Controlling Cytoplasmic c-Fos Controls Tumor Growth in the Peripheral and Central Nervous System

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Controlling Cytoplasmic c-Fos Controls Tumor Growth in the Peripheral and Central Nervous System

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Abstract Some 20 years ago c-Fos was identified as a member of the AP-1 family of inducible transcription factors (Angel and Karin in Biochim Biophys Acta 1072:129–157, 1991). More recently, an additional activity was described for this protein: it associates to the endoplasmic reticulum and activates the biosynthesis of phospholipids (Bussolino et al. in FASEB J 15:556–558, 2001), (Gil et al. in Mol Biol Cell 15:1881–1894, 2004), the quantitatively most important components of cellular membranes. This latter activity of c-Fos determines the rate of membrane genesis and consequently of growth in differentiating PC12 cells (Gil et al. in Mol Biol Cell 15:1881–1894, 2004). In addition, it has been shown that c-Fos is over-expressed both in PNS and CNS tumors (Silvestre et al. in PLoS One 5(3):e9544, 2010). Herein, it is shown that c-Fos-activated phospholipid synthesis is required to support membrane genesis during the exacerbated growth characteristic of brain tumor cells. Specifically blocking c-Fos-activated phospholipid synthesis significantly reduces proliferation of tumor cells in culture. Blocking c-Fos expression also prevents tumor progression in mice intra-cranially xeno-grafted human brain tumor cells. In NPcis mice, an animal model of the human disease Neurofibromatosis Type I (Cichowski and Jacks in Cell 104:593–604, 2001), animals spontaneously develop tumors of the PNS and the CNS, provided they express c-Fos (Silvestre et al. in PLoS One 5(3):e9544, 2010). Treatment of PNS tumors with an antisense oligonucleotide that specifically blocks c-Fos expression also blocks tumor growth in vivo. These results disclose cytoplasmic c-Fos as a new target for effectively controlling brain tumor growth.

Keywords Cytoplasmic c-Fos · Brain tumors · Membrane biogenesis · Phospholipid synthesis · Regulation

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Introduction

The expression of the proto-oncogene *c-fos* is rapidly and transiently induced in response to a plethora of stimuli [1–3]. c-Fos heterodimerizes with *jun* proteins thus forming AP-1 transcription factors that are imported into the

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nucleus and regulate the expression of target genes involved in the initiation of DNA synthesis in response to growth factors [1, 4, 5]. In addition, an increasing number of reports show the presence of c-Fos and of other immediate early genes in the cytoplasm. Such is the case in light-stimulated retina ganglion cells [6, 7], in growing NIH 3T3 cells [8], in frog primary spermatogonia [9], in PC12 cells induced to differentiate [10] and in tumors of the nervous system [11]. An additional, AP-1 independent activity has been ascribed to cytoplasmic c-Fos: it associates to components of the endoplasmic reticulum (ER) and activates the overall synthesis of phospholipids in cells induced to differentiate or to grow, all cellular processes that demand high rates of membrane biogenesis [6–8, 10].

c-Fos associated to the ER activates only specific enzymes of the pathway of synthesis of these lipids [12, 13]. Activation of the synthesis of polyphosphoinositides involves the activation of CDP-diaylglycerol synthase and phosphatidyl inositol 4-Kinase II α but not of phosphatidyl inositol synthase or of phosphatidyl inositol 4-Kinase II ß. Co-immunoprecipitation and fluorescence resonance energy transfer (FRET) assays evidenced a physical interaction between c-Fos and the enzymes it activates. Kinetic parameters (K_m and V_{max}) of the activated enzymes shows a more than doubling of the V_{max} of each reaction performed in the presence of c-Fos as compared to those in the absence of c-Fos with no substantial modification of the K_m [13]. By contrast, no association could be evidenced between c-Fos and the enzymes not activated by this protein nor was any modification found in the kinetic parameters of these enzymes when assayed in the presence and the absence of c-Fos [13].

c-Fos also activates the overall metabolic labeling of another ubiquitous membrane component, the glycolipids, in differentiating PC12 cells [14]. Enzyme determinations showed that c-Fos activates the enzyme glucosylceramide synthase (GlcCerS), the product of which, GlcCer, is the first glycosylated intermediate in the pathway of synthesis of glycolipids. By contrast, the activities of GlcCer galactosyltransferase 1 and lactosylceramide sialyltransferase 1 are essentially unaffected by c-Fos. Co-immunoprecipitation experiments evidenced that c-Fos participates in a physical association with a V5-tagged version of glucosylceramide synthase but not with glucosylceramide galactosyltransferase 1 and lactosylceramide sialyltransferase 1. Furthermore, c-Fos increases the V_{max} of glucosylceramide synthese without significantly modifying the K_m of the reaction; no variations in the kinetic parameters of glucosylceramide galactosyltransferase 1 and lactosylceramide sialyltransferase 1 assayed in the presence and the absence of c-Fos were observed [14].

