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Original Article

I-Kappa-B Kinase-epsilon Activates Nuclear Factor-kappa B and STAT5B and Supports Glioblastoma Growth but Amlexanox Shows Little Therapeutic Potential in These Tumors

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Aim: This study aims to analyze the role of I-kappa-B kinase (IKK)-epsilon in glioblastoma (GBM).

Methods: A series of *in vitro*, *in vivo*, microarray, and immunohistochemical assessments were performed to evaluate the biological effects of IKK-epsilon on cell signaling, radiation sensitivity, and patient survival in GBM condition.

Results: IKK-epsilon was strongly expressed in 75% of 195 primary GBM samples but did not correlate with patient survival. No correlation was established between the copy number, messenger RNA (mRNA) expression, and protein expression in 38 fresh tumor samples, nor between IKK-epsilon mRNA expression and survival in 543 GBM of the TCGA repository. *In vitro*, IKK-epsilon contributed to the growth and migration of glioma cells, independent of their $EGFR_{VIII}$ status. IKK-epsilon activated nuclear factor (NF)-κB and STAT5B *in vitro*, confirming the observed correlation surgical GBM samples. IKK-epsilon silencing did not alter the sensitivity of GBM cells to ionizing radiation. Amlexanox, inhibitor of IKK-epsilon and TBK1, poorly (IC50 > 100 μM) decreased cell growth and increased NF-κB activity in GBM cells, *in vitro,* notably due to TBK1 inhibition. *In vivo*, oral amlexanox failed to inhibit the growth of intracerebral U87 GBM xenografts in nude mice.

Conclusion: The results confirm a moderate pro-oncogenic role of IKK-epsilon in GBM, but question the potential of amlexanox as a therapeutic drug.

Key words: Amlexanox, glioblastoma, IKBKE, I-kappa-B kinase epsilon

INTRODUCTION

Glioblastoma (GBM), the prominent type of primary brain tumor, remains uniformly lethal despite aggressive multimodality treatment.[1,2] As a result, the current focus of research is on deciphering the mechanism of cell growth and therapeutic resistance of GBM cells, uncovering key regulators thereof and assessing their potential as therapeutic targets. While much progress has been made in understanding the biology of these tumors,[3,4] therapeutic clinical trials based on these discoveries have thus far been disappointing.[5,6] Besides the emergence of therapeutic resistance to these drugs or the associated unforeseen side effects,[7] the existence of redundant mechanisms that promote tumor growth and compensate for each other's inhibition certainly play a role in these failures. Besides, the enthusiasm elicited by preclinical discoveries should never lead to aggrandize the therapeutic potential of novel targets, to correctly inform the patients

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on the potential risk/benefit ratios of the offered experimental therapies. Confirmatory data and reproducibility of the findings are thus mandatory before progressing with translational clinical trials.

Several recent articles have reported the implication of inhibitor of nuclear factor (NF) IκB kinase subunit epsilon (IKK-epsilon) in the growth and invasion of malignant gliomas,[8-10] which was earlier known for its role in mammary malignancies.[11-13] In breast cancer, CK2 is shown to induce functional IKK-epsilon, which in turn mediates NF-κB activation.[14] We have previously shown the oncogenic role of both CK2 and NF-κB in GBMs.[15-17] In lieu with this, we sought to analyze the contribution of IKK-epsilon in glioblastoma progression and therapeutic resistance, and further assessed the therapeutic potential of amlexanox, an orally bioavailable inhibitor of IKK-epsilon, in GBM treatment.

METHODS

Genetic analyses

The GISTIC 2.0 copy number (CN) data and Agilent-based messenger RNA (mRNA) expression data of 543 GBM samples of the TCGA repository were obtained from the UCSC Cancer Genomics Browser (accessed in September 2015). Threshold CN values were used to assess the correlations with mRNA expression data using Pearson correlation tests. CN analysis and mRNA expression analyses were also run on 38 GBM samples obtained from the University Medical Center of Utrecht (UMCU), using Affymetrix SNP 6.0 arrays and the broad institute version of the GISTIC2.0 algorithm method, and Affymetrix U133 plus 2.0 RNA chips. These fresh frozen surgical samples were obtained at the UMCU following written informed consent of the patients, following approval of their collection by the relevant ethical committee (protocol #16-348).

