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Boswellic acid formulations are not suitable for treatment of pediatric high-grade glioma due to tumor promoting potential

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ABSTRACT

Background and aim: Pediatric high-grade gliomas (pedHGG) comprise a very poor prognosis. Thus, parents of affected children are increasingly resorting to complementary and alternative medicine (CAM), among those Boswellia extracts. However, nothing is known about the therapeutic effectiveness of their active substances, Boswellic acids (BA) in pedHGG. Thus, we aimed to investigate if the three main Boswellic acids (BA) present in Boswellia plants, alpha-boswellic acid (α-BA), beta-boswellic acid (β-BA) and 3-acetyl-11-keto-beta-boswellic acid (AKBA) hold any promising potential for treatment of affected pedHGG patients.
Experimental procedure: Histone 3 (H3)-wildtype and H3.3K27M-mutant pedHGG cell lines were treated with BA, either alone or in combination with radio-chemotherapy with temozolomide. Cell viability, stemness properties, apoptosis, *in ovo* tumor growth and the transcriptome was investigated upon BA treatment.
Results and conclusion: Interestingly, α-BA and β-BA treatment promoted certain tumor properties in both pedHGG cells. AKBA treatment reduced cell viability and colony growth accompanied by induction of slight anti-inflammatory effects especially in H3.3K27M-mutant pedHGG cells. However, no effects on apoptosis and in ovo tumor growth were found. In conclusion, besides positive anti-tumor effects of AKBA, tumor promoting effects were observed upon treatment with α-BA and β-BA. Thus, only pure AKBA formulations may be used to exploit any potential positive effects in pedHGG patients. In conclusion, the use of commercially available supplements with a mixture of different BA cannot be recommended due to detrimental effects of certain BA whereas pure AKBA formulations might hold some potential as therapeutic supplement for treatment of pedHGG patients.

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1. Introduction

Pediatric high-grade gliomas (pedHGG) are very aggressive brain tumors in childhood. Historically, pedHGG included glioblastoma (GBM) and diffuse intrinsic pontine glioma (DIPG). With the introduction of molecular diagnostic parameters into the most

Abbreviations

BA	boswellic acid
α-BA	alpha-boswellic acid
β-BA	beta-boswellic acid
AKBA	3-acetyl-11-keto-beta-boswellic acid
pedHGG	pediatric high-grade glioma
DIPG	diffuse intrinsic pontine glioma
CAM	complementary and alternative medicine
H3	histone 3
K27 M	Lysin 27 substituted by Methionine
GBM	glioblastoma
CNS	central nervous system
WHO	world health organization
DMG	diffuse midline glioma
TMZ	temozolomide
GSEA	gene set enrichment analyses
COX-2	Cyclooxygenase 2

recent editions of the WHO (world health organization) classification of central nervous system (CNS) tumors, pediatric GBM have been redefined and are now found among different pediatric subtypes of diffuse high-grade gliomas.[1] DIPG were reclassified as well and are now predominantly subsumed as diffuse midline glioma (CNS WHO grade 4), H3K27-altered (DMG). Despite intensive therapeutic efforts, prognosis remains very poor with an overall survival rate of less than 15% for all diffuse pedHGG and even less than 2% for DIPG patients.[2] Of note, the epigenetically relevant histone H3K27M-mutation confers to a similarly poor survival rate in children with DMG, irrespectively if the pons like in DIPG or other midline locations are affected.[3] The persistent poor prognosis is supported by the lack of effective therapeutic options; standard treatment has not changed prognosis over decades and still consists of irradiation mostly in combination with chemotherapy such as temozolomide (TMZ).[4] Therefore, due to their desperate situation, parents often tend towards complementary and alternative medical (CAM) treatment options.[5] Boswellic acids are one of the most commonly used CAM by parents to treat their children suffering from DIPG.[6]

Boswellic acids (BA) including α-Boswellic Acid (α-BA), β-Boswellic Acid (β-BA), and 3-*O*-Acetyl-11-Keto-β-Boswellic Acid (AKBA) are extracted from *Boswellia serrata*, *carteri* and *sacra* plants.[7] The pentacyclic triterpenic acids can modulate several molecular targets, thereby harbouring anti-cancer and anti-inflammatory as well as neuroprotective and analgesic activities.[7] Thus, BA had been intensively studied *in vitro* and *in vivo* as potential anti-cancer agents. *In vitro*, BA reduced cell viability and clonogenicity of adult glioblastoma cells and a potential synergistic effect with irradiation and TMZ was observed.[8,9] BA were also shown to cross the blood brain barrier in a rat model.[10] Moreover, Boswellia oleogum resin efficiently reduced radiochemotherapy-induced edema of brain tumor patients, most probably due to their anti-inflammatory properties.[11] Altogether, BA might serve as a successful therapeutic treatment option for glioma patients without severe adverse effects.[12,13] However, corresponding studies in pedHGG have not been carried out yet. Given the increasing number of parents treating their affected child with BA, there is an obvious need to further elucidate the potential of BA as therapeutic approach for pedHGG. The present study, therefore, investigated time potential therapeutic effects of the three main BA, α-BA, β-BA and AKBA, in pedHGG and DIPG cells for the first time.

