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Fos-related antigen-1 (Fra-1) is a regulator of glioma cell malignant phenotype

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Malignant gliomas are aggressive cancers, characterized by innate resistance to therapies and short patient survival.[1] However, the prognosis of these tumors is changing, with the advent of molecular-targeted therapies aimed at the oncogenic signaling associated with tumor specific proliferation, invasion and angiogenesis pathways. There is renewed hope that the unprecedented amount of basic science knowledge acquired in recent years will translate into improved clinical outcomes.

AP-1 (activating protein-1) is a collective term referring to dimeric transcription factors composed of Jun, Fos or ATF (activating transcription factor) subunits that bind to a common DNA site, the AP-1-binding site.[2] There is a high level of complexity in the dimer's formation, as pairing can consist of both homodimers and heterodimers of Jun (v-Jun, c-Jun, JunB, JunD), Fos (v-Fos, c-Fos, FosB, Fra-l, Fra-2) or activating transcription factor (ATF2, ATF3/LRF1, B-ATF) bZIP (basic region leucine zipper) proteins.[3] In addition, the heterodimers display distinguishable DNA binding specificities from each other and from their parental homodimers,[3] raising the question of the different biological functions each and every one of these dimers play both in normal physiology and in the pathophysiology of human diseases.[4] AP-1 has been implicated in a large variety of biological processes including cell differentiation, proliferation,[5] apoptosis[6] and oncogenic transformation.[7] AP-1 activity is modulated by interactions with other transcriptional regulators and is further controlled by upstream kinases that link AP-1 to various signal transduction pathways.[8]

AP-1 is constitutively activated in glial tumors,[9] and directly associates with resistance to chemotherapy,[10] and decreased response to apoptotic signals.[11] Malignant gliomas have an abundance of AP-1 stimulating signals, including epidermal growth factor receptor,[12] and platelet-derived growth factor receptor,[13] and contain mutations in the p53 gene,[14] which contribute to phosphoinositide-3-kinase (PI3K)/Akt and Ras/mitogen-activated protein kinase activation. PTEN (phosphatase and tensin homologue deleted on chromosome ten), a tumor suppressor gene commonly inactivated in glioblastoma, downregulates AP-1, while aberrant PTEN expression contributes to AP-1 constitutive activation.[15] Interestingly, curcumin, a naturally occurring polyphenol derived from the root of the rhizome, *Curcuma longa*, inhibits AP-1 activation and sensitizes glioma cells to several clinically utilized chemotherapeutic agents (cisplatin, etoposide, camptothecin and doxorubicin) and radiation.[16]

The members of Fos protein family are subdivided in two main groups according to their ability to transform rodent fibroblasts: transforming (c-Fos, FosB) and non-transforming (Fra-l and Fra-2).[17] Fra-1 (also named FOSL1), albeit unable to transform, is involved in the malignant progression of multiple tumor types, including non-small cell lung carcinoma[18] and colorectal carcinoma.[19] Silencing or knockdown of Fra-1 expression reverses