Tissue microarrays, immunohistochemistry, and scoring

Formalin-fixed, paraffin-embedded archived tumor tissues of 195 GBM patients, treated in the UMC Utrecht between 2005 and 2009, along with their clinical record, were retrospectively collected under IRB approval (protocol #16-348). The need for informed consent was obtaineded by the ethical committee of the UMC Utrecht, according to Dutch law for retrospective studies. Histology of each specimen was reviewed by a senior clinical neuropathologist and reviewed on H and E stained sections. For each patient, 2–3 tissue cores were placed on recipient arrayed paraffin blocks with use of a manual arrayer (Beecher Instruments). Immunohistochemical staining was performed on 4-μm sectioned tissue microarray (TMA) slides, which were deparaffinized in xylene and rehydrated with graded alcohol solutions. After peroxidase blocking, antigen retrieval was achieved by incubation in citrate buffer (pH 6) for 12 min at 126°C. Slides were incubated with anti-STAT5B (Abcam ab194380), phospho-P65 (Santa cruz sc-101752), or IKK-epsilon (Novus Biological NB100-56524) antibodies for 1 h at room temperature. Protein expression evaluator was blinded to the clinical data and immunostaining was evaluated under light microscopy as the percentage of positive cells within the entire tissue surface and scored as: 1 = 0%–25%, 2 = 26%–50%, 3 = 51%–75%, and 4 = 76%–100%.

Cell cultures, reagents, and IκB kinase-epsilon knock-down

The genetic profile of human LN18 and U87 malignant glioma cells (ATCC) was verified using CGH (Affymetrix SNP6.0 arrays) and TP53 sequencing (ion torrent). GM2 and GM3 primary GBM cells were obtained from fresh samples of human GBM and cultured as published previously[16] and characterized using GFAP immunohistochemistry (IHC), TP53 sequencing, and CGH analysis. $U87_{VIII}$ cells were kindly provided by Dr. M. Broekman (UMCU), the expression of EGFRVIII in which was confirmed by next-generation sequencing. Cells were grown in 5% CO_2 in DMEM (Life Technologies) supplemented with 10% FBS (Gibco) and 1% of 5 mg/mL penicillin–streptomycin (Gibco) solution at 37°C and maintained at early passages. For knockdown experiments, following manufacturer's instructions (Dharmacon using Dharma FECT), 70% confluent cultured cells were transfected with 25 nM of SMART pool human IKK-epsilon small interfering RNA (siRNA) (M-003723-02-) or SMART pool human TBK1 siRNA (M-003788-02-), while control pool nontargeting #1 (D-001810-10-05) transfected cells served as control. Then, assays were performed at timings described in the results section.

Quantitative real-time polymerase chain reaction assay

RNAs were extracted with the RNeasy Mini Kit (Qiagen) and quantified using a spectrophotometer (Nanodrop 1000, Isogen). Reverse transcription reaction on 500 ng total RNA was then performed using the Reverse Transcription Core Kit (Eurogentec). Polymerase chain reaction (PCR) was carried out using KAPA SYBR® Fast quantitative PCR kit (Sopachem) in the Lightcycler 480 (Roche). The real-time primers of each gene are as follows: for IKBKE, 5' ACC-AGC-TCT-CCG-GAT-TT 3' and 5' GCA-GAG-CAG-AGC-CAA-TTA 3' or 5' GTG-ACT-AAG-GAC-GCT-TGA-TAC 3' and 5' GCA-GAT-TCA-CAA-GCT-GGA-TA 3', for B-actin, 5' AAC-CCC-AAG-GCC-AAC-CGC-GAG-A 3' and 5' CAG-TGT-GGG-TGA-CCC-CGT-CA 3'.

Western blot

Whole-cell lysates were obtained using lysis sodium dodecyl sulfate 1% buffer containing protease (Santa Cruz) and phosphatase inhibitors (Thermo Scientific). Western blot analysis was performed in polyacrylamide 10% gels and run for 1 h 30 at 100 V then transferred to polyvinylidene difluoride membrane (Roche) for 1 h at 100 V. All primary antibodies were incubated overnight at 4°C and the dilution recommended by the manufacturer was used. Antibodies: IKK-epsilon: Novus Biological, NB100-56524; GAPDH: Sigma, PLA0125; TBK-1: Cell signaling, #3013.

Cell survival assays

Twenty-four hours posttransfection (siRNA control or siRNA IKK-epsilon) of cells, as explained above, 2500 cells were seeded in 96-well plate and cultured up to 24 h, 48 h, or 72 h before subjecting to MTS assay (One solution cell proliferation assay, Promega). For amlexanox treatment, 2500 cells were seeded in 96-well plate and let to adhere overnight before being treated with amlexanox or with dimethyl sulfoxide (DMSO) that served as control. MTS assay was performed 24 h after these treatments.

