字日期： 1999 年 02 月 04 日
苏州大学附属第二医院神经外科
知情同意书

患者：宋大妹，□男 □女性，36岁，住院号53225。

根据目前的病史、体格检查、辅助检查（影像学资料等），初步诊断为脑胶质瘤术后复发，并拟行手术切除肿瘤。

手术日期拟定于1999年07月12日。

鉴于手术中所切除的脑肿瘤组织具有进一步科学研究的价值，为此，我们在取得肿瘤组织标本后，拟对其进行一系列的相关研究，包括基因芯片、相关分子病理检测等，并就所取得的成果发表相应的文章。为此，希望得到患方的同意，取得对这些组织标本的处置权。

【患方意见】医生已就上述内容向我们进行了详细、明确的解释；我们也就所关心的问题与医生进行了讨论，并得到了满意的答复。为此，我们在完全知情并自愿的情况下签署本同意书，同意将手术所取得的组织标本进行上述研究并发表相应的文章和申请成果奖。

患者姓名：宋大妹 身份证号：320523631020146
被委托人：沈桂通 关系：丈夫
医生签字：李小军 患者或被委托人签字：沈桂通
签字日期：1999年07月11日
苏州大学附属第二医院神经外科

知情同意书

患者 宋大妹，△男 □女 性，36 岁，住院号 76707。

根据目前的病史、体格检查、辅助检查（影像学资料等），初步拟诊为 脑胶质瘤二次术后复发，并拟行手术切除肿瘤。

手术日期拟定于 2001 年 07 月 11 日。

鉴于手术中所切除的脑肿瘤组织具有进一步科学研究的价值，为此，我们在取得肿瘤组织标本后，拟对其进行一系列的相关研究，包括 基因芯片、相关分子病理检测 等，并就所取得的成果发表相应的文章。为此，希望得到患方的同意，取得对这些组织标本的处置权。

【患方意见】医生已就上述内容向我们进行了详细、明确的解释：我们也就所关心的问题与医生进行了讨论，并得到了满意的答复。为此，我们在完全知情并在自愿的情况下签署本同意书，同意将手术所取得的组织标本进行上述研究并发表相应的文章和提交成果奖。

患者姓名： 宋大妹 身份证号： 320523631020146

被委托人： 沈桂通 关系： 丈夫

医生签字： 金淑兰 患者或被委托人签字： 沈桂通

签字日期： 2001 年 07 月 11 日
知情同意书

患者 赵云霞，□男 ■女 性，52 岁，住院号 123481。
根据目前的病史、体格检查、辅助检查（影像学资料等），初步拟诊为 颅内占位性病变：胶质瘤 ，并拟行手术切除肿瘤。
手术日期拟定于 2004 年 10 月 13 日。

鉴于手术中所切除的脑肿瘤组织具有进一步科学研究的价值，为此，我们在取得肿瘤组织标本后，拟对其进行一系列的相关研究，包括 干细胞培养、相关病理学检测 等，并就所取得的成果发表相应的文章。为此，希望得到患方的同意，取得对这些组织标本的处置权。

【患方意见】医生已就上述内容向我们进行了详细、明确的解释；我们也所关心的问题与医生进行了讨论，并得到了满意的答复。为此，我们在完全知情并在自愿的情况下签署本同意书，同意将手术所取得的组织标本进行上述研究并发表相应的文章和申请成果奖。

患者姓名： 赵云霞 身份证号： 320504520915102
被委托人： 顾雄康 关 系： 丈夫
医生签字： [签字] 患者或被委托人签字： [签字]
签字日期： 2004 年 10 月 13 日
知情同意书

患者：潘淋淋，□男 ■女性，18 岁，住院号：138252。

根据目前的病史、体格检查、辅助检查（影像学资料等），初步拟诊为中孕，□并拟行引产术。
手术日期拟定于 2005 年 07 月 16 日。

鉴于引产后的死亡胚胎，特别是其脑组织，具有进一步科学研究的价值，为此，我们在取得死胎标本后，拟对其进行一系列的相关研究，包括干细胞培养、相关病理学检测等，□并就所取得的成果发表相应的文章。为此，希望得到患方的同意，取得对引产后死胎标本的处置权。

【孕妇意见】医生已就上述内容向我进行了详细、明确的解释，我也就所关心的问题与医生进行了讨论，并得到了满意的答复。为此，我在完全知情并在自愿的情况下签署本同意书，□同意将引产后的死胎标本进行上述研究并发表相应的文章及申请成果奖。

孕妇姓名：潘淋淋 身份证号：342425198702272429
被委托人：□/ □ 关系：□/ □
医生签字：□/ □ 孕妇或被委托人签字：潘淋淋
签字日期：2005 年 07 月 15 日