2. Methods

2.1. Cell culture and treatment

HSJD-DIPG-007 (herein referred to as DIPG-007) and HSJD-GBM-001 (herein referred to as GBM-001) cells were kindly provided by Angel M. Carcaboso (Pediatric Hematology and Oncology, Hospital Sant Joan de Deu/Institut de Recerca, Sant Joan de Deu, Barcelona, Spain) and cultured as previously described as gliomaspheres or under differentiation conditions.[14] DIPG-007 cells which harbor the H3.3K27M mutation and, therefore, qualify as DMG cells according to the latest WHO classification. GBM-001 represent H3K27-wildtype tumors, i.e. non-DMG pedHGG.

PedHGG are characterized by heterogeneous cellular phenotypes which we try to represent by applying different cell culture conditions in this study: highly resistant pedHGG cells are represented in the present study by gliomaspheres which are less susceptible to chemotherapeutics, irradiation, and other therapeutic approaches and resemble stem-like cells with an increased self-renewal ability. More differentiated, non stem-like pedHGG cells are represented by cells growing as adherent monolayers. Gliomaspheres and monolayer pedHGG cells often respond differentially to treatment.[14]

Cells were treated with alpha-boswellic acid (α-BA), beta-boswellic acid (β-BA), 3-acetyl-11-keto-beta-boswellic acid (AKBA) (Extrasynthese, Genay, France) and Temozolomide (Selleckchem, Munich, Germany) dissolved in DMSO, as indicated. Radiation treatment at a dose 8 Gy was delivered by a RS 225 X-Ray Research System (Gulmay Medical Systems, Xstrahl Ltd, Camberley, Surrey, UK) operated at 200 kV, 15 mA and with 2-mm 5 filtration.

2.2. Functional cell assays

Cell viability evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)- assays, sphere- and colony formation were carried out as previously described.[15,16]

Briefly, 1×10^5 cells/ml were seeded in triplicates under spheroid conditions or as adherent monolayers and treated with indicated increasing concentrations of α-BA, β-BA, AKBA and DMSO, as indicated. After incubation for 72 h, cells were incubated with 1 mg/ml MTT for 4 h, lysed in DMSO-lysis buffer (33% DMSO, 5% formic acid, 62% isopropanol) and absorbance was measured at 550 nm.

For colony and sphere formation assay self-renewal assays, 5000 cells/ml were seeded in triplicates and treated with α-BA, β-BA, AKBA and DMSO, as indicated. For colony formation, cells were incubated under differentiation, for sphere formation under stem cell-like gliomasphere conditions for five (HSJD-DIPG-007) or eight days (HSJD-GBM-001), and fixed and stained using crystal violet staining solution (9.6% crystal violet solution, 19.2% formaldehyde (min. 37%), 19.2% methanol, 19.2% PBS). Self-renewal assays were analyzed using ImageJ tool "Particle analyzer".

All experiments were performed with at least three independent biological cell passage replicates. P values < 0.05 were considered as significant and determined by student's t-tests.

2.3. Western blotting

Western blotting was performed as previously described[15] and according to standard protocols. Briefly, cells of at least three independent biological replicates were used and treated, as previously indicated, and lysed using 1x cytoplasmic extract buffer (10 mM HEPES, 60 mM KCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 0.075% NP40). After incubation and centrifugation, the cytoplasmatic fraction was collected and nuclear proteins were

obtained using nuclear extract buffer (20 mM Tris Cl, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 1 mM PMSF, 25% glycerol); Western Blotting and detection of the proteins of interest (POI) was performed using specific antibodies against COX-2 (12282S) and Caspase 3 (9662S) purchased from Cell Signaling (Leiden, The Netherlands), cleaved Caspase-3 (ab90437) purchased from abcam (Cambridge, UK) and anti β-Actin-Peroxidase (AC-15) purchased from Sigma-Aldrich (Darmstadt, Germany). Acrylamide gels for detection of POI and the housekeeping protein β-actin were run separately with same amount of protein lysates to avoid multiple use of membranes and thereby occurrence of unspecific bands. β-actin was used as a housekeeping protein due to its most stable and unchanged expression within the DIPG and HGG cell lines studied with and without treatment.

2.4. Chicken chorioallantoic membrane assays

Chicken chorioallantoic membrane assays were performed as described previously.[15] Briefly, 3×10^6 of 24 h pretreated cells with AKBA and vehicle were implanted onto a 10 days old chicken embryo CAM in 40 μl extracellular matrix (ECM) supplemented with AKBA or vehicle and incubated for further seven days. Tumors were analyzed using ImageJ. At least two different cell line passages were used. Overall, at least eight grown tumors per condition and cell line were analyzed. P values < 0.05, determined by student's t-tests, were considered as significant.