Clonogenic assays

Forty-eight hours after transfection (siRNA control or siRNA IKK-epsilon), as explained above, 500 cells were seeded in 6-well plate followed, or not, with gamma radiation at doses of 2 or 4 Gy (Gammacell 40 Exactor irradiator). The cells were then left to grow for 7 days and then fixed with 4% PFA and stained with cresyl violet before counting. For amlexanox treatment, cells were seeded and let to adhere for few hours before to be treatment with amlexanox or DMSO before irradiation and processing as described above.

Luciferase reporter gene assay

Cells were seeded at a density of 2500 cells in 96-well plate and cotransfected using TransIT-2020 transfection reagent (Mirus) with: (1) a luciferase-coupled reporter gene for NF-κB or for STAT5B and (2) a Renilla luciferase reporter driven by a constitutive promoter. Twenty-four hours posttransfection, cells were treated with amlexanox or for siRNA experiments, and cells were transfected with the control or IKK-epsilon siRNA, for 24 or 48 h, using the Dharmafect (Dharmacon) system, according to the manufacturer's instructions. Cells were then lysed and luciferase activity was measured with Dual Luciferase Assay System (Promega,) using a Victor luminometer (PerkinElmer), as per the manufacturer's instructions. The relative NF-κB or STAT5B luciferase activity was normalized to that of the Renilla.

Migration assays

For Boyden chamber assays, 48-h post-siRNA transfection (siRNA control or siRNA IKK-epsilon), a calculated number of cells, in serum-free medium with 0.1% BSA, were seeded into collagen type I (50 μg/mL)-coated upper chamber of transwell (8 um, Corning). The lower chamber received the medium with 1.5% FBS and 1% BSA, to serve as chemoattractant. After 6 h of incubation to support migration, cells were fixed and stained with crystal violet and counted under a light microscope.

In vivo experiments

Six-week-old female immunodeficient athymic Balb-c/nude mice, obtained from Charles River® animal facilities (Charles River Laboratories®, UK), were engrafted intracranially – in the right striatum, with 75.000 U87 cells suspended in 2 uL phosphate-buffered saline. All animals were cared following the guidelines of the Belgium Ministry of Agriculture in agreement with EC laboratory animal care and use regulation (86/609/CEE, CE of J n°L358, 18 December 1986), and also following agreement of the local animal experiment Review Board/Ethical Committee. One week following engraftment, amlexanox or vehicle (DMSO) was administered daily by oral gavage at a dose of 25 mg/kg for 2 weeks. As one animal died immediately following surgery, a total of 14 and 15 mice formed the control and the amlexanox groups, respectively. One week after completion of the treatment, the mice were sacrificed; brains were harvested and fixed in 4% paraformaldehyde followed by sucrose before immunohistological processing. Tumor volume was assessed using the ellipsoidal formula, $\pi/6 \times l \times w \times h$.

Statistical analysis

Statistical analyses were performed using the Prism 5.0 (Graphpad Inc., CA, USA) and the SPSS 24 softwares (IBM Corporation, Armonk, New York, USA). Unpaired *t*-test, Mann–Whitney *U*-test, or two-way ANOVA, with Dunnett's or Sidak's multiple comparisons tests were performed as appropriate. The overall patient survival was calculated using Kaplan–Meier method. Results were expressed as means ± standard deviation and considered significant at a two-sided $P < 0.05$.

RESULTS

IκB kinase-epsilon copy number and expression in human glioblastomas, and patient survival

IKK-epsilon was expressed in 50%–100% of tumor cells (i.e., IHC score of 3 or 4), in 77.2% of the assessed 195 GBM samples. There was no significant difference in the overall survival of these 77.2% GBM patients, compared to the rest of the 22.8% patients who expressed lower levels of this kinase [Figure 1].

The CN for IKBKE (IKK-epsilon gene) was normal in 79.5%, reduced in 4%, and amplified in 16.4% of 543 human GBMs from the TCGA data repository and failed to establish any correlation with patient survival (data not shown). The CN of IKBKE did not correlate with its mRNA expression in these tumors (Pearson's correlation coefficient = 0.001, $P = 0.972$), and the mRNA expression of IKBKE did not correlate with survival in these patients (Kaplan–Meier estimates based on a median split of the mRNA expression values, log-rank $P = 0.421$). Copy number and mRNA expression data were available for a series of 38 additional tumors from our center. IKBKE was amplified in 34% of these tumors and was normal in the remaining tumors. The CN did not correlate with the mRNA expression in these tumors, while the mRNA expression inversely correlated with the protein expression of IKK-epsilon in the tumors, as assessed by immunochemistry (Pearson correlation coefficient = −0.353, $P = 0.03$).

