Reviewer's report

Title: Molecular fingerprinting reflects different histotypes and brain region in low grade gliomas.

Version: 1 Date: 14 May 2013

Reviewer: Federico Roncaroli

Reviewer's report:

Barla et al have profiled 40 paediatric tumours including pilocytic astrocytomas (PA) and mixed glioneuronal tumours (GG) using an Affymetrix array platform. Their aims were to characterize supratentorial vs. infratentorial lesions, identify a gene expression profile that is characteristic of PA and distinguish between supratentorial and infratentorial cases, and to discriminate GG and PA. Candidate genes were then validated with qPCR.

The author’s main conclusions are that supra and infratentorial PAs show different expression profile and that GG are strikingly separated from PA.

Comments

Analysis of expression microarray data is methodologically sound but I think the study is flawed with inaccuracies and is not free of limitations.

There is definitively a problem with the classification or at least the definition of glioneuronal tumours. The term “glial mixed tumours” is inaccurate. It does not exist anywhere in the WHO classification and tumours with a neuronal / ganglionic component are not “low grade gliomas”. The group of LGGs only includes WHO grade I and grade II astrocytomas, grade II oligodendrogiomas and mixed oligo-astrocytomas.

In materials and methods section (page 4), the authors state that they only selected PA but in table 1, case 28 is a reported as WHO grade II fibrillary astrocytoma. This would mean that analysis was performed on 27 PAs, as stated at page 8. If so, why including 1 example of diffuse astrocytoma? Has the diffuse astrocytoma been included between the 10 supratentorial astrocytomas compared with 12 GG?

One of the 27 PA patients has NF1 (case 24 in Table 1). Was it necessary to include this case in a much larger cohort of sporadic PAs? Because it has been done, it would have been interesting to compare its profile with that of sporadic tumours, given the known molecular differences between NF1-related and sporadic PAs.

On a less relevant note, was the NF1-related PA in the optic nerve / chiasm?

The group of GG seems to contain gangliogliomas and desmoplastic infantile gangliogliomas; if so, in what proportion. Histotypes should be specified.
The authors demonstrate that molecular fingerprinting differs between supratentorial and posterior fossa PA. Though interesting, this observation is not original being first published by Sharma MK et al Cancer Res 2007, 67:890-900. A recent study published by Lambert SR et al Acta Neuropathol 2013 May 10. [Epub ahead of print] should also be discussed and compared with the current dataset.

The second conclusion of this study is that 70 genes can discriminate between PAs and GG. This result is interesting but not surprising. PAs and GG are different tumours that composed of different cell types. Also I am not sure of the real, practical value of discriminating PA from GG based on gene fingerprint / signature in order to "develop clinically predictive systems for accurate diagnostic and planning treatment strategies".

Can the authors support this statement with references?

If any, the study would have benefited form further work on tissue to prove on tissue, perhaps using simple immunohistochemistry, that one or some differentially expressed genes can improve the diagnostic accuracy of between supratentorial PAs and GG.

The KIAA1549:BRAF fusion rearrangements has not been assessed in sporadic PAs. I see this as a limitation of this study. Comparison between PAs with and without such rearrangements would have added value to the results and helped to further unveil the complexity of PAs.

One of most pressing questions in paediatric neuro-oncology is to understand why up to 20% of PAs recur and why some behave aggressively. Unfortunately, this study provides no data on outcomes and does not address this point.

The English is good but it should be either British or American rather than mixed.

Minor comments

Introduction

The fact that normal / reactive astrocytes are heterogeneous does not necessarily mean that neoplastic cells in PA and GG have the same degree of heterogeneity

Methods

The sentence “In order to prevent intratumoral heterogeneity and the presence of secondary phenomena, we avoided tissue sampling on marginal areas and we carefully considered homogeneous zones” is unclear.

Intratumoral heterogeneity is something that nobody can prevent. In particular PAs contain microglial cells, macrophages and often show microvascular proliferation, all contributing to intratumoural heterogeneity. Do the authors mean that they wanted to avoid sampling normal tissue?
What does "secondary phenomena" mean?

Extensive dissemination was an exclusion criterion. This contradicts the point made in the introduction indicating a need of predictors of poor outcome for PAs.

The sentence “The intrinsic constitutional variations among individuals may introduce inter-tumoral genetic differences, causing a too high background noise in the dataset” is unclear. What does this mean?

Results

The collagen gene family was significantly represented in supratentorial tumour. Is this because desmoplastic infantile gangliogliomas were included?

Discussion

This investigation has focused on the identification of a specific gene signature based on high-throughput techniques that provide a genome-wide snapshot of LGG with respect to both distinct lesion site in the brain and histotype. I feel this is an overstatment

The authors state that "Although an abundance of data is available on gene expression profiles of LGG, they are often conflicting. Indeed, statistical methods for evaluation and interpretation of microarray data are still evolving. We successfully adopted an analysis workflowable to overcome a major criticality in high-throughput studies, that is to find robust, reproducible and biologically sound results”. Have the authors replicated their analysis using publicly available datasets?

“From a biological point of view, it is remarkable that the ganglial cell tumours are strikingly separated from PA, allowing us to look differently at GG, in which, generally, the glial component catches the attention of the pathologists and contributes to grading” I do not quite understand this sentence

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare I have no competing interests