2.5. RNA-sequencing and data analysis

RNA was isolated from three independent biological replicates of HSJD-DIPG-007 cells after treatment with AKBA or DMSO for 48 h using the ReliaPrepTM RNA Cell Miniprep System (Promega, Walldorf, Germany) according to the manufacturer's instructions. Sequence images were transformed with Illumina software Base-Caller to BCL files, which was demultiplexed to fastq files with bcl2fastq v2.20. The sequencing quality was asserted using FastQC.[17] Sequences were aligned to the reference genome Homo sapiens (GRCh38.p12, https://www.ensembl.org/Homo_sapiens/Info/Index) using the RNA-Seq alignment tool[18] (version 2.5) allowing for 2 mismatches within 50 bases. Subsequently, read counting was performed using featureCounts.[19] Read counts were analyzed in the R/Bioconductor environment (version 3.6.2, www.bioconductor.org) using the DESeq2[20] package version 1.24. Candidate genes were filtered using an absolute log2 fold-change >1 and FDR-corrected p-value <0.05. Differentially expressed genes that were potentially regulated after AKBA treatment were selected for gene set enrichment analysis (GSEA) embedded into R package WebGestaltR.[21] Sequence data were deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE214897. cDNA synthesis and qPCR was performed as described previously, using the PowerUp SYBR Green Mastermix according to manufacturer's instructions (Thermo Fisher Scientific, Osterode am Harz, Germany).[15]

2.6. Data and statistical significances

All data are represented as mean of at least three independent biological replicates and their respective standard error of the mean (SEM). Unless stated otherwise significances were calculated using student's t-test and were defined as p*/# values ≤ 0.05, p**/## values ≤ 0.01, p***/### values ≤ 0.005.

3. Results and discussion

3.1. α-BA, β-BA, and AKBA with and without temozolomide and/or irradiation exert differential effects on cell viability of pedHGG cells in dependence on tumor cell phenotype

Cell viability inhibiting activity of BA strongly depends on chosen cell lines and on the BA itself.[8] Here, alpha-boswellic acid (α-BA), beta-boswellic acid (β-BA), and 3-acetyl-11-keto-beta-boswellic acid (AKBA) (Fig. 1A) widely regarded as the three most effective BA[7,22] were used in increasing concentrations to treat GBM-001 (non-DMG) and DIPG-007 (DMG) cells.

In the line of previous reports,[14] pedHGG cells grown under stemness conditions as gliomaspheres, overall responded less effective to BA treatment than differentiated adherent monolayer pedHGG cells (Fig. 1B, C and D). Gliomaspheres are characterized by stronger stem cell potential and marked CD133 expression which is known to contribute to therapy resistance.[23,24]

Moreover, treatment of gliomaspheres with β-BA even led to a slight, but significant increase of cell viability of both GBM-001 and DIPG-007 cells whereas α-BA treatment showed similar effects only in DIPG-007 cells. As already reported for adult GBM cells[8,25] the most efficient effects in pedHGG cells were observed using AKBA, independently of the cellular phenotype and cell line investigated (Fig. 1B and C).

In the present study, treatment with higher dosages of AKBA alone was highly effective and almost completely abolished viability of pedHGG gliomaspheres and monolayers (Fig. 1). However, these high concentrations used in this study (Fig. 1), are not reached in patients' brains and not achievable for brain tumor treatment in patients anyway[26,27]: Determination of biologically available BA concentrations in a volunteer revealed that 786 mg oral application of Boswellia extracts, three times daily for 10 days was efficient to obtain AKBA plasma levels of max. 0.1 μmol/L.[26] Another study found steady-state plasma levels of only 0.04 ± 0.01 μM of AKBA but 6.35 ± 1.0 μM of β-BA after administration of 800 mg for 4 weeks daily.[27] These higher values reached for β-BA are supposed to be due to their higher amounts in the extracts. In accordance and to be able to detect any effects and potential differences in combination with TMZ and/or irradiation doses of 10 and 20 μM of α-BA, β-BA and AKBA were used for further experiments.