In the present report, we show that c-Fos-activated lipid synthesis enables the high rates of membrane genesis required for tumor growth both in the CNS and the PNS. Specifically blocking c-Fos-activated phospholipid synthesis significantly reduces proliferation of tumor cells in culture and prevents tumor progression in mice intra-cranially xeno-grafted human brain tumor cells. In animals that spontaneously develop PNS and CNS tumors, treatment of PNS tumors with c-Fos antisense oligonucleotide blocks tumor growth in vivo. The lipid-activating portion of c-Fos that is, from the N-terminus up to the Basic Domain (BD, amino acids 1-139) [10] is capable of fully supporting tumor cell proliferation in primed cells.

Materials and Methods

Cell Cultures

T98G, NB41A3, C6 and U87M cells (ATCC) were grown at 37 °C in 5 % CO_2 in DMEM (Sigma-Aldrich) supplemented with 0.04 mM glutamine plus 10 % FBS (Invitrogen). Cells grown 48 h post-seeding were serum depleted for another 48 h to arrest in G_0 and induced to re-enter growth by feeding of 10 % FBS.

Phospholipid Labeling

In vitro phospholipid labeling capacity of cultured cells or of the stated sub-cellular fractions was performed as described previously [10] by incubation at 37 °C for 60 min in a final volume of 80 µl containing 140 mM NaCl, 4.5 mM KCl, 0.5 mM $MgCl_2$, 5.6 mM Glucose, HEPES 64 mM pH 7.4, 3 µCi of γ^{32}P-ATP (3,000 Ci/mmol, Perkin Elmer Life Sciences) and when stated, recombinant c-Fos [1 µg/ng of initial total homogenate or the protein recovered in the membrane fraction (MF) or the supernatant fraction (SF) starting with 1 ng of homogenate protein] suspended in 3 µl of 300 mM imidazole/8 M urea or 3 µl of c-Fos vehicle for controls. Reactions were initiated by addition of 100 µg of total homogenate or the corresponding MF, SF or stripped fractions and stopped by the addition of trichloro acetic acid-phosphotungstic acid (5–0.5 % final concentration). ^{32}P-phospholipid labeling was quantified as described previously [15].

Cell Treatments with Cellular Effectors

Oligonucleotide treatment: Cells in G_0 or at the indicated times after initiation of serum treatment were fed *c-fos* mRNA Morpholino antisense (5′-CCA TGA TGT TCT CGG TTT CAA CGC-3′) or sense oligonucleotide according to manufacturer's instructions (Gene Tools) during 3 h, then medium replaced by oligonucleotide-free medium and continued under the indicated experimental conditions for the times stated.

Blocking of AP-1-c-Fos nuclear import: Cells were fed the peptide NLSP of sequence AAVALLPAVLLALLAPVQRKRQKLMP that carries the AP-1-signal-dependent nuclear import sequence (underlined) preceded by a cell-permeation sequence to deliver the functional domain [16] (Biosynthesis). NLSP was fed to cells suspended in 5 µl of medium at the times indicated.

Protein Analysis

Ten µg of protein were subjected to SDS-PAGE on 12 % poly acryl amide gels as described previously [8]. Blocked membranes were incubated with rabbit c-Fos Ab (Santa Cruz, sc-52), goat calnexin (Santa Cruz, sc-6465) or mouse DM1A mAb raised against α-Tubulin (Sigma, T9026) in a 1/5,000 dilution, washed for 15 min twice in PBS-Tween and then incubated with a secondary, biotin conjugated antibody (Vector), dilution 1/15,000, raised against each corresponding primary antibody for immunodetection. Samples were incubated with streptavidin-peroxidase conjugated (Amersham), dilution 1/60,000 and immunodetection performed using ECL plus (Amersham).