Altogether, these results show that neither IKK-epsilon CN nor its expression can be prognostic markers of GBM and that the expression of IKK-epsilon is not the result of its genetic amplification.

IκB kinase-epsilon knockdown affects glioblastoma proliferation

We transfected LN18, U87, $U87_{VIII}$, GM2, and GM3 GBM cells with SMART pool human IKK-epsilons iRNA, which effectively knocked down the IKK-epsilon mRNA and its protein expression, as confirmed by qRT-PCR and Western blot, respectively, within 48 h of the transfection [Figure 2a]. The clonogenic proliferation was reduced by 38.1% to 77.9% in all GBM cells after IKK-epsilon knockdown ($P < 0.001$) [Figure 2b]. Likewise, IKK-epsilon silencing also moderately decreased the exponential cell-growth ofseveral of the assessed cell types, as measured by MTS assay 72–96 h posttransfection [Figure 2c].

IκB kinase-epsilon knockdown affects glioblastoma cell migration

Using Boyden chamber assay, we observed a small decrease (8.9%–39.65%) in the migration of all the assessed GBM cell types

following si-IKK epsilon transfection, as compared to controls. However, this decrease in migration reached significance only in U87GBM cell line with the number of replicates performed ($n = 4$, $P < 0.05$) [Figure S1].

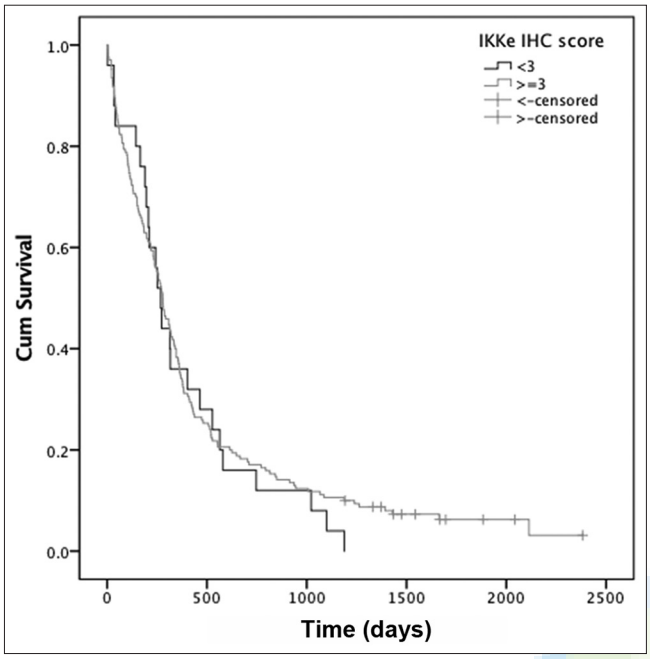

Figure 1. IκB kinase-epsilon expression in human glioblastomas and patient survival. Kaplan-Meier overall survival curves for glioblastoma IKBKE expression score < 3 and glioblastoma IKBKE expression score > 3 in a population of 195 glioblastoma patients

IκB kinase-epsilon knockdown, NF-κB activity, and STAT5B activation in glioblastomas

IKK-epsilon is a known regulator of NF-κB activation in several cancer types.[14,18] In line with these reports, luciferase reporter gene activity assay showed significant decrease in NF-κB activity after si-IKK-epsilon transfection in our GBM cell lines [Figure 3a]. In our clinical series of GBM tumors, the nuclear expression of phosphorylated-P65 was significantly higher in tumors expressing a high level of IKK-epsilon (higher than 3 on our scale) compared to tumors that only expressed a low level of this kinase ($P = 0.026$, Mann–Whitney *U*-test, $n = 192$).

As IKK-epsilon was also reported to regulate STAT proteins in other cell types, using our TMA slides, we sought to explore whether the IKK-epsilon expression in GBM would correlate with the STAT3 and STAT5B activation, the most important members of this transcription factors family in GBMs. While there was no correlation between IKK-epsilon and STAT3 expression (data not shown), a significant correlation was seen between IKK-epsilon expression and the nuclear expression of phospho-STAT5B in the studied tumors ($P = 0.031$, Mann–Whitney *U*-test, $n = 192$). *In vitro*, knockdown of IKK-epsilon reproducibly ($n = 4$) and significantly decreased STAT5b activity in our cell cultures, as assessed by reporter assay [Figure 3b].

