A comparative in vitro study of human glioma cell lines: ^{11}C-Methionine uptake and immunocytochemistry approaches

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INTRODUCTION

Glioma are the 33% of all primary brain tumours. However, most of the functional and structural properties of their cellular components remain unknown. PET neuroimaging with ^{11}C-Methionine gives valuable clinical information but knowing the tumour gradation is still difficult. PET multitracer approach for tumour staging is of great interest. Immunocytochemistry allows the assessment and comparison of glial cell markers expression giving complementary information to functional studies. PET and immunocytochemistry were performed to improve our knowledge of the cellular biology of gliomas *in vitro*, and allowed comparing their metabolic behaviour with the expression of different markers of glial cells.

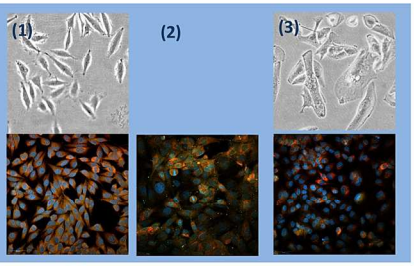
MATERIALS AND METHODS

CELL LINES

1.LN229(ATCC®CRL-2611™)
Glioblastoma multiforme
Grade IV

2. M059K(ATCC®CRL-2365™)
Glioblastoma multiforme
Grade IV radio-resistant

3. SW1783 (ATCC®HTB-13™)
Astrocytoma Grade III



^{11}C-METHIONINE synthetized in CUDIM

PRIMARY ANTIBODIES: mouse polyclonal anti-MAOB (abcam 88510, 1:100 dilution); rabbit polyclonal anti-GFAP (Sigma G9369, 1:400 dilution)

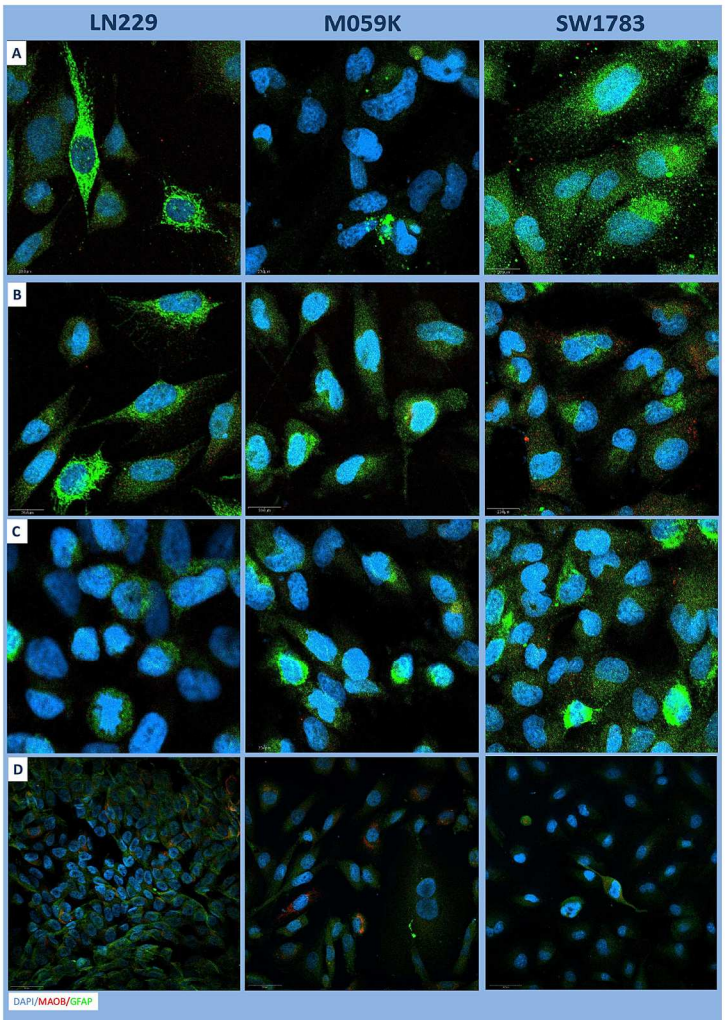
SECONDARY ANTIBODIES: (1:500 dilution): Alexa Fluor 546 goat anti-mouse IgG (Invitrogen A21123); Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen A21206)

OTHER REAGENTS AND SOLUTIONS:
DAPI (Sigma D9417); FORSKOLIN (Sigma F6886); Dichloroacetate (DCA)

INSTRUMENTS:
Nikon Eclipse light microscopy
Olympus FV300 Upright laser confocal microscopy

^{11}C-MET Internalization studies: cells were seeded at 300.000/well. Nonspecific binding (NSB) was assessed with cold methionine. ^{11}C-MET was added, followed by 40min incubation. After glycine acid treatment and buffer washes, cells were lysed and the internalized activity was calculated.

Immunocytochemistry: was performed in control conditions or after inducing astrocyte reactivity. Astrocyte cultures were used as positive controls. Samples of each cell line were exposed 20min to 4% paraformaldehyde and then, 20min to 0.3% Triton X-100. The samples were incubated overnight with primary antibodies (1:100 polyconal Ab anti-monoamine oxidase B together with 1:400 polyclonal anti-glial acidic fibrillary protein). After washed, cells were incubated with fluorescent conjugated secondary antibodies and imaged by confocal microscopy.



A: Control
B: DCA 20mM x 24h
C: pH 6,5 X 24h
D: Forskolin
E: Astrocyte primary culture
F: Reactivity with Forskolin

RESULTS and DISCUSSION

Cell line	*^{11}C MET Uptake*
LN229	92.93±2.1
M059K	80.69±0.7
SW1783	56.35±8.4

The ^{11}C-MET uptake seems to be higher for the more malignant cell lines, although it is necessary to perform more assays in order to confirm our results . MAO-B activity has been reported to be increased in reactive gliosis as well as GFAP. According to our results, these astrocyte cell lines have a moderate expression of both markers , compared with the primary culture of astrocytes in control or reactive conditions. It would be interesting to asses the activity of MAO-B since it has been found that could be increased in tissue samples of malignant glioma.

CONCLUSIONS AND REFERENCES

Our results confirm that the more aggressive glioma cells have a higher protein metabolism. This correlates with a lower expression of MAO-B and GFAP, probably as result of dedifferentiation. Further studies using PET multitracers (i.e. ^{11}C-MET, ^{11}C-DED) might be useful for staging of gliomas.

1. *Gabilondo et al* (2008) Monoamine oxidase B activity is increased in human gliomas. Neurochem Int. 52(1-2):230-234.
2. *Olivera et al* (2011) Neonatal astrocyte damage is sufficient to trigger progressive striatal degeneration in a rat model of glutaric acidemia-I. PlosOne 6(6):e20831