In vivo 1H MRS, ex vivo HR-MAS and genetic biomarkers for Oligodendroglial tumours differentiation

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Introduction

Oligodendroglial Tumours (OT) are constituted by oligodendrogliomas or pure tumors (OD), and Oligoastrocytomas or mixed tumors (OA) and their respective Anaplastic grade (AOD, AOA). According to WHO classification, pure and mixed classes are considered differential tumour entities. Both classes share genetic-molecular alterations but at different frequencies. Unfortunately, histopathological classification has a subjective component and it is limited in treatment management. It has been observed that Oligodendrogliomas are one of the most chemosensitive solid tumours and that loss of chromosome (LOH) 1p was tightly associated with chemotherapy response

Purpose

The aim of this study was correlating in vivo (1H MRS) and ex vivo (HR-MAS) NMR metabolic profiles, gene expression with genetic and chromosomal aberrations in order to investigate whether metabolic alterations and gene expression profiling could be used to classify OT in an objective manner.

Material and methods

In vivo 1H MRS spectra were acquired following eTUMOUR protocols [1,2]. PRESS sequence with TE 30 and 136 ms and TR 2000 ms were used for SV acquisition for 23 lesions identified as oligodendroglial tumours by histology. jMRUI program was used for quantitative analysis of SV.

HR-MAS 1H NMR spectra and consequent biochemical profile determination were obtained for samples of human oligodendroglial tissues for 15 OD, 3AOD and 7 OA. The amount of human oligodendrogial tissue analysed for each subject ranged from 15 to 40 mg. The whole HR-MAS study was performed at 4 C. HR-MAS spectra were recorded in a Bruker AVANCE spectrometers at 500 and 600 MHz. Samples were spun at 4 and 5kHz. Three different types of spectral editing were obtained by recording 1D 1H pre-saturation, 1D 1H NOESY and 1D 1H CPMG (30 ms echo time) experiments. 2D 1H TOCSY and 2D 13C-HSQC experiments were also recorded on selected samples for assignment purposes [1, 2]. 1D spectra were processed with 0.3 Hz line broadening. Central position of Alanine doublet (1.478ppm) was used for spectral referencing purposes. All samples were analyzed by post-HRMAS histopathology to assess the tissue integrity and double validate histological diagnosis. Aliphatic (0.5 and 4.50 ppm) and Aromatic (4.5 and 9.5 ppm) regions were evaluated and normalized to total spectra area. Spectra were binned into 0.01 ppm buckets to reduce the impact of misalignment and standard statistical analysis were applied for metabolic profile differentiation among the oligodendrogial tumour subtypes.

Microarray analysis was used to determine the expression of ~47.000 transcripts in a set of 16 OD, 3AOD, 7OA and 3AOA. LOH at 1p, 19q and 10q was assessed by quantitative Real Time PCR microsatellite analysis. EGFR and CDK2NA were studied by multiplex Real Time PCR, and TP53 mutations were screened by SSCP-PCR and confirmed by sequencing. Genes more differentially expressed among molecular groups were selected and evaluated by unsupervised analysis (PCA and Hierachical Clustering). 17 genes identified in our screen were validated by Semiquantitative RT-PCR using SYBER-Green.

Supervised learning approaches were used to build a two class prediction model based on their histology class, pure or mixed. We performed an evaluation of 3 algorithms (DLDA, 1-NN, and PAM) and 8 different prediction model were built in each one (2, 5, 10, 20, 35, 50, 75, 100 features). The training error of this prediction models were determined using CV-10, and LOO. Finally, the best number of genes that result in the smallest cross-validation error were selected.

Results:

LOH for 1p, 10q, and 19q was seen in 45%, 45%, and 41% of OT respectively. EGFR amplification, and CDKN2A deletion was found in 14% and 17% respectively. Expression profile showed that OT with 1p/19q loss, express high levels of genes related to neurogenesis meanwhile OT with 1p/19q retention over expressed genes related to immune response and inflammation. No Gene-Ontology based functional enrichment was found in 94 more significant and differentially expressed genes among defined histological classes. We identified 72 features frequently used by predictors, the Average Mixed and Pure Predictive Value was 79.64%, and 94.38% respectively. 37 metabolites previously identified and assigned [3] were quantified and evaluated by Unsupervised (PCA and Hierachical Clustering) and supervised statistically analysis. Likewise, effects caused by spectrometers and anatomical tumour location were taken into account. OT with worst prognosis and lower survival presented increased levels of Phosphocholine, Choline, Fatty acids and Alanine, Figure 1. In the same way OT harboring 1p/19q ROH present higher glutathione levels as compared with 1p/19q allelic deletion. These metabolites provide biological insights related to behaviour and tumour evolution; moreover, mentioned metabolites could be used to monitor tumour progression "in vivo" by MRS as a non invasive diagnostic tool. In vivo MMA/Cr and MMB/Cr ratios showed a significant statistical difference between grades of oligodendroglial tumours. A trend between mI/Cr ratio and LOH and ROH in 1p/19q has been observed.

Conclusions

More functionally significant and differentially expressed features (transcripts or metabolites) were detected among molecular status than defined histological classes. Gene expression profile was decisively conditioned by 1p/19q allelic deletions. Detected alterations could reveal the divergent response showed by this molecular subgroup in chemotherapy treatment. Molecular predictors could be complementary to pathological diagnosis. In vivo metabolic profiles show statistical differences between oligodendroglial grades and a correlation between mI/Cr and LOH/ROH has been detected.

References

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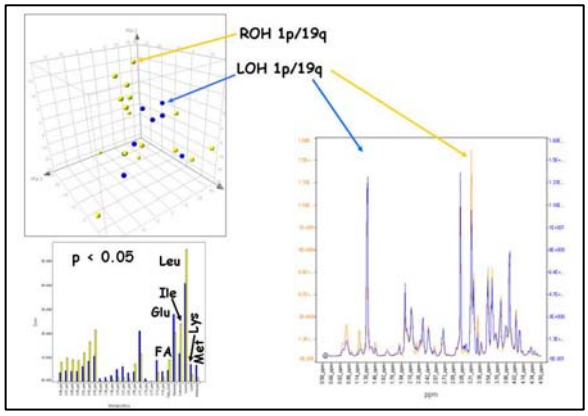

Figure 1.- Correlation between ex vivo metabolic profiles and genetic status (LOH and ROH 1p/19q) for oligodendrogial tumours