IκB kinase-epsilon knockdown does not sensitize glioblastoma cells to ionizing radiation

IKK-epsilon has been reported to modulate the sensitivity of GBM cells to UV irradiation.[18] Using clonogenic assays, we however did not find any evidence of the altered sensitivity

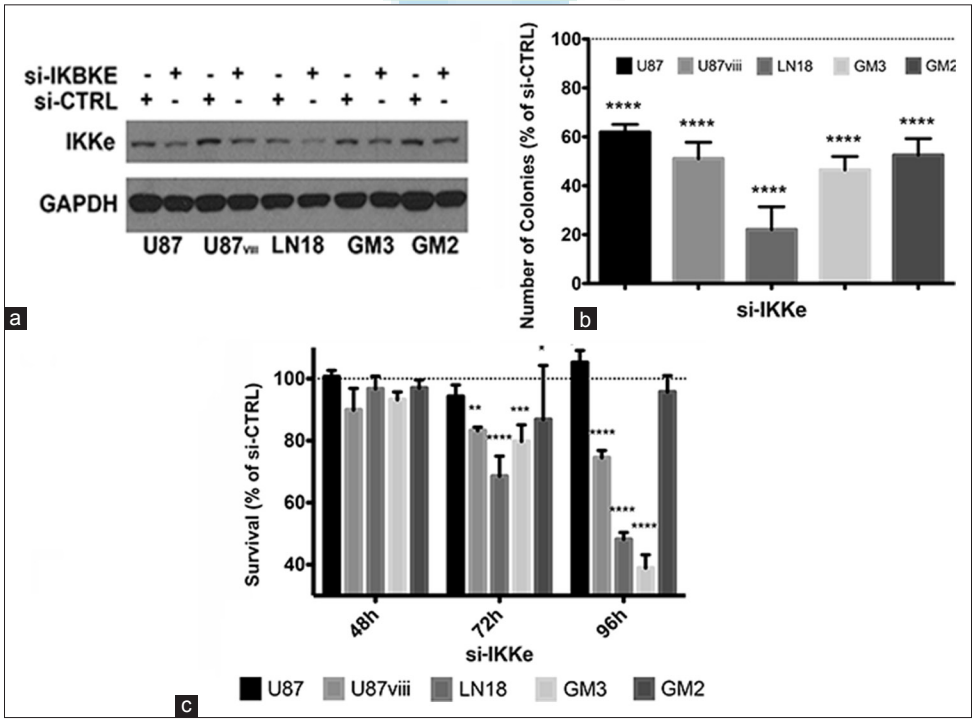

Figure 2. IκB kinase-epsilon knockdown affects glioblastoma proliferation. (a) IκB kinase-epsilon protein level expression 48 h after si-RNA transfection evaluated by Western blot. (b) Colony-forming assay after IκB kinase-epsilon inhibition, expressed in percent of the number of colonies grown following control si-RNA treatment. (c) Exponential cell growth after IκB kinase-epsilon silencing-MTS assay. Data are shown as the mean ± standard deviation, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

