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A Radial Glia Gene Marker, Fatty Acid Binding Protein 7 (FABP7), Is Involved in Proliferation and Invasion of Glioblastoma Cells

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Abstract

Glioblastoma multiforme (GBM) is among the most deadly cancers. A number of studies suggest that a fraction of tumor cells with stem cell features (Glioma Stem-like Cells, GSC) might be responsible for GBM recurrence and aggressiveness. GSC similarly to normal neural stem cells, can form neurospheres (NS) in vitro, and seem to mirror the genetic features of the original tumor better than glioma cells growing adherently in the presence of serum. Using cDNA microarray analysis we identified a number of relevant genes for glioma biology that are differentially expressed in adherent cells and neurospheres derived from the same tumor. Fatty acid-binding protein 7 (FABP7) was identified as one of the most highly expressed genes in NS compared to their adherent counterpart. We found that down-regulation of FABP7 expression in NS by small interfering RNAs significantly reduced cell proliferation and migration. We also evaluated the potential involvement of FABP7 in response to radiotherapy, as this treatment may cause increased tumor infiltration. Migration of irradiated NS was associated to increased expression of FABP7. In agreement with this, in vivo reduced tumorigenicity of GBM cells with down-regulated expression of FABP7 was associated to decreased expression of the migration marker doublecortin. Notably, we observed that PPAR antagonists affect *FABP7* expression and decrease the migration capability of NS after irradiation. As a whole, the data emphasize the role of FABP7 expression in GBM migration and provide translational hints on the timing of treatment with anti-FABP7 agents like PPAR antagonists during GBM evolution.

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Introduction

Gliomas are the most common primary malignancy in the central nervous system (CNS). These tumors exhibit histological resemblance to glial cells. They are classified into WHO grades I to IV [1] with grade III and grade IV (glioblastoma multiforme, GBM) representing the more malignant tumors.

Despite improvements in therapeutic strategies the median survival times of high grade gliomas remain low [2]. The development of novel, more efficacious therapies for this highly complex disease are therefore required.

Recent findings have paved the way towards a better understanding of the biology of glioblastoma. In particular, it has been suggested that many tumors contain a subpopulation of cancer cells possessing stem cell properties. These "cancer stem-like cells" were reported to contribute to invasion and chemoresistance of glioblastoma tumors [3,4]. They are defined as cells that demonstrate stem cell properties (self renewal/multi differentiation capacity), grow as neurospheres, and are functionally associated with increased aggressiveness in terms of invasion/reduced differentiation (more flexible to adapt to different environments), and increased chemoresistance. More importantly, when injected in vivo they are able to partially recapitulate the phenotype of the tumor of the patient from which they are derived [5]. Although there is no unanimity around the exact role and nature of cancer stem cells, many studies converge in showing that under specific culture conditions GBM cells tend to form spheres that contain stem-like cells [6–8]. Whether these cells are pure cancer stem cells remains a matter of debate and in the absence of markers that differentiate stem from non-stem cells [9,10] the question will remain unanswered. However, Lee et al. [11] have demonstrated that cells derived from patient tumors cultured in stem-promoting conditions as neurospheres, maintain the pheno- and geno-type of the original tumor better than the same cells cultured as adherent cells under classical, serum-containing conditions. Also in the current study we find that neurospheres, display typical characteristics (invasion, migration, proliferation)

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the inhibition (compared to scrambled, Panel A). In this cell line silencing of FABP7 caused an in vitro growth arrest. Ten days after plating shFABP7 NS, cells appeared small and disrupted (compared to scrambled NS, Panel B) or attached to the plate showing signs of differentiation, suggesting that the efficient inhibition of FABP7 expression in this NS line impacts on biological functions.
(TIF)

Figure S3 Immunohistochemistry analysis of glioblastoma cell lines engrafted into mouse brain. Photomicrograph of H&E (a, d, g), Ki67 (b, e, h) and FABP7 (c, f, i) stained sections obtained from DBTRG AC-derived (a, b, c), DBTRG NS-derived (d, e, f) and BT138 NS-derived (g, h, i) orthotopic xenografts. Asterisk (*) = Necrotic areas. Arrowheads (>): Pseudopalisading cells. Scale bar = 100 μm.
(TIF)