Immunofluorescence Analysis

Cells grown on round, acid-washed poly-lysine (1 mg/ml) coated cover slips were fixed, blocked and immunolabeled as described [10]. Rinsed cells were fixed at 37 °C for 10 min in 3 % paraformaldehyde, 4 % sucrose in 10 mM PBS, washed twice and permeabilized with 0.25 % Triton X-100 in PBS for 10 min at 37 °C. Washed cover slips were blocked with 1 % BSA/0.1 % Tween 20 (v/v) in 10 mM PBS (blocking buffer) for 2 h and incubated overnight at 4 °C in blocking buffer containing c-Fos Ab (Santa Cruz, sc-52) dilution 1/500, α-Tubulin (DM1-A, Sigma Aldrich) dilution 1/1,000 and calnexin (Santa Cruz) dilution 1/500 antibodies. Washed cells were incubated with Alexa 546 and Alexa 488 (Molecular Probes) each diluted 1/1,000 in blocking solution, washed, mounted in ProLong Antifade (Molecular Probes) and visualized on a confocal laser scanning microscope LSM 5 (Carl Zeiss).

Cell Proliferation Assay

Cells were grown on 96 well plates and proliferation followed for the stated time with CyQuant cell proliferation kit (Molecular Probes) according to manufacturer's instructions.

Intracranial Cell Transplantation

3.7×10^4 T98G cells in 3 µl of DMEM were injected intracranially into the right caudate putamen area of 4- to 6-week old Nu/Nu nude mice, following general anesthesia [17]. Six days later, c-Fos antisense oligonucleotide or sense/scrambled oligonucleotide (350 nmol per 7.8 ul DMEM per day, for 28 days) or vehicle was infused continuously through osmotic minipumps (Alzet) inserted under the neck skin connected to a cannulae implanted at cell injection site. Twenty-eight days later, mice under general anaesthesia were perfused intracardially with 4 % paraformaldehyde/PBS 0.1 M. Removed brains were immersed 3 days in 0.1 M PBS/30 % sucrose, crio-sectioned at 30 µm thickness and stained with haematoxylin/eosin for histopathological analysis. Immunocytochemical examination was performed as described [11].

Treatment of PNS Tumors in NPcis Mice

Mice with spontaneously developed tumors of ~600 mm^3 of volume received once every 3 days, a 50 µl intratumoral injection containing DMEM (control) or 1.5 µmol of ASO or SO resuspended in DMEM. Tumor volume was measured with a digital caliber on days 0, 4, 8, 10, 16 and 20 of initiating treatment. Injections and tumor volume measurements were performed double-blind.

Results

The possibility that c-Fos activates phospholipid synthesis was examined in cultures of brain tumor cell lines derived from a neuroblastoma (NB41A3), a glioma (C6) and a glioblastoma multiforme (U87M) and confirmed in T98G cells that also derive from a glioblastoma multiforme [18, 19]. Cells were induced to proliferate and grow by feeding of FBS (fetal bovine serum) (+FBS) as compared to fasted (−FBS) sister cells. Figure 1a shows a ~3-fold activation of phospholipid synthesis and abundant c-Fos expression as determined by western blot (Fig. 1b) in T98G cells cultured +FBS during 4 days as compared to −FBS cells. In addition, cell number was about fourfold higher in +FBS with respect to −FBS cells at 4 days in culture (Fig. 1c). Inhibition of c-Fos expression with a c-Fos mRNA antisense oligonucleotide (ASO) abrogated phospholipid synthesis activation and reduced proliferation rates to those of –FBS cells whereas the corresponding sense oligonucleotide (SO) had no effect (Fig. 1). These results were also a constant in the other brain tumor cell lines examined (Supplementary Fig. 1, 2 and 3).

Confirmation that c-Fos activates phospholipid synthesis was obtained by comparing phospholipid labeling in vitro in membranes prepared from T98G cells grown +FBS and −FBS. Phospholipid labeling was activated in total homogenate (TH) and in the membrane fraction (MF) obtained from +FBS after centrifugation at 100,000×*g* for 1 h as compared to −FBS cells (Fig. 2a). Western blot