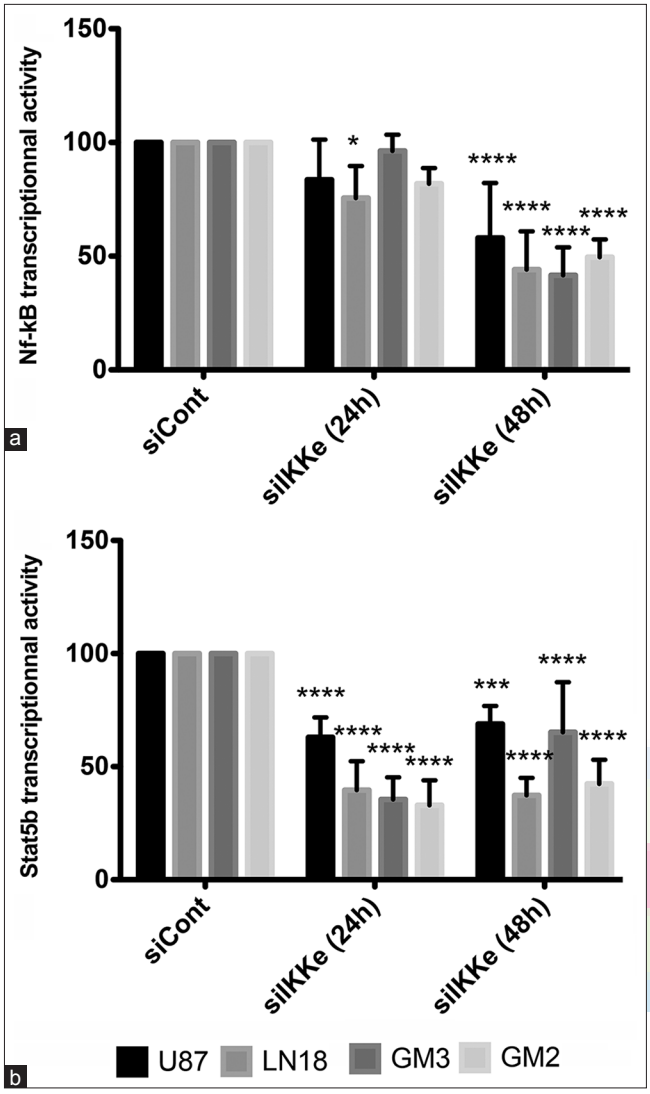

Figure 3. IκB kinase-epsilon knockdown, NF-κB activity and STAT5B activation in glioblastoma. Luciferase reporter gene assay using cells co-transfected with (i) luciferase-coupled reporter gene for NF-κB (a) or for STAT5b (b), (ii) a Renilla luciferase reporter, and (iii) siRNA against IκB kinase-epsilon or siRNA control. Transcriptional activity is expressed in percent of the respective luciferase transcriptional activity in control si-RNA-treated cells. Data are shown as the mean ± standard deviation, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

of IKK-epsilon knockdown GBM cells to ionizing radiation (gamma rays) [Figure 4].

Amlexanox on NF-κB and STAT5B activation and proliferation and tumorigenicity in glioblastomas

Amlexanox is a reportedly small, orally available, selective pharmacological inhibitor of IKK-epsilon. At concentrations between 40 and 100 μM, amlexanox effectively decreased GBM cell survival in our cell-culture experiments, as assessed by MTS assay ($P < 0.001$) [Figure 5a]. Of note, the IC50 was not reached for any of the cell lines, despite these concentrations being far higher than those required to inhibit IKK-epsilon effectively.[19] In clonogenic assay, amlexanox (50 μM) also significantly reduced the formation of colonies by 18%–58% in GBM

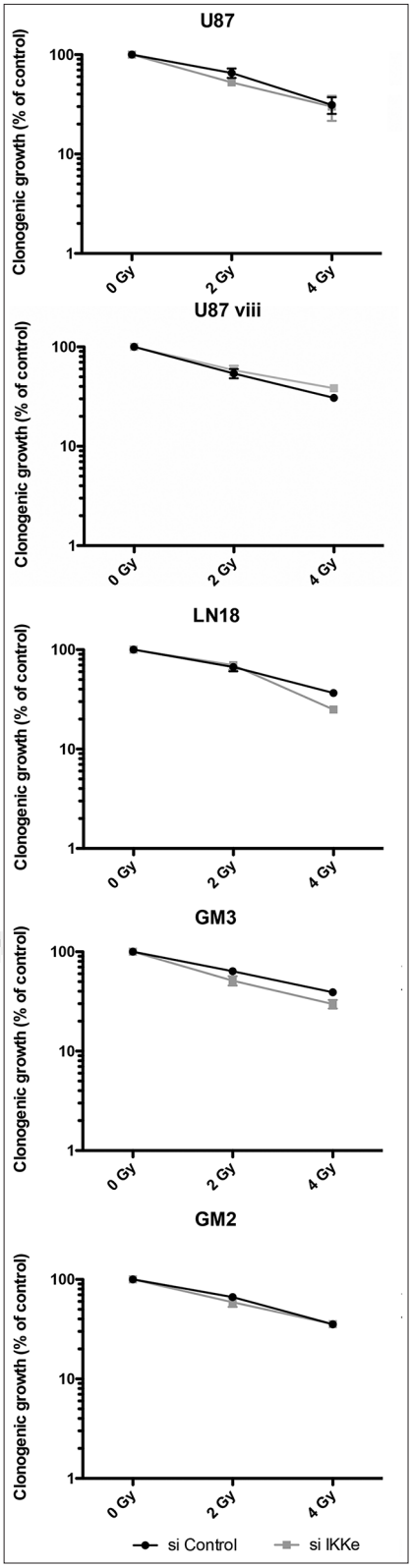

Figure 4. IκB kinase-epsilon knockdown does not sensitize glioblastoma cells to ionizing radiation. Clonogenic assays of glioblastoma cells transfected with si-CTRL and si-IKKe followed by gamma radiation (0, 2, or 4 Gy). Numbers of colonies for each si-RNA are expressed in percent of the number of colonies growing from nonirradiated cells treated with the same si-RNA. Dose-response curves were compared between si-CTRL and si-IKKe conditions using two-way ANOVA (N. S. in all cell types)