Figure S4 Histochemistry analysis of brains from tumor-bearing mice. Whole brain photomicrograph of Ki67 staining performed in BT138 NS (a) and DBTRG NS (b) generated tumors. In the lower panels are highlighted the different tumor burden of the two tumors. Scale bar = 100 μm.
(TIF)

File S1 This file includes supporting material, methods and relative references.
(DOC)

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Author Contributions

Conceived and designed the experiments: ADR SP. Performed the experiments: ADR MR SP VM MCS. Analyzed the data: ADR LM EM FM. Contributed reagents/materials/analysis tools: ADR SP AB GF. Wrote the paper: ADR PT.

References

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, et al. (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114: 97–109.
2. Hau P, Stupp R, Hegi ME (2007) MGMT methylation status: the advent of stratified therapy in glioblastoma? Dis Markers 23(1–2): 97–104.
3. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, et al. (Identification of a cancer stem cell in human brain tumors. Cancer Res 63(18): 5821-5828.
4. Llaguno SRA, Chen J, Parada LF (2009) Signaling in Malignant Astrocytomas: Role of Neural Stem Cells and Its Therapeutic Implications. Clin Cancer Res 15: 7124–7129.
5. Vescovi AL, Galli R, Reynolds BA (2006) Brain tumour stem cells Nature Reviews Cancer Vol6 : 425–436.
6. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, et al. (2004) Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 64(19): 7011–21.
7. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, et al. (2004) Identification of human brain tumour initiating cells. Nature 432(7015): 396–401.
8. Tunici P, Bissola L, Lualdi E, Pollo B, Cajola L, et al. (2004) Genetic alterations and in vivo tumorigenicity of neurospheres derived from an adult glioblastoma. Mol Cancer 3: 25.
9. Wang J, Sakariassen PØ, Tsinkalovsky O, Immervoll H, Bøe SO, et al. (2008) CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. Int J Cancer 122(4): 761–8.
10. Lottaz C, Beier D, Meyer K, Kumar P, Hermann A, et al. (2010) Transcriptional profiles of CD133+ and CD133- glioblastoma-derived cancer stem cell lines suggest different cells of origin. Cancer Res 70(5): 2030–40.
11. Lee J, Kotliarova S, Kotliarov Y, Li A, Su Q, et al. (2006) Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 9(5): 391–403.
12. Shimizu F, Watanabe TK, Shinomiya H, Nakamura Y, Fujiwara T (1997) Isolation and expression of a cDNA for human brain fatty acid-binding protein_B-FABP. Bochim Biophys Acta 1354(1): 24–28.
13. Oeemig JS, Jørgensen ML, Hansen MS, Petersen EI, Duroux L, et al. (2009) Backbone and sidechain 1H, 13C and 15N resonance assignments of the human brain-type fatty acid binding protein (FABP7) in its apo form and the holo forms binding to DHA, oleic acid, linoleic acid and elaidic acid. Biomol NMR Assign 3(1): 89–93.
14. Feng L, Hatten ME, Heintz N (1994) Brain lipid-binding protein (BLBP): a novel signaling system in the developing mammalian CNS. Neuron 12(4): 895–908.
15. Taylor M D, Poppleton H, Fuller C, Su X, Liu Y, et al. (2005) Radial glia cells are candidate stem cells of ependymoma Cancer Cell, 8: 323–335.
16. Liang Y, Diehn M, Watson N, Bollen AW, Aldape KD, et al. (2005) Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme PNAS, 102: 5814–5819.
17. Mita R, Coles JE, Glubrecht DD, Sung R, Sun X, et al. (2007) B-FABP–Expressing Radial Glial Cells: The Malignant Glioma Cell of Origin? Neoplasia 9: 734–744.
18. Liang Y, Bollen AW, Aldape KD, Gupta N (2006) Nuclear FABP7 immunoreactivity is preferentially expressed in infiltrative glioma and is associated with poor prognosis in EGFR-overexpressing glioblastoma. BMC Cancer 6: 97.