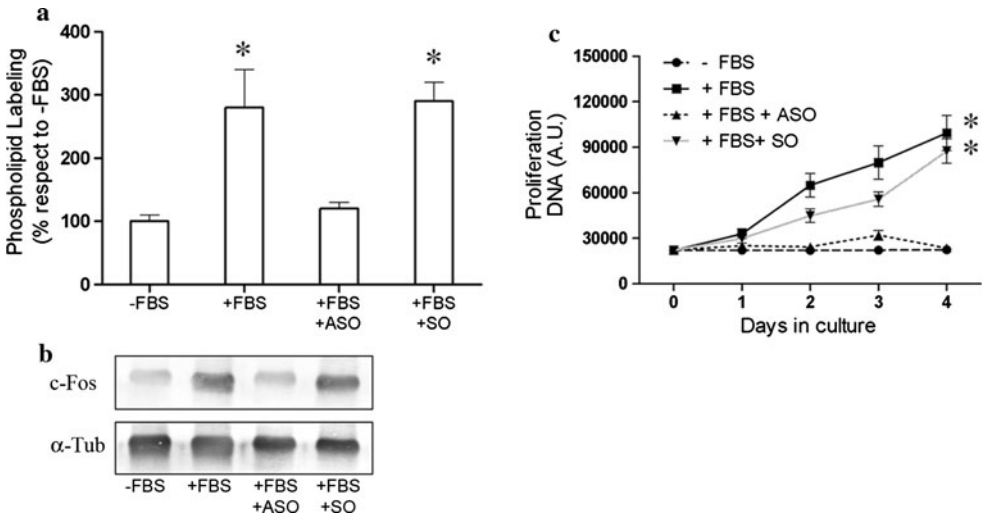

Fig. 1 Activated phospholipid synthesis and proliferation in c-Fos-expressing T98G tumor cells. T98G cells were cultured 4 days +FBS, −FBS, +FBS + ASO or +FBS + SO. Then cells were examined for: (**a**) in vitro ^{32}P-phospholipid labeling capacity (mean % phospholipid labeling ± SD; n = 4 experiments in duplicate; −FBS cell values = 100 %; *$p < 0.0001$ determined by Students two tailed t test. Assays contained 100 µg of the corresponding total homogenate as enzyme source); (**b**) c-Fos content by Western blotting; α-tubulin was used as a loading control; (**c**) cell proliferation expressed as arbitrary units (AU) of DNA; shown is one experiment of 5 performed in quintuplicate, all with essentially the same results (10,000 cells were plated for each proliferation assay)

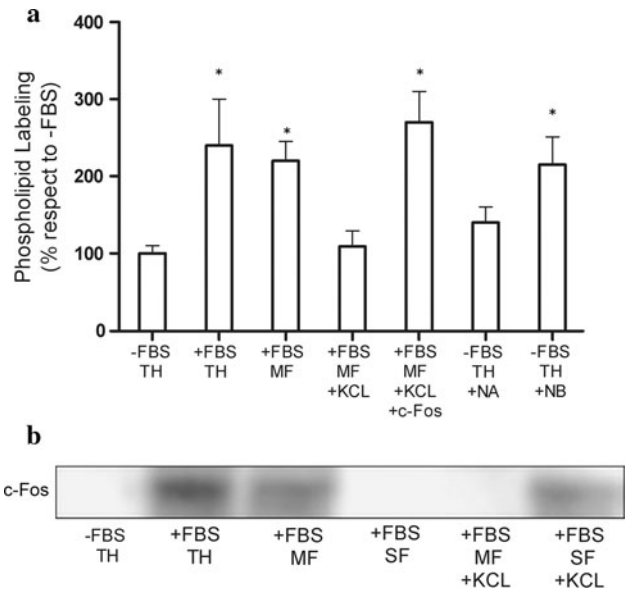

Fig. 2 Membranes from +FBS T98G cells show elevated c-Fos content and activated phospholipid labeling. **a** TH, MF and KCl-stripped MF (MF + KCl) prepared from 100 µg of total homogenate of T98G cells cultured 4 days in the absence (−FBS) or the presence (+FBS) were assayed for phospholipid labeling capacity (mean % phospholipid labeling ± SD; n = 3 experiments in triplicate; -FBS TH values = 100 %; *$p < 0.0001$ determined by Students two tailed t test) in the absence or the presence of recombinant c-Fos (+c-Fos) or of the c-Fos deletion mutants NA or NB added at 1 ng/µg of TH protein. **b** Western blot showing c-Fos content in the samples used in (**a**). For comparative purposes, equal volumes of each subcellular fraction prepared from 100 µg of total homogenate were loaded

examination showed that the elevated content of the c-Fos initially found in the TH of +FBS cells was recovered in the MF whereas the 100,000×*g* supernatant fraction (SF) was practically devoid of c-Fos (Fig 2b). Releasing of c-Fos from MF of these +FBS cells by stripping with 1 M KCl (MF + KCl) (Fig. 2b) resulted in −FBS cell levels of phospholipid labeling whereas addition of recombinant c-Fos restored phospholipid labeling activation (Fig. 2a, compare 4th and 5th columns). Furthermore, the addition of the c-Fos deletion mutant NB (aa 1–159) that includes the basic domain contained in aa 139–159 of full length c-Fos, activates phospholipid synthesis whereas the deletion mutant NA (aa 1–139) which lacks this basic domain has no effect (compare last two columns of Fig. 2a) [10]. c-Fos released from +FBS after stripping MF with KCL is recovered in the SF (last two lanes of Fig. 2b).