By using lower concentrations of 10 μM BA, DIPG-007 responded additively to either TMZ or irradiation alone but not to combined radiochemotherapy (Fig. 2 and data not shown). In comparison to DIPG-007 cells, GBM-001 cells showed sensitization to TMZ in combination with the different BA supporting the concept of a higher chemotherapy resistance in H3K27M-mutant cells than in H3K27-wildtype cells.[16,28] However, additive effects of BA and irradiation were similar to those observed in DIPG-007 cells. Combination of BA, TMZ, and irradiation did not produce stronger effects than BA plus TMZ alone. Synergistic effects, defined as 20% differences of observed vs. additive effects, were only found in the present study after treatment with β-BA (Fig. 2) confirming previous reports in adult GBM cells.[8] The limited additive effects of BA in combination with irradiation were also in concordance with similar reports for lower doses of AKBA in adult GBM cells.[9]

3.2. Boswellic acids do not impair stemness associated characteristics of pedHGG cells

PedHGG display an aggressive clinical course characterized by a marked treatment resistance, which is usually connected to the presence of stem cell-like characteristics in pedHGG cells (summarized by[29]). Accordingly, we also investigated in the present
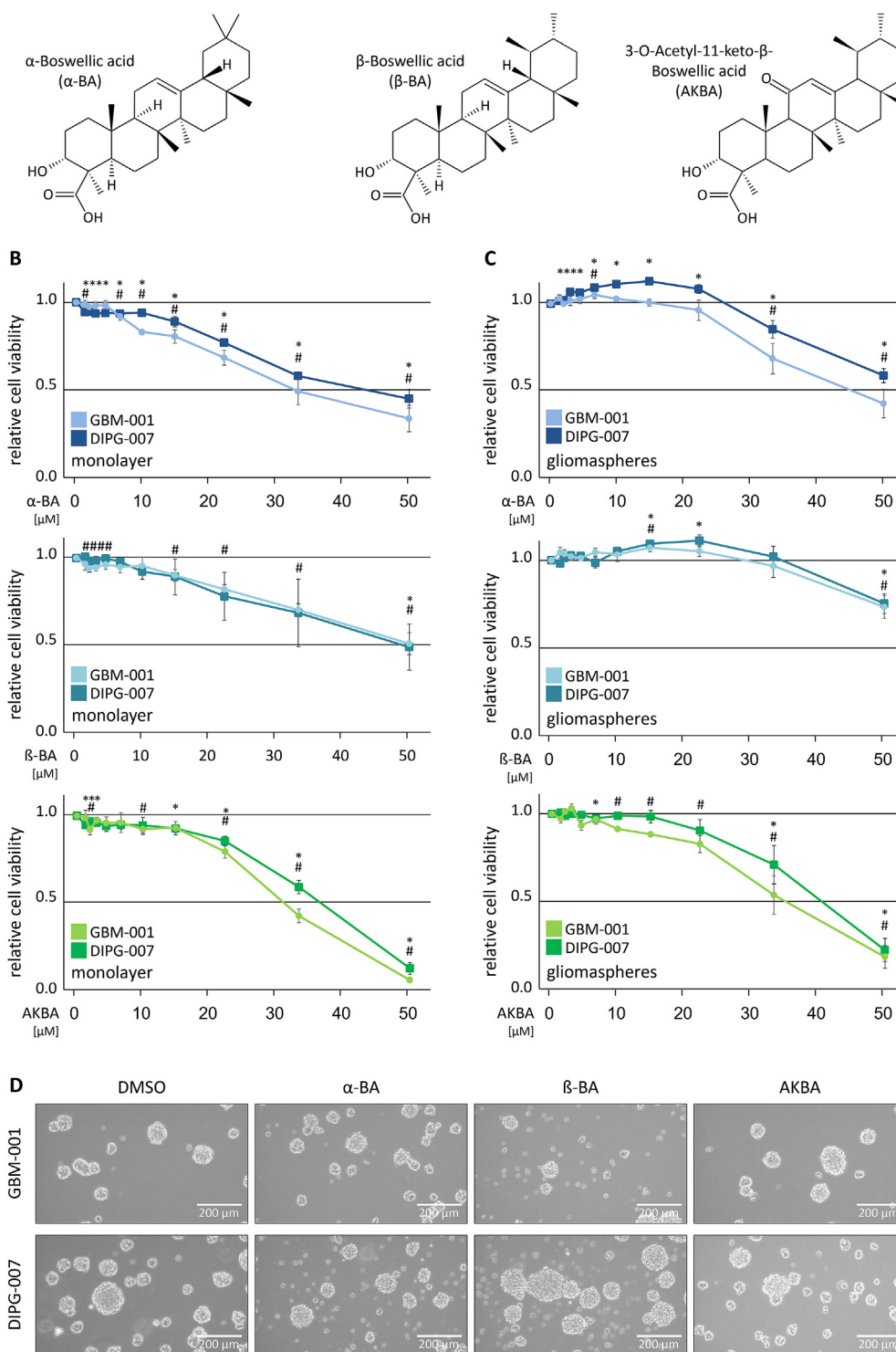
Fig. 1. Cell viability of pedHGG cells after treatment with different derivatives of boswellic acids: Structural formula of (A) alpha-boswellic acid (α-BA), beta-boswellic acid (β-BA), and 3-acetyl-11-keto-beta-boswellic acid (AKBA) and cell viability of (B) more differentiated, adherent monolayer cell culture and (C) gliomaspheres under stem-cell supporting conditions after 72 h of treatment with different BA, as indicated. p*/# values ≤ 0.05 were considered significant in comparison to non-treated *DIPG-007 or #GBM-001 control cells, respectively. (D) Brightfield images of gliomaspheres treated for 72 h with the different BA, as indicated, scale bar 200 μm.

study if BA treatment alter pedHGG colony formation and clonogenicity differentially under gliomasphere (i.e. stem cell-like) and under adherent monolayer (=non stem cell-like) conditions.