19. Kaloshi G, Mokhtari K, Carpentier C, Taillibert S, Lejeune J, et al. (2007) FABP7 expression in glioblastomas: relation to prognosis, invasion and EGFR status. J Neurooncol 84(3): 245–8.
20. Hemmati HD, Nakano I, Lazareff JA (2003) Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci Usa 100(25): 15178–15183.
21. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, et al. (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5(10): R80.
22. Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, et al. (2003) Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 15;31(4): e15.
23. Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19(2): 185–193.
24. Pelizzola M, Pavelka N, Foti M, Ricciardi-Castagnoli P (2006) AMDA: an R package for the automated microarray data analysis. Bmc Bioinformatics 7: 335.
25. Smyth GK (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 3:Article3.
26. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, et al. (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9(3): 157–73.
27. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, et al. (2010) Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 17(5): 510–22.
28. Daou MC, Smith TW, Litofsky NS, Hsieh CC, Ross AH (2005) Doublecortin is preferentially expressed in invasive human brain tumors. Acta Neuropathologica, 110(5): 472–80.
29. Masui K, Mawatari S.-ya, Suzuki SO, Iwaki T (2008) Evaluation of sensitivity and specificity of doublecortin immunostatining for the detection of infiltrating glioma cells. Brain Tumor Pathology, 25(1): 1–7.
30. Bisgrove DA, Monckton EA, Packer M, Godbout R (2000) Regulation of brain fatty acid-binding protein expression by differential phosphorylation of nuclear factor I in malignant glioma cell lines. J Biol Chem 275(39): 30668–30676.
31. Josephson R, Müller T, Pickel J, Okabe S, Reynolds K, et al. (1998) POU transcription factors control expression of CNS stem cell-specific genes. Development 125(16): 3087–3100.
32. Arai Y, Funatsu N, Numayama-Tsuruta K, Nomura T, Nakamura S, et al. (2005) Role of Fabp7, a downstream gene of Pax6, in the maintenance of neuroepithelial cells during early embryonic development of the rat cortex. J Neurosci 25(42): 9752–9761.
33. Feige JN, Gelman L, Michalik L, Desvergne B, Wahli W (2006) From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. Prog Lipid Res 45(2): 120–159.
34. Montemayor C, Montemayor OA, Ridgeway A, Lin F, Wheeler DA, et al. (2010) Genome-wide analysis of binding sites and direct target genes of the orphan nuclear receptor NR2F1/COUP-TFI. Plos One 5(1): e8910.
35. Godbout R, Bisgrove DA, Shkolny D, Day RS 3rd (1998) Correlation of B-FABP and GFAP expression in malignant glioma. Oncogene 16(15): 1955–62.
36. Li A, Walling J, Kotliarov Y, Center A, Steed ME, et al. (2008) Genomic changes and gene expression profiles reveal that established glioma cell lines are poorly representative of primary human gliomas. Mol Cancer Res 6(1): 21–30.
37. Bazan NG (2005) Neuroprotectin D1 (NPD1): A DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress. Brain Pathol 15: 159–166.

38. Stillwell W, Wassall SR (2003) Docosahexaenoic acid: membrane properties of a unique fatty acid. Chem Phys Lipids 126(1): 1–27.
39. Mita R, Beaulieu M J, Field C, Godbout R (2010) Brain Fatty Acid-binding Protein and ω-3/ω-6 Fatty Acids. J Biol Chem 285(47): 37005–37015.
40. Brun M, Coles JE, Monckton EA, Glubrecht DD, Bisgrove D, et al. (2009) Nuclear Factor I Regulates Brain Fatty Acid-Binding Protein and Glial Fibrillary Acidic Protein Gene Expression in Malignant Glioma Cell Lines. J Mol Biol 391(2): 282–300.
41. Slipicevic A, Jørgensen K, Skrede M, Rosnes AK, Trøen G, et al. (2008) The fatty acid binding protein 7 (FABP7) is involved in proliferation and invasion of melanoma cells. BMC Cancer 8: 276–288.