Cytoplasmic c-Fos Sustains Tumor Cell Proliferation

The participation of nuclear and cytoplasmic c-Fos in driving tumor cell proliferation and growth was examined in +FBS T98G cells grown in the presence or the absence of an AP-1 Nuclear Localization Sequence Peptide (NLSP) that blocks the nuclear import of c-Fos as an AP-1 dimmer [10, 16]. Figure 3a shows that, as expected, at 30 h of cell priming with FBS, the number of cells roughly doubled. Feeding of NLSP at 0 or 6 h after FBS blocked cell proliferation whereas at 9 h it was no longer effective, indicating that nuclear AP-1-c-Fos is required to trigger

proliferation only at early stages of cell proliferation. By contrast, cytoplasmic c-Fos is required at all time points because blocking of its expression by the addition of ASO to the culture medium at any time after feeding FBS, + or − NLSP specifically blocks proliferation (Fig. 3a).

The importance of phospholipid synthesis activation during proliferation and growth was examined in fasted T98G cells transfected to express c-Fos or its deletion mutants NA or NB. Removal of FBS from the culture medium at 6 h results in non-proliferating cells, irrespective of these being or not transfected to express c-Fos or any of its deletion mutants (Fig. 3b). However, if FBS is removed from the medium at 9 h, proliferation continues normally provided cells are transfected to express c-Fos or its deletion mutant NB that activates phospholipid synthesis. No proliferation is observed in NA-transfected cells that, as shown in Fig. 2a, also show no phospholipid synthesis activation. These results and the previous observation that no proliferation or phospholipid synthesis activation is observed in primed T98G cells transfected to express the phosphomimetic version of c-Fos, Y10/30E [19], indicate the need of AP-1 formation to trigger the genomic events for proliferation and growth whereas cytoplasmic c-Fos is required to sustain growth.

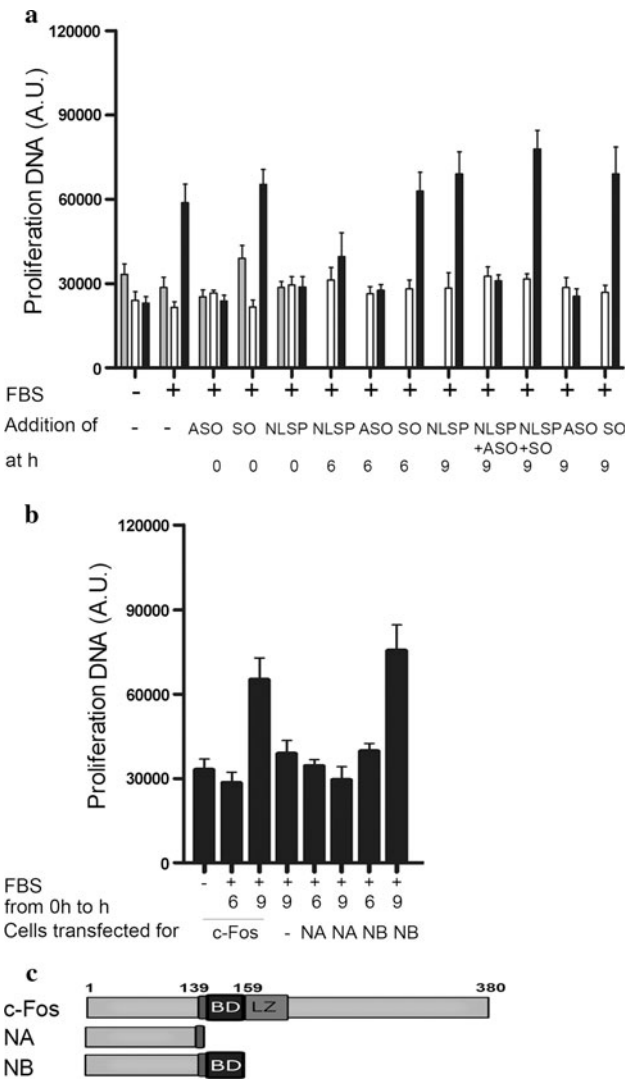

Fig. 3 Nuclear AP-1-c-Fos and cytoplasmic c-Fos are required at early and late stages of cell proliferation, respectively. **a** T98G cells cultured + or − NLSP, ASO or SO added at the indicated times after FBS addition, were examined for proliferation at 0 h (*Grey bars*), 18 h (*White bars*) or 30 h (*Black bars*). **b** Proliferation of cells transfected to express c-Fos, NA or NB were cultured +FBS for 6 h or 9 h as indicated, then medium replaced by −FBS medium and cultures continued to complete 30 h. Proliferation is expressed as arbitrary units (AU) of DNA; results are the mean ± SD of 3 experiments performed in quintuplicate; 10,000 cells were plated for each proliferation assay. Note that only c-Fos- and NB- transfected cells proliferate after elimination of FBS at 9 h of culturing that is, proteins with phospholipid synthesis activating capacity. **c** Schematic representation of the c-Fos and c-Fos mutants used in (**b**)