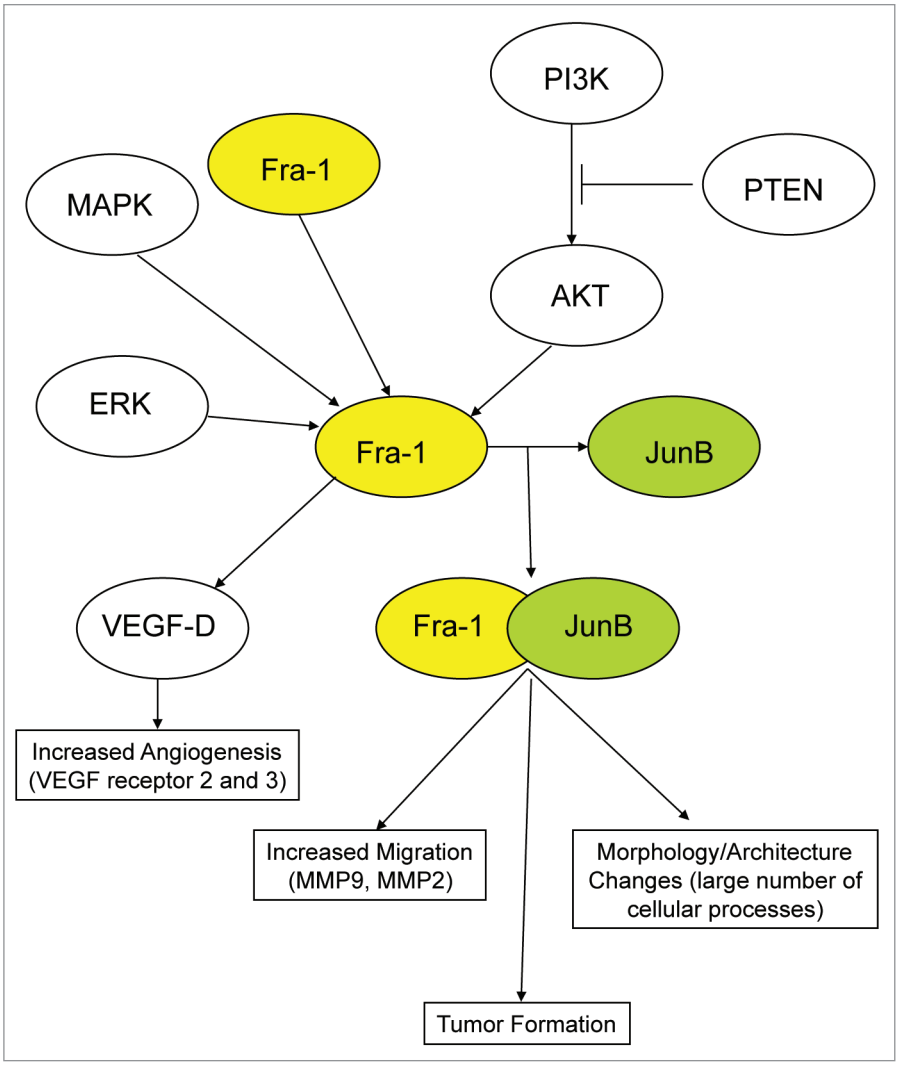

Figure 1. Fra-1 is a key regulator of multiple oncogenic pathways in malignant gliomas. Fra-1 activation is caused by multiple pathways involved in malignant glioma progression, such as Map kinases (MAPK), growth factors (ERK) and the PI3K/AKT cascade. In addition, Fra-1 binds to its gene promoter and auto activates its transcription. In turn, Fra-1 stimulates JunB production and pairs with JunB. The Fra-1-JunB complex formation is associated with cell morphology changes which promote invasion. Fra-1 also stimulates neoangiogenesis through the VEGF-D expression.

the malignant phenotype.[20] In murine models, Fra-1 deletion results in severe growth retardation and embryonic lethality, because this transcription factor is critical for extra-embryonic tissue development, namely the normal vascularization of the placenta.[21] However, if Fra-1 deletion is restricted only to the embryo, these mice subsequently display reduced bone mass formation and osteopenia.[22] Fra-1 overexpression results in osteosclerosis[23] and can lead to lung tumors.[24] In glioma cells, Fra-1 modulates the malignant phenotype by altering the cell shape and anchorage, and its overexpression confers tumorigenicity in non-tumorigenic glioma cell lines.[25]

Malignant gliomas have long been known to be one of the most densely vascularized tumors.[26] Malignant gliomas express high levels of VEGF, a key regulator of angiogenesis. Levels of VEGF expression correlate with both tumor grade and microvessel density.[27-29] These findings suggest that therapeutic strategies targeting angiogenesis may provide an effective approach to suppress malignant glioma growth. Avastin (bevacizumab, Genentech Inc., South San Francisco, CA), a humanized, monoclonal, anti-vascular endothelial growth factor (VEGF) antibody has obtained accelerated FDA approval for the treatment of recurrent glioblastoma (GBM).[30] In this context, VEGF-D, the most recently described member of the VEGF family, has an important role to play.[31] Previously, Debinski et al. have shown that VEGF-D is ubiquitously expressed in GBM under the tight regulation of Fra-1, which in turn activates VEGF receptors 2 and 3, which are very important for tumor angiogenesis.[32] This was the initial report that Fra-1 is a potential novel target for malignant gliomas.