Colony formation was only significantly impaired in DIPG-007 cells upon BA treatment, especially upon doses of 20 μM α-BA and AKBA. Colony formation of GBM-001 cells tended to remain

unchanged. In contrast, at higher dosage of α-BA, colony formation was even increased (Fig. 3A) pointing to detrimental effects of α-BA in pedHGG cells.

When investigating sphere formation, pedHGG cells remained unaffected by α-BA and AKBA in both cell lines. Strikingly, β-BA appeared to induce increased sphere formation, especially in DIPG-
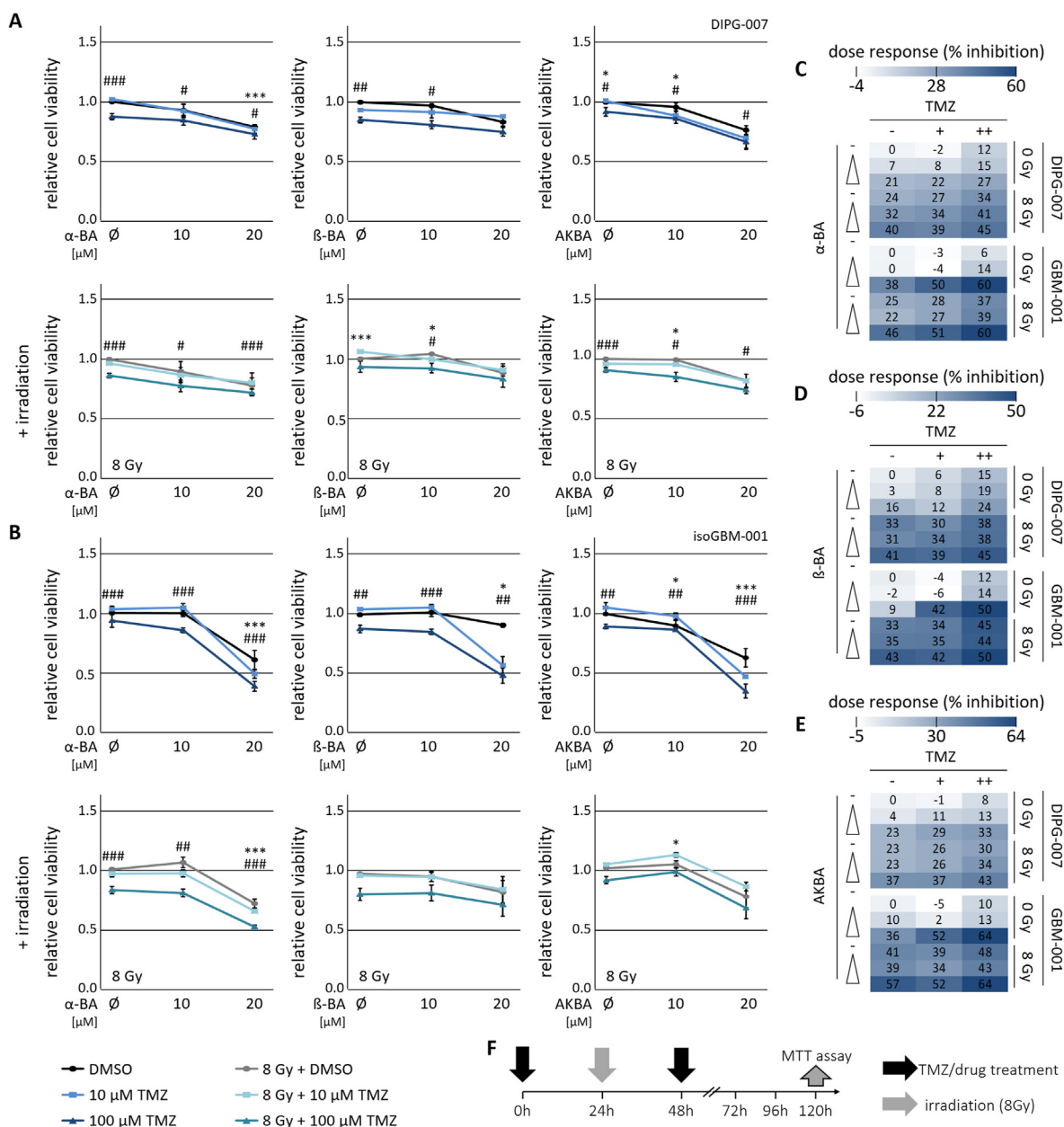
Fig. 2. Effects of BA in combination with irradiation (8 Gy) and temozolomide (TMZ): (A) DIPG-007 and (B) GBM-001 pedHGG cells were (F) treated twice with TMZ and BA and irradiated once with 8 Gy, as indicated. After 120 h total treatment, cell viability was assessed. Control treated cells (DMSO or DMSO + irradiation) were set to 1 to be able to visualize the additional effect of TMZ treatment. (C–E) color-coded dose response (% inhibition) of pedHGG cells to indicated treatments. Control treated cells (DMSO or DMSO + irradiation) $\triangleq$ 0% inhibition. p values were assessed in comparison to the respective control cells (ø): p values were calculated respective to DMSO/BA-treated cells in comparison to cells receiving *10 μM TMZ and #100 μM TMZ.