Validation of c-Fos requirement for tumor growth was obtained in vivo in nude mice xeno-grafted T98G cells intracranially [17]. On day 7 of engraftment, animals were separated into 3 groups and received, at the engraftment site, either ASO or SO or vehicle until sacrifice, 30 days later. Brain tissue samples were examined, double blind, for the presence of tumor (Fig. 4a). Tumors developed in 9 out of 10 vehicle-, in 7 out of 8 SO-, and in 0 out of 9 ASO-injected animals. In addition, brain samples immunostained for c-Fos and the ER marker calnexin showed abundant c-Fos staining in vehicle and SO animals whereas ASO mice showed no detectable c-Fos expression. Furthermore, in vehicle and SO animals, c-Fos co-localized with the ER (Fig. 4b) supporting the importance of c-Fos activated phospholipid synthesis for tumor progression. Furthermore, the levels of ER-immunoblotting found in the ASO-treated samples were similar to those found in brain from non-xeno-grafted mice as determined by WB (Fig. 4c). These observations are in accordance with previous results showing significantly higher levels of expression of ER markers in actively growing brain tumor cells with respect to normal brain tissue [20].

Given the results described above, the relevance of cytoplasmic c-Fos for tumor growth was validated in vivo in the animal model of the human syndrome Neurofibromatosis Type I (NF1), the NPcis mouse [21]. The primary clinical feature of NF1 is the development of benign peripheral nerve sheath tumors termed neurofibromas, which are composed primarily of neoplastic Schwann cells and non-neoplastic stromal cells. Between 15 and 50 % of NF1 patients develop some type of glioma, although they are often indolent in nature [21, 22]. NPcis mice bare, on a C57BL/6J background, a disrupted allele of both the trp53 and the nf1 tumor suppressor genes that are located on chromosome 11 at 7 cm of distance from each other. Consequently, both genes usually segregate together. Loss of heterozygosis determines the spontaneous development