Jun B is an essential gene for the normal embryonic development. In trophoblasts, the lack of JunB causes a deregulation of proliferin, matrix metalloproteinase-9 (MMP-9) and urokinase plasminogen activator (uPA) gene expression, resulting in a defective neovascularization of the decidua and subsequent death of embryos. Jun B involvement in cancer, however, is more complicated as it seems that it can both promote and hinder carcinogenesis. JunB is amplified or overexpressed in cervical cancer cell lines[34] and in tumor samples from patients with primary cutaneous T-cell lymphomas.[35] However, Jun B can also suppress tumorigenesis,[24,36] and can maintain replicative senescence as a balance against excessive proliferation and carcinogenesis.[37] The preferential association of Fra-1 and Jun B was shown to be related with malignant transformation of JB6 epithelial cells, with gain of JunB and Fra-1 binding complexes in all transformed cells, which might contribute to a more proliferative phenotype.[38] The heterodimer Fra-1-JunB is able to regulate the expression of multiple genes including the interleukin 2 (IL-2),[39] and cyclin A,[40] which in turn promote neoplastic cell growth and proliferation.

In the last issue of *Cancer Biology & Therapy,* Debinski and Gibo present their research work showing that the Fra-1-JunB partnership is maintained in glioma, and that this heterodimer binds to the AP-1 site in the JunB promoter. Their interest on the Fra-1-JunB interaction stems from their previously published work, where they document that JunB is 7-fold overexpressed in response to ectopic Fra-1 expression in H4 malignant glioma cells.[25] Such

efforts to clarify the AP-1 interactions in malignant glioma might ultimately elucidate the importance of this pathway in gliomagenesis and create the ability to find a therapeutic target that can affect multiple cancer processes such as vascularization, migration and proliferation.

The authors use multiple, elegant techniques to highlight the important characteristics of this interaction. First, with the use of shRNA and siRNA, they show that Fra-1 silencing deprives cells of extended cellular processes and impairs cell migration, suggesting that Fra-1 plays an important role in glioma invasion in the brain. These morphologic changes were not fully replicated with JunB knock-down, suggesting that the two genes do not have identical functions in glioma cells.

The most important contribution is the elucidation of the Fra-1 and JunB interaction at both protein and gene levels. By employing Fra-1 immunoprecipitation, a significant increase of JunB was noted. The Fra-1-JunB complex is not the only AP-1 heterodimer detected, as c-Jun and JunD were also detected in the cell lysate. Almost all the immunoreactive Fra-1 was phosphorylated, which is the modification required for complex formation. A similar finding was demonstrated for JunB. Chromatin immunoprecipitation of the AP1 binding sites on the *junB* promoter resulted in both Fra-1 and Jun-B precipitation on the AP-1 canonical site, and only of JunB on the noncanonical site. This is another proof of the intricacy of AP-1 regulated mechasnisms, as Fra-1 can regulate JunB in cooperation with JunB, but JunB can also transactivate itself.

A few unsolved questions will need further scientific enquiry. First and foremost, we are still to determine the roles of the different Fra-1 heterodimers in malignant glioma. We now know more about the interaction with JunB, but the importance of Fra-1 interactions with c-Jun and JunD are unknown. If different complexes have different functions—and possibly some of them promote oncogenesis while some others inhibit it—we still need to determine how to affect this fine balance in order to identify potential drug targets. Secondly, the gene targets of Fra-1-JunB in malignant glioma remain to be determined: multiple genes were shown to be potential targets for the AP-1 complexes in both embryogenesis and in cancer—and the targets differ between different malignancies. However, the Fra-1-JunB downstream targets are still elusive in gliomas.

The holy grail of glioma therapy is to find a potent, targeted treatment. Inhibition of multiple molecular pathways seemed promising in in vitro and in animal models. However, many of them failed to improve patients survival.[41] As curcumin-an AP-1 inhibitor seems to hold initial promise, it would be interesting to be able to develop specific compounds targeted to Fra-1 and other important AP-1 proteins. This avenue of research might permit further refinements of our approaches to an almost universally deadly disease.