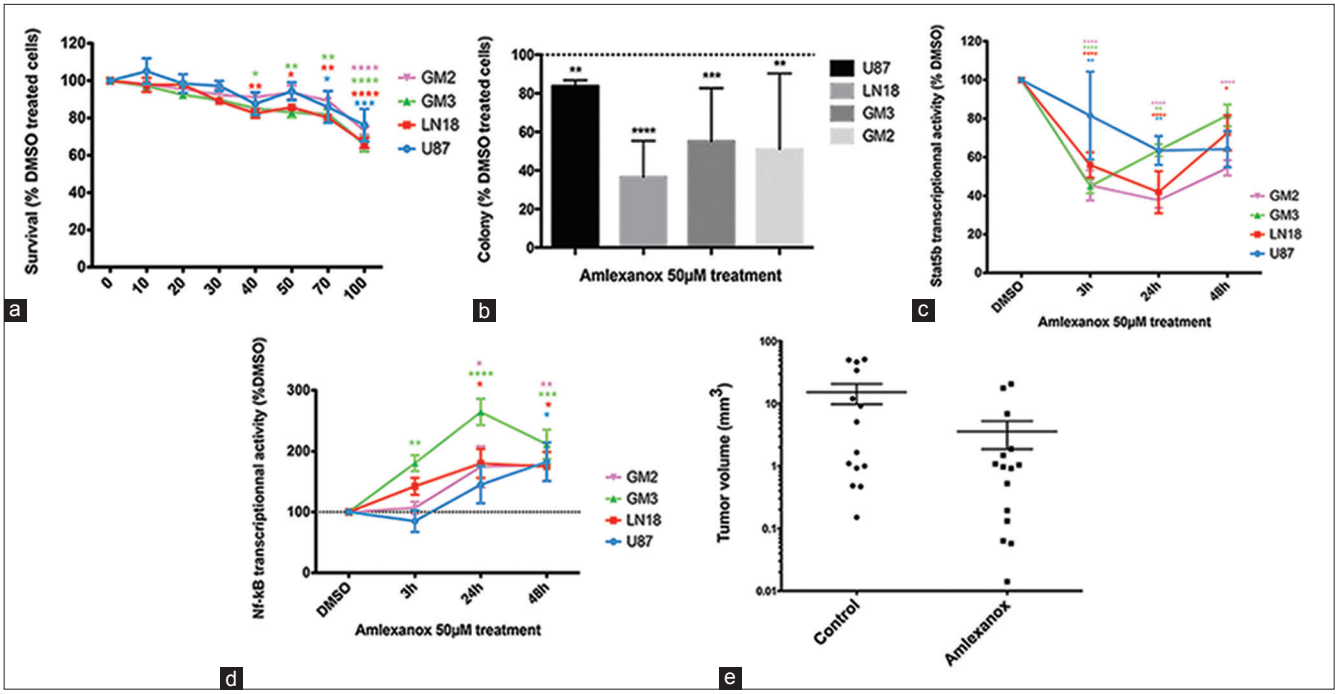

Figure 5. Amlexanox, NF-κB and STAT5B activation, proliferation and tumorigenicity in glioblastomas. (a) Cell Survival after amlexanox treatment (24 h)-MTS assay, survival expressed in percent of dimethyl sulfoxide treated cells. (b) Colony forming after amlexanox treatment, surviving fraction expressed in percent of the dimethyl sulfoxide treated cell colony. Luciferase reporter gene assay using cells co-transfected with (i) luciferase-coupled reporter gene for NF-κB (d) or for STAT5b (c) and (ii) a Renilla luciferase reporter treated with 50 µM amlexanox or dimethyl sulfoxide as control. Transcriptional activity expressed in percent of dimethyl sulfoxide treated cells. Data are shown as the mean ± standard deviation, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (e) Tumor volume compared between two groups of mice treated by oral gavage of amlexanox-25 mg/kg or vehicle. Representative pictures are provided in Figure S3

cells ($P < 0.01$) [Figure 5b]. Further, amlexanox significantly decreased STAT5B activity in GBM cells [Figure 5c]. In sharp contrast to IKK-epsilon knockdown, however, it significantly enhanced NF-κB activity in these cells until 48 h after amlexanox treatment [Figure 5d]. Finally, amlexanox did not alter the sensitivity of GBM cells to ionizing radiation (data not shown).

Despite a trend seen in *in vitro* experiments, the effect was not apparent *in vivo*, evidenced by no significant difference in the growth of intracranial U87 tumor xenografts between mice that were gavage-fed with amlexanox (25 mg/kg) or with control vehicle ($P = 0.063$, Mann-Whitney *U* test) [Figures 5e and S2].