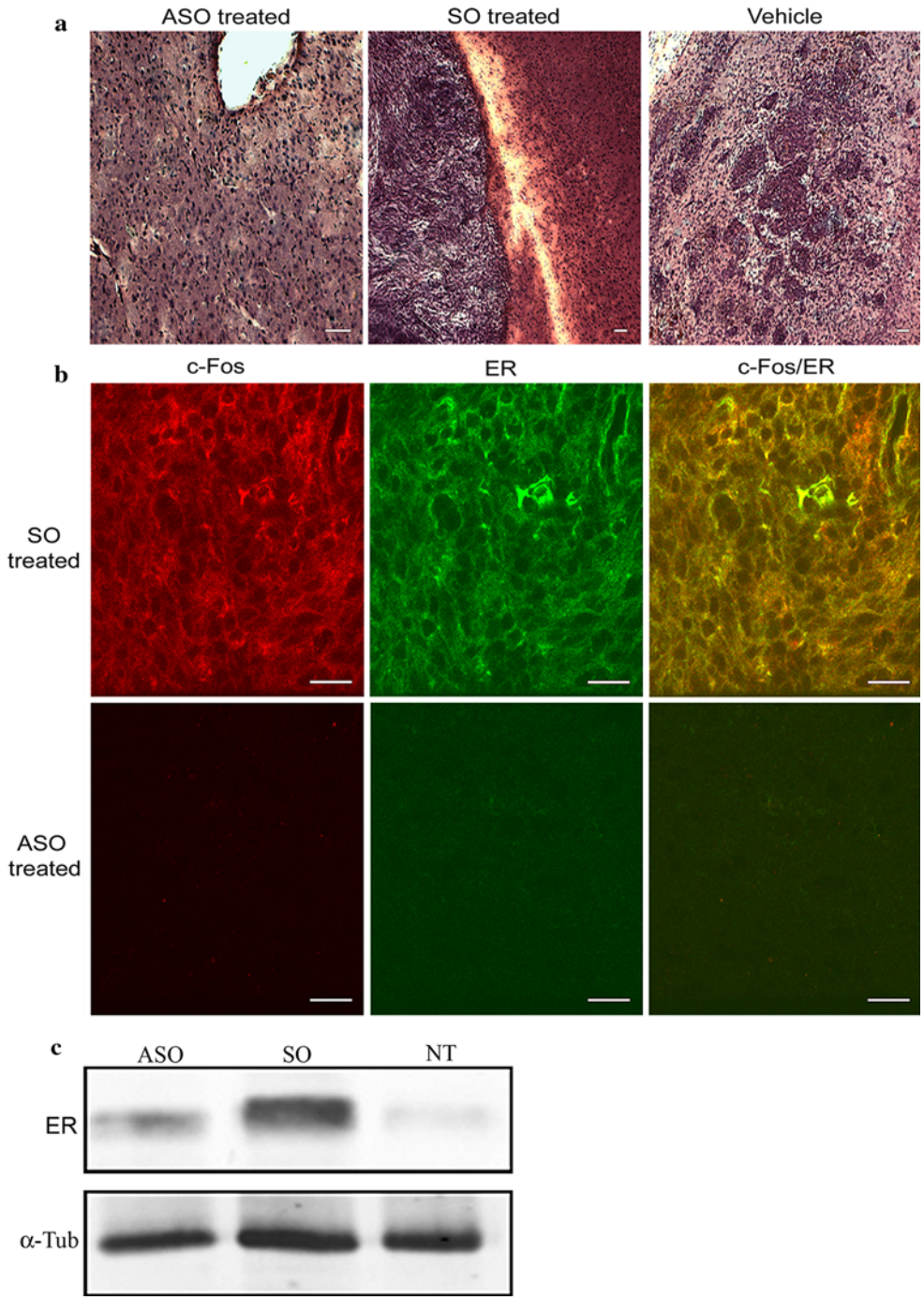

Fig. 4 Abundant c-Fos expression and c-Fos/ER co-localization in xenografted T98G cells from SO- or vehicle-treated animals; no c-Fos expression or tumor growth in ASO-treated mice. Nude mice intracranially xenografted T98G cells were treated during 28 days with ASO, SO or vehicle as indicated. **a** Haematoxylin/eosin-stained fixed brain slices were examined double-blind for histopathological determination of tumor growth (*bar* 50 μm) or **(b)** were immunolabeled for c-Fos (*left column*) and calnexin (*middle column*). c-Fos/ER co-localization can be evidenced only in tumors treated with SO (or vehicle, not shown) in merged images (*right column*). *Bar* 20 μm. **c** WB showing ER immunolabeling (calnexin) in ASO-, SO or non-treated (NT) mice; α-tubulin labeling was used as a loading control. Tumor growth was observed only in c-Fos-expressing brains (SO- and vehicle-treated) (**a**) which also showed c-Fos/ER co-localization (**b**)

of CNS and PNS tumors with a close to 100 % penetrance by the age of 6–7 months [23]. The histological pattern of the CNS tumors resembles that of a glioblastoma multiforme whereas PNS tumors show the histological characteristics of human Malignant Peripheral Nerve Sheath Tumors (MPNST's) [24, 25].

We previously examined c-Fos in CNS and PNS tumors from 6-month-old NPcis mice with clear signs of PNS

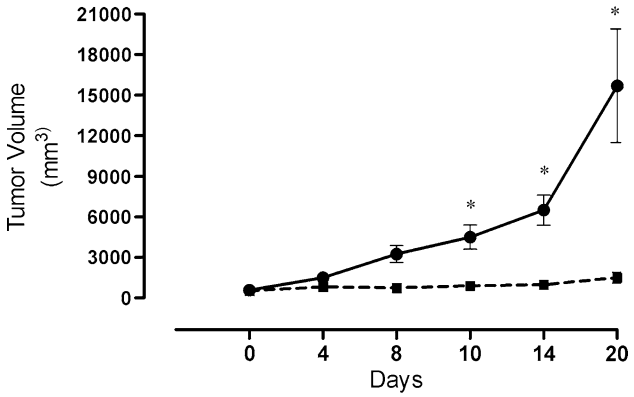

Fig. 5 In NPcis mice, tumors grow significantly slower upon blocking of c-Fos expression as compared to SO-treated or vehicle-treated tumors. NPcis mice with tumors of $\sim$ 600 mm^3 received an intra-tumor injection containing either c-Fos antisense oligonucleotide (ASO, *filled rectangle*) or sense oligonucleotide (SO, *filled circle*) or vehicle once every 3 days. Tumor growth was determined by measuring tumor volume with a digital caliber on days 0, 4, 8, 10, 16 and 20 of initiating treatment. Animal handling and treatment was performed double blind. Results are the mean of 6 animals in each group. No differences in tumor volume between SO- and vehicle-treated animals was found so results from both groups were pooled. *$p < 0.001$ as determined by Student's two tailed t test