References

1. Primary Brain Tumors in the United States. Statistical Report 1997–2001. Central Brain Tumor Registry of the United States 2004–2005.
2. Karin M, Liu Zg, Zandi E. AP-1 function and regulation. Curr Opin Cell Biol 1997; 9:240-6.
3. Hai T, Curran T. Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc Natl Acad Sci USA 1991; 88:3720-4.
4. De Cesare D, Vallone D, Caracciolo A, Sassone-Corsi P, Nerlov C, Verde P. Heterodimerization of Jun with ATF-2 and c-Fos is required for positive and negative regulation of the human urokinase enhancer. Oncogene 1995; 11:365-76.
5. Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1991; 1072:129-57.
6. Colotta F, Polentarutti N, Sironi M, Mantovani A. Expression and involvement of c-fos and c-jun protooncogenes in programmed cell death induced by growth factor deprivation in lymphoid cell lines. J Biol Chem 1992; 267:18278-83.
7. Jochum W, Passegue E, Wagner EF. AP-1 in mouse development and tumorigenesis. Oncogene 2001; 20:2401-12.
8. Huang C, Schmid PC, Ma WY, Schmid HH, Dong Z. Phosphatidylinositol-3-kinase is necessary for 12-O-tetradecanoylphorbol-13-acetate-induced cell transformation and activated protein 1 activation. J Biol Chem 1997; 272:4187-94.
9. Antonyak MA, Kenyon LC, Godwin AK, James DC, Emlet DR, Okamoto I, et al. Elevated JNK activation contributes to the pathogenesis of human brain tumors. Oncogene 2002; 21:5038-46.
10. Potapova O, Gorospe M, Bost F, Dean NM, Gaarde WA, Mercola D, et al. c-Jun N-terminal kinase is essential for growth of human T98G glioblastoma cells. J Biol Chem 2000; 275:24767-75.
11. Potapova O, Gorospe M, Dougherty RH, Dean NM, Gaarde WA, Holbrook NJ. Inhibition of c-Jun N-terminal kinase 2 expression suppresses growth and induces apoptosis of human tumor cells in a p53-dependent manner. Mol Cell Biol 2000; 20:1713-22.
12. Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. Nature 1985; 313:144-7.
13. Goussia AC, Agnantis NJ, Rao JS, Kyritsis AP. Cytogenetic and molecular abnormalities in astrocytic gliomas (Review). Oncol Rep 2000; 7:401-12.
14. Sidransky D, Mikkelsen T, Schwechheimer K, Rosenblum ML, Cavanee W, Vogelstein B. Clonal expansion of p53 mutant cells is associated with brain tumour progression. Nature 1992; 355:846-7.
15. Koul D, Shen R, Shishodia S, Takada Y, Bhat KP, Reddy SA, et al. PTEN downregulates AP-1 and targets c-fos in human glioma cells via PI3-kinase/Akt pathway. Mol Cell Biochem 2007; 300:77-87.
16. Dhandapani KM, Mahesh VB, Brann DW. Curcumin suppresses growth and chemoresistance of human glioblastoma cells via AP-1 and NFkappaB transcription factors. J Neurochem 2007; 102:522-38.
17. Tulchinsky E. Fos family members: regulation, structure and role in oncogenic transformation. Histol Histopathol 2000; 15:921-8.
18. Risse-Hackl G, Adamkiewicz J, Wimmel A, Schuermann M. Transition from SCLC to NSCLC phenotype is accompanied by an increased TRE-binding activity and recruitment of specific AP-1 proteins. Oncogene 1998; 16:3057-68.
19. Mann B, Gelos M, Siedow A, Hanski ML, Gratchev A, Ilyas M, et al. Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. Proc Natl Acad Sci USA 1999; 96:1603-8.
20. Milde-Langosch K. The Fos family of transcription factors and their role in tumourigenesis. Eur J Cancer 2005; 41:2449-61.
21. Schreiber M, Wang ZQ, Jochum W, Fetka I, Elliott C, Wagner EF. Placental vascularisation requires the AP-1 component fra1. Development 2000; 127:4937-48.
Eferl R, Hoebertz A, Schilling AF, Rath M, Karreth F, Kenner L, et al. The Fos-related antigen Fra-1 is an activator of bone matrix formation. EMBO J 2004; 23:2789-99.
23. Jochum W, David JP, Elliott C, Wutz A, Plenk H Jr, Matsuo K, et al. Increased bone formation and osteosclerosis in mice overexpressing the transcription factor Fra-1. Nat Med 2000; 6:980-4.
24. Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 2003; 3:859-68.
25. Debinski W, Gibo DM. Fos-related antigen 1 modulates malignant features of glioma cells. Mol Cancer Res 2005; 3:237-49.
26. Brem S, Cotran R, Folkman J. Tumor angiogenesis: a quantitative method for histologic grading. J Natl Cancer Inst 1972; 48:347-56.
27. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 1992; 359:845-8.
28. Schmidt NO, Westphal M, Hagel C, Ergun S, Stavrou D, Rosen EM, et al. Levels of vascular endothelial growth factor, hepatocyte growth factor/scatter factor and basic fibroblast growth factor in human gliomas and their relation to angiogenesis. Int J Cancer 1999; 84:10-8.
29. Samoto K, Ikezaki K, Ono M, Shono T, Kohno K, Kuwano M, et al. Expression of vascular endothelial growth factor and its possible relation with neovascularization in human brain tumors. Cancer Res 1995; 55:1189-93.
30. Chamberlain MC. Emerging clinical principles on the use of bevacizumab for the treatment of malignant gliomas. Cancer 116:3988-99.
31. Achen MG, Jeltsch M, Kukk E, Makinen T, Vitali A, Wilks AF, et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). Proc Natl Acad Sci USA 1998; 95:548-53.
32. Debinski W, Slagle-Webb B, Achen MG, Stacker SA, Tulchinsky E, Gillespie GY, et al. VEGF-D is an X-linked/AP-1 regulated putative onco-angiogen in human glioblastoma multiforme. Mol Med 2001; 7:598-608.