007 where even a dose-dependent effect was observed (Fig. 3B). However, expression of the stemness-associated marker OCT4 remained unchanged in GBM-001 cells after BA treatment for 72 h including β-BA (data not shown). Moreover, in particular the proportion of large spheres increases after β-BA treatment in both cell lines, indicating a stronger effect on proliferation, rather than sphere formation and thereby self-renewal ability itself. The proliferation promoting activity of β-BA leading to sphere growth is strongly supported by the above observation that cell viability may also increase by lower concentration of β-BA (Fig. 1C).

Since BA treatment at higher doses resulted in reduction of pedHGG viability (Fig. 1), we further aimed to investigate if BA induces apoptosis by determination of Cleaved Caspase 3 expression levels. In contrast to the previously reported apoptosis-inducing ability of BA[25,30] we did not find any hint for α-BA-, ß-BA- or AKBA-induced apoptosis in DIPG-007 cells at lower doses of 10 and 20 μM, respectively (Fig. 3C).

We also aimed investigate if the relatively strong reduction of viability and colony formation induced by AKBA (Figs. 1 and 3) in DIPG-007 cells showed a correlate in altered gene expression as determined by mRNA-sequencing analysis. Surprisingly, we did not find any significantly altered gene expression (p adjust <0.05, FC ≥ 1.5 and ≤ 0.75) after 48 h of AKBA treatment (Supplemental Fig. 1). Accordingly, no alteration of acetylation and trimethylation at H3K27 or acetylation at H4 was detected by Western blot analysis (data not shown).
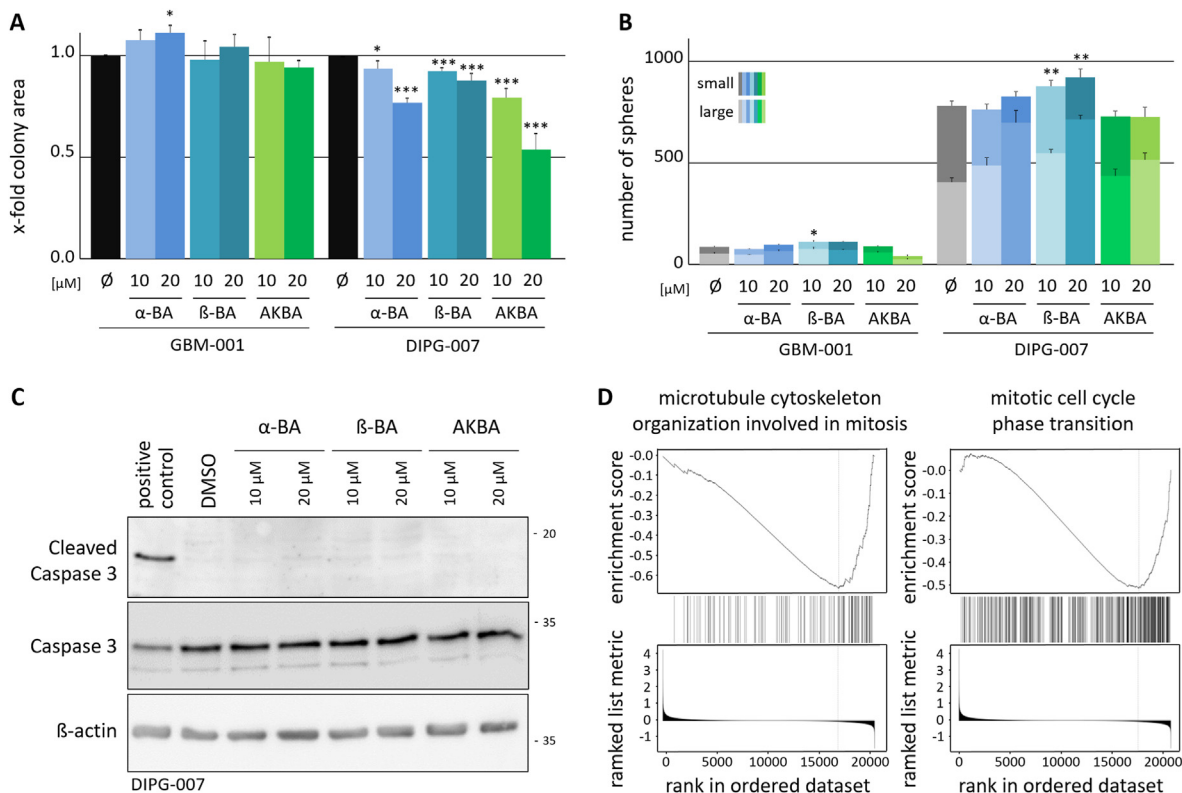
Fig. 3. | Boswellic acids do not impair stemness associated characteristics: Clonogenicity assays after treatment with BA, as indicated, assessing (A) colony formation under differentiation conditions and (B) sphere formation under gliomasphere conditions favoring stem-like tumor cells. p values with respect to none-treated control cells (ø). (C) Western Blot analysis determines apoptosis after BA treatment, as indicated, using Cleaved Caspase 3, β-actin served as loading control, representative replicate of n = 2. (D) Gene set enrichment analysis (GSEA) of all genes expressed after 20 μM AKBA treatment using WebGestalt.[21]