tumor burden. As expected, high levels of c-Fos were observed in brain cortex from NPcis animals contrasting with the lack of expression in the same area of their WT littermates. PNS tumor samples also clearly show abundant c-Fos that was found co-localizing with the ER marker calnexin [11]. Consequently, to determine the importance of c-Fos expression on tumor development, NPcis mice bearing tumors of $\sim$600 mm^3 were treated with c-Fos ASO or SO oligonucleotide or with vehicle once every 3 days and tumor volume measured on days 0, 4, 8, 10, 16 and 20 of initiating treatment. As no differences in growth between SO and vehicle-treated animals were observed, data from both groups were pooled and compared with ASO-treated animals. Fig. 5 shows that in animals treated with ASO, tumors grow significantly slower than their counterpart animals treated either with SO or vehicle (Fig. 5).

Discussion

A rigorous control of the c-Fos-dependent activation of lipid synthesis must be expected because of the importance it has on the cell's outcome. Up to date, at least two distinct levels of control have been described. One is the strict control imposed by the cell on the levels of c-Fos expression: the induction of c-Fos expression in response to extracellular stimuli is a very well known phenomenon that has been extensively documented (reviewed in [1–3]). The other level of control of the lipid activating capacity of c-Fos is imposed by the cell on c-Fos by regulating the phosphorylation state of its tyrosine (tyr) residues #10 and #30: tyr-phosphorylated c-Fos neither associates to the ER nor does it activate phospholipid synthesis [18, 19]. The small amounts of c-Fos present in quiescent cells is tyr-phosphorylated, is dissociated from the ER membranes and does not activate lipid synthesis. However, upon induction of the cell to re-enter growth, c-Fos expression is rapidly induced, it is found dephosphorylated, associated to the ER and activating phospholipid synthesis. The kinase c-Src phosphorylates these c-Fos tyr residues whereas the phosphatase TC45 (TC-PTP) dephosphorylates them, thus enabling c-Fos/ER association and activation of phospholipid synthesis [19].

Herein, we have shown that tumor growth depends on c-Fos expression both in cells in culture and in tumors of the CNS and the PNS; specifically blocking c-Fos expression blocks tumor growth in cultured cells, in xenographed tumor cells and in spontaneously developed tumors. We previously showed that abundant c-Fos expression is observed in 100 % of the 156 human brain malignant tumors examined contrasting with the non-detectable levels of c-Fos in non-pathological brain specimens [11]. Furthermore, in NPcis mice, knock out for c-Fos, no tumor development is observed contrasting with the development of tumors in 71.4 % of their NPcis littermates, c-Fos +/+ or +/−. No substantial changes in the content of AP-1 transcription factors were found between *fos*−/− and *fos* +/+ mice [11].

Despite the progress in our understanding of the molecular and genetic mechanisms that underlie tumorigenesis in the CNS and the prediction of the behavior of some human brain cancers that tumor histology is starting to achieve [26], the statistics showing a median survival of less than one year for one of the most aggressive human cancers, the glioblastoma multiforme, has not changed significantly over the past two decades [27]. The finding that all the human brain tumor species show non-nuclear c-Fos expression irrespective of their growing environment, i.e. in culture, in ablated specimens or in intracranially xeno-graphed mice contrasts with the lack of significant expression in non-proliferating tumor cells in culture and in the normal, non-pathological brain [28]. Taken together, these results point to a new and highly potent cytoplasmic foundation for tumors. They also add a new target for the reduction of tumor growth by directing therapies such as antisense strategies, towards blocking of cytoplasmic c-Fos activation of phospholipid synthesis because the most promising target gene candidates for this therapy are those that become up-regulated during and are causally related to cancer progression [29]. This is clearly the case for cytoplasmic c-Fos. Finally, c-Fos stands as an

ideal target for brain cancer treatment given its high expression in this pathological state with no significant levels found in healthy tissue, as shown in this report and in many others [1]. Taken together, our results show that c-Fos can be blocked specifically and efficiently in tumor cells thus blocking tumor progression leaving intact the non-transformed ones, what has become the Holy Grail in cancer study.

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Conflict of interest The authors declare no conflict of interest.

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