33. Schorpp-Kistner M, Wang ZQ, Angel P, Wagner EF. JunB is essential for mammalian placentation. EMBO J 1999; 18:934-48.
34. Choo KB, Huang CJ, Chen CM, Han CP, Au LC. Jun-B oncogene aberrations in cervical cancer cell lines. Cancer Lett 1995; 93:249-53.
35. Mao X, Orchard G, Lillington DM, Russell-Jones R, Young BD, Whittaker SJ. Amplification and overexpression of JUNB is associated with primary cutaneous T-cell lymphomas. Blood 2003; 101:1513-9.
36. Deng T, Karin M. JunB differs from c-Jun in its DNA-binding and dimerization domains and represses c-Jun by formation of inactive heterodimers. Genes Dev 1993; 7:479-90.
37. Konishi N, Shimada K, Nakamura M, Ishida E, Ota I, Tanaka N, et al. Function of JunB in transient amplifying cell senescence and progression of human prostate cancer. Clin Cancer Res 2008; 14:4408-16.
38. Yang S, Misner B, Chiu R, Meyskens FL Jr. Common and distinct mechanisms of different redox-active carcinogens involved in the transformation of mouse JB6P$^+$ cells. Mol Carcinog 2008; 47:485-91.
39. Ehret A, Li-Weber M, Frank R, Krammer PH. The effect of HIV-1 regulatory proteins on cellular genes: derepression of the IL-2 promoter by Tat. Eur J Immunol 2001; 31:1790-9.
40. Casalino L, Bakiri L, Talotta F, Weitzman JB, Fusco A, Yaniv M, et al. Fra-1 promotes growth and survival in RAS-transformed thyroid cells by controlling cyclin A transcription. EMBO J 2007; 26:1878-90.
41. Kesari S, Ramakrishna N, Sauvageot C, Stiles CD, Wen PY. Targeted molecular therapy of malignant gliomas. Curr Oncol Rep 2006; 8:58-70.