However, gene set enrichment analyses (GSEA) using WebGestalt[21] revealed several significantly dysregulated pathways upon AKBA treatment in DIPG-007 cells. Strongest downregulation of genes affected mitosis, protein localization, and stem cell differentiation whereas strongest upregulation was associated with endoplasmatic reticulum and metabolic processes (Fig. 3D and Supplemental Fig. 2) reflecting our previous results indicating a lower colony-forming ability of DIPG-007 cells. Interestingly, previous investigations also reported that β-BA targets the ribosomal machinery and thereby inhibits protein synthesis.[31] Since viability reduction upon AKBA treatment does not appear to result from relevant gene expressional changes, it seems that AKBA may involve similar mechanisms in the investigated pedHGG cells. However, this question has to be addressed in future studies.

3.2. AKBA has no impact on pedHGG tumor growth in vivo/in ovo but reduces inflammation marker expression

BA are also regarded to display anti-inflammatory activity *in vitro* and *in vivo* (summarized in Ref. 11) which may explain the suggested anti-edema effect of BA in brain tumors (summarized in Ref. 32).

Thus, we asked if AKBA, the most efficient BA in our hands, might not only reduce pedHGG tumor growth but also inflammation *in vivo*. To this end, we performed chicken chorioallantoic membrane assays and inoculated chicken eggs with DIPG-007 tumor cells. Although AKBA treatment was efficient to reduce DIPG-007 cell viability and sphere formation *in vitro*, no significant change in tumor growth *in vivo/in ovo* was observed after preceding treatment with AKBA (Fig. 4A and B).

As surrogate parameter for a potential anti-inflammatory effect, we investigated the impact of AKBA treatment on the inflammation marker Cyclooxygenase 2 (COX-2) *in vivo/in ovo*. Using immunohistochemistry, no differences in Cyclooxygenase 2 (COX-2) expression in both cell lines were detected within chorioallantoic membrane tumors treated and non-treated with AKBA (data not shown). In contrast, COX-2 protein expression was significantly reduced in DIPG-007 cells but not in GBM-001 cells (Fig. 4C and D) after AKBA treatment. However, treatment with α-BA and β-BA had no significant effect on COX-2 expression.

These data may support any potential benefit of AKBA as therapeutic adjuvant for pedHGG patients to reduce tumor edema by anti-inflammatory effects interfering in the COX-2 pathway, but not of α-BA and β-BA. However, the anti-inflammatory effects observed in our pedHGG *in vitro* and *in vivo* models did not indicate a similarly strong effect as propagated in the literature (Efferth und Oesch 2022).

In addition, commercially available BA supplements used by parents and caretakers are composed of different BA derivatives at low doses. If the detrimental effects of α-BA and β-BA treatment observed *in vitro* are reflected *in vivo* and if a combination of BA as present in BA supplements leads to comparable effects, needs to be further investigated.

