Author’s response to reviews

Title: ADAR2 editing activity in newly diagnosed versus relapsed pediatric high-grade astrocytomas

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Version: 4 Date: 17 December 2012

Author’s response to reviews: see over
**Point-by-point**

CASE REPORT titled "ADAR2 editing activity in newly diagnosed versus relapsed pediatric high-grade astrocytomas" (MS: 1468846291761783).

Referee: Jacques Grill

**MAJOR POINT:**

1) With respect to the role of RNA editing in pediatric astrocytomas, the authors do not bring new data to support the findings of the first version of their manuscript. If editing by ADAR2 plays a role in astrocytoma progression, similar loss of ADAR2 editing activity should be observed in recurrent low-grade glioma that are often operated more than once than it has been shown here in 4 patients with supratentorial high-grade gliomas. This would strengthen the statement of the authors. This does not mean as said in their answer to my comments to repeat another study on low-grade astrocytomas with or without relapse but analyse patients with matched samples at diagnosis and at relapse (a frequent case in low-grade astrocytomas).

Our previous studies (Cenci et al, 2008; Galeano et al, 2010) showed that different editing profiles exist in pediatric low and high-grade astrocytomas. Indeed, a significant loss of ADAR2 activity is present in high-grade tumors, while the low-grade astrocytomas display only a slight (some time undetectable) decrease of ADAR2 editing levels compared to controls. For this reason we believe that RNA editing in low-grade astrocytomas (at diagnosis and recurrence) would show a different molecular scenario from the high-grade astrocytomas considered in the present study and deserve an independent study.

The only possibility to bring additional data supporting further our findings on pediatric high-grade astrocytomas would be to increase the number of pediatric patients with high-grade astrocytoma. However, the small number of pediatric patients with high-grade astrocytomas enrolled in our study is mainly due to:

- The rarity of high-grade astrocytomas in children;
- Difficulty to get, from the same patient, tumor tissues both at diagnosis and at recurrence,
- Necessity to collect tumor samples, from different patients, developed within the same brain region (in this Case Report we concentrated on supratentorial tumors as our “Case 4” is a supratentorial astrocytoma), as editing change depending on different brain areas (Paupard et al., 2000; Cenci et al., 2008)

Thanks to the Referee’s comment, we have included the following paragraph in Discussion (page 12):

“The small size of patient cohort analyzed in this study is mainly due to the rarity of high-grade astrocytomas in children, together to the difficulty in collecting tumor samples from the same patient both at diagnosis and at recurrence. Additionally, as RNA editing profiles change depending on different brain areas [20, 31], we needed to collect tumor samples from different patients developed within the same brain region (supratentorial astrocytomas).”

2) regarding ADARB1/ADAR2 expression in pediatric high-grade gliomas, a quick look at http://hgserver1.amc.nl/cgi-bin/r2/main.cgi, the web tool compiling gene expression data in pediatric tumors, would tell the authors that ADAR2 expression is higher in grade II than in grade IV. However, there does not seem to exist difference with respect to survival. These data merit to be discussed with respect to the results presented here.

As suggested by the Referee, we have used the “R2: microarray analysis and visualization platform” (http://t2.amc.nl) and searched for ADAR2 mRNA expression in different datasets of
brain tumors/high-grade astrocytomas/GBMs. Of note, only some of these datasets included clinical information such as patient outcome or survival and only one dataset was specific for pediatric gliomas (Tumor Glioma pediatric - Paugh - 53 - MAS5.0 - u133p2). According to this specific array, there is not a statistically significant correlation between \textit{ADAR2} mRNA expression and patients survival (alive/dead).

Thanks to the Referee’s comment, we have included this data in Discussion (page 13):

“Following the observation of \textit{ADAR2} upregulation in a peculiar Case (Case 4), we asked whether a possible correlation exist between \textit{ADAR2} mRNA expression and pediatric patient survival, interrogating available datasets. We analyzed a glioma array specific for pediatric patients in which the clinical outcome was also reported (http://r2.amc.nl, dataset Paugh-53-MAS5.0-u133p2). We observed that, at least in this dataset, there is not a statistically significant correlation between \textit{ADAR2} levels and outcome, even if a slight decrease of \textit{ADAR2} expression is reported for patients died of disease compared to patients alive.”

\textit{MINOR POINT:}

\textit{The paper from Ishiuchi in Nature Medicine 2002 does not state that editing events within \textit{GluR-B} inhibit glioma cell migration in vivo. It shows that Ca(2+)-permeable AMPA receptors have crucial roles in growth of glioblastoma. The expression of these receptors were manipulated with adenoviruses constructs not editing. The sentence should be corrected in the introduction at page 4 line 13.}

We are really sorry to disagree with the Referee regarding this minor point.

Indeed, Ishiuchi et al. did prove that editing at the GluR-2 (or GluR-B) Q/R site in its second hydrophobic segment of this receptor (AMPA channel) is essential for glioma cell migration, as also reported by several papers (for example Choudhury Y et al., 2012; Tan BZ et al., 2009).

From Ishiuchi et al:

“Cell migration was markedly inhibited when the expression of \textit{GluR2} in the tumor cells caused them to adopt a flattened shape and retracted their processes. In contrast, overexpression of Ca2+-permeable AMPARs by adenoviral-mediated delivery of \textit{GluR2(Q)} elongated cellular processes and appeared to promote their migratory behavior.”

“The unique properties of GluR2 can be traced to a single amino-acid residue in the second hydrophobic segment. This residue is arginine (R) in GluR2, whereas the corresponding site is occupied by glutamine (Q) in the other subunits. When the arginine at this critical site (the Q/R site) is replaced with glutamine, the homomeric receptors assembled from the mutant GluR2(Q) show high Ca2+ permeability”

In particular, Ishiuchi et al overexpressed either the GluR2 cDNA contained an arginine (R) residue at 100% or the GluR2(Q) cDNA containing a glutamine (Q) residue, as genomically coded. It is well known that this specific aminoacid change (Q/R) in the GluR-2 (B) is a consequence of an A-to-I RNA editing event mediated by ADAR2 (Higuchi et al 2000).