DISCUSSION

In line with the recent reports in gliomas,[18] and in other cancer types,[20] IKK-epsilon was shown to be highly expressed in more than 75% of the assessed GBM samples. High IKK-epsilon expression did however not associate with any survival disadvantage in these patients. There was also no correlation between the CN, the mRNA expression, and the protein expression of IKK-epsilon in the studied tumors, nor was there any correlation between the IKBKE amplification, mRNA expression, and patients' survival in 543 GBM samples data obtained from the TCGA repository of tumors. Likewise, we found that 75%–100% of the astrocytes in nontumoral brain samples ($n = 10$) obtained from epilepsy surgeries expressed IKK-epsilon in IHC (data not shown). Together, these results suggest that IKBKE is not a major driver of glioma oncogenesis. However, under *in vitro* culture conditions, IKK-epsilon contributed to the growth and the migration of glioma cells, independent of their $EGFR_{VIII}$ status. These results are in line with the recent findings that IKK-epsilon contributes to the EMT transition, clonogenic proliferation, migration, and invasion in GBM cells.[8-10,20] The Hippo and the NF-κB pathways have been reported to play a major role in this pathology of IKK-epsilon in gliomas. Our results, from our 195 surgical GBM samples, indeed confirm that IKK-epsilon modulates the activation of NF-κB *in vitro* and *in vivo*. While other mechanisms strongly contribute to NF-κB activation in GBMs, such as IKBKA deletions of receptor tyrosine kinase hyperactivity,[21,22] the modulation of NF-κB activity by IKK-epsilon might help GBMs to resist against the drugs that target these primary mechanisms. Interestingly, IKK-epsilon also regulated the activation of STAT5B in our GBM cell cultures, and its expression significantly correlated with the activation of this transcription factor in our surgical samples. While IKK-epsilon has previously been reported to phosphorylate STAT1,[23] up to our knowledge, this is the first study to report that it can regulate STAT5B, the major isoform of STAT5 in gliomas and a purported regulator of cell proliferation, migration, and invasion in these tumors.[24-26]

Despite its contribution to clonogenic cell proliferation, IKK-epsilon silencing did not alter the sensitivity of the cultured GBM cells to ionizing radiation. This contrasts with the reported contribution of IKK-epsilon to UV-induced cell toxicity[18] but is rather relevant given the importance of ionizing radiation, rather than UV radiation, in the clinical treatment of these tumors.[1]

Amlexanox is a small, orally available, specific inhibitor of IKK-epsilon and TBK1.[19] The molecule was indeed effective in decreasing cell growth in our GBM cell culture experiments, but with a low efficacy, as the IC50 was not reached for the

drug concentrations up to 100 μM, which is far higher than that needed to inhibit 50% of the kinase activity (2 μM). In contrast to IKK-epsilon silencing, amlexanox actually increased the NF-κB activity in the treated GBM cells, rather than inhibiting it. As amlexanox can inhibit both IKK-epsilon and TBK1, we speculated if TBK1 could play a role in the amlexanox-induced NF-κB activity. Indeed, IKK-epsilon and TBK1 are known to play different roles in the activation of IRF3,[27] a known negative regulator of NF-κB gene transcription,[28] and TBK1 can exert a direct inhibitory action on the NIK/IKK/NF-κB axis.[29] In line with this hypothesis, TBK1 silencing using specific siRNA resulted in an increase in NF-κB activity in LN18 and GM3 cells, similar to amlexanox induced the NF-κB activation [Figure S3]. However, the later was more effective, especially in U87 and GM2 cells, indicating the possible involvement of other mechanisms.

In vivo, amlexanox, given by oral gavage, failed to inhibit the growth of intracerebral U87 GBM xenografts in nude mice. This contrasts with a prior report where, following intraperitoneal injections, amlexanox reduced the growth of subcutaneous U87 GBM xenografts, by merely 50% in volume and tumor weight, in nude mice.[30] The reason could be the use of less relevant subcutaneous tumor injection sites (preclinical and clinical studies are performed through this safe *per os* mode of administration[19,31]), and a 4-fold higher dose of amlexanox (100 mg/kg/d) through intraperitoneal route. In our experiment, we indeed used an oral dose of 25 mg/kg/day, shown to be safe and more than sufficient to induce potent systemic effects in mice (while 30 mg/kg was found to be the "no effect level for toxicity" dose in mice after chronic intake (FDA–NDA 20-511).[19,32-34]

In conclusion, our results confirm a moderate pro-oncogenic role of IKK-epsilon in GBM, but seriously question the potential of amlexanox, the IKK-epsilon inhibitor, as a therapeutic drug against these tumors.

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Conflicts of interest

There are no conflicts of interest.

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