4. Conclusion

BA treatment induced a relatively strong variety of different therapeutic effects in the pedHGG cell models used, i.e., GBM-001 as prototype for H3 wildtype tumors and DIPG-007 as H3K27M-mutant tumor. Unfortunately, the effects observed were not
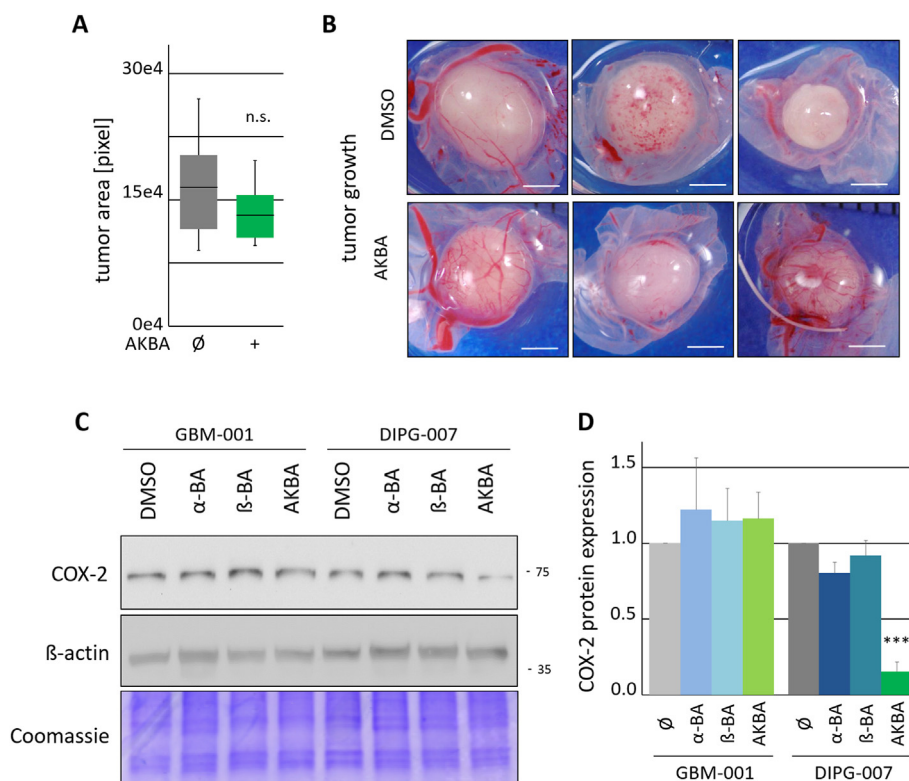
Fig. 4. | AKBA has no significant impact on DIPG tumor growth *in vivo/in ovo*. (A + B) AKBA treated DIPG-007 cells show only very slightly reduced not statistically significant tumor growth in chicken chorioallantoic membrane assays. DIPG-007 cells were pre-incubated with AKBA (n = 9) or DMSO (n = 8) for 24 h and inoculated on chicken chorioallantoic membrane. After five days tumor growth was (A) quantified, and (B) brightfield images were taken, scale bar 2 mm. (C) Protein expression of the inflammation marker COX-2 analyzed by western blotting (representative replicate of n = 4) and (D) quantification of COX2 protein expression relative to β-actin expression, which served as loading control.

consistently beneficial in regards to tumor treatment. Whereas all BA showed dose-dependent reduction of viability in GBM-001 and DIPG-007 cells with only high concentrations, growth promoting effects were observed using lower concentrations of α-BA and β-BA depicted by cell viability in both cell lines. Moreover, tumor-propagating effects as indicated by increased colony formation were additionally found upon α-BA treatment in GBM-001 cells and increased sphere formation upon β-BA, in both, but most efficiently in DIPG-007 cells. Of note, in patients tumor cell-propagating concentrations are earlier achieved during therapy than higher concentrations that mediate anti-tumor effects. Thus, patients would rather suffer than benefit from therapeutic approaches with BA. Any relevant chemotherapy sensitizing effect was only observed upon all three BA in GBM-001 cells, but not in DIPG-007 cells. In opposite, only DIPG-007 cells responded to AKBA treatment with reduced inflammation marker expression *in vitro* which was not observed in GBM-001 cells. These differential effects among different tumor derived cell lines point again to the all-over insufficient effects of BA treatment.

The highly mixed therapeutic efficacy with a marked inconsistency of beneficial and detrimental effects mediated by the three different BA in H3 wildtype and H3K27M-mutated pedHGG cells does not allow recommending the uncritical use of BA in pedHGG patients. Commercially available BA supplements are usually composed of different BA derivatives at low doses. The use of pure AKBA formulations instead of BA mixtures may help to exploit any left potential positive effects on tumor viability and stemness as well as on inflammation-mediated peritumoral edema in pedHGG patients. However, because even positive effects were very inconsistently mediated in different pedHGG cell lines, future studies should examine a broad range of cell lines.

Most importantly, Boswellia formulations cannot be recommended as a complementary therapeutic adjuvant for the treatment of pedHGG patients, and parents and caretakers of affected children should be aware of potential unintended effects.

Authors' contributions

All authors contributed to the study conception and design and discussion. Material preparation, data collection and analysis were performed by MW, PB, KK, FEK, MS and CK. The first draft of the manuscript was written by MW and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Informed consent was obtained from all authors of the study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2023.07.007.

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