

LETTER TO THE EDITOR

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GSK3-beta as a candidate therapeutic target in soft tissue sarcomas

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Abstract

Soft tissue sarcoma (STS) is a predominantly fatal rare malignancy with inadequate treatment options. Glycogen synthase kinase 3 β (GSK-3 β) is an emerging target in human malignancies. Its therapeutic relevance in STS is unknown. We analyzed the prognostic impact of GSK-3 β gene and protein expression in two independent cohorts of patients with STS. We then treated STS cell lines and mice xenografts with a novel GSK-3 inhibitor 9-ING-41 alone or in combination with chemotherapy. We demonstrated that 9-ING-41 treatment induced significant STS cells apoptosis and was synergistic *in vivo* when combined with chemotherapy. Mechanistically, 9-ING-41 induces significant apoptosis of STS cells via suppression of NF- κ B-mediated X-linked inhibitor of apoptosis protein (XIAP) expression. These data support the inclusion of patients with STS in clinical studies of 9-ING-41 alone and in combination with chemotherapy.

Keywords Glycogen synthase kinase 3 β , Soft tissue sarcomas, 9-ING-41

To the Editor

The prognosis of patients with advanced STS is extremely poor with a median overall survival of less than 18 months [1, 2]. Identification of new therapeutic strategies is therefore an important medical need.

Glycogen synthase kinase-3 β (GSK-3 β) has been shown to play an important role in tumor progression particularly through the modulation of oncogenes, cell cycle regulators and mediators of epithelial–mesenchymal transition [3]. Recent studies have also demonstrated that aberrant overexpression of GSK-3 β promotes tumor growth and chemotherapy resistance in various solid tumors including pancreatic, colorectal and prostate

cancer through differential effects on pro-survival NF- κ B and c-Myc pathways as well as on TNF-related apoptosis-inducing ligand (TRAIL) and p53-mediated apoptotic mechanisms [4–6]. GSK-3 β represents therefore an important therapeutic target in human malignancies.

9-ING-41 is a small molecule potent selective GSK-3 β inhibitor with antitumor activity in several epithelial tumor models as shown by the interim results of the first clinical trial reported in patients with refractory cancers. Five other phase 2 clinical trials are ongoing in salivary gland carcinoma, myelofibrosis, pancreatic adenocarcinoma and pediatric patients with advanced malignancies (<https://clinicaltrials.gov/ct2/results?cond=&term=9ING41&cntry=&state=&city=&dist=>) [7–9]. We report here the first study investigating the therapeutic potential of GSK-3 β targeting with 9-ING-41 in STS (see Additional file 1: Methods).

We first used the Gene Expression Profiling Interactive Analysis (GEPIA), a web-based interactive database that compiles the standardized analysis of RNA-Seq data from 9736 tumors and 8587 normal samples based on The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. We found that

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(See figure on next page.)

Fig. 1 **A** Examples of GSK3 β staining intensity in soft tissue sarcomas. GSK3 β was evaluated semi-quantitatively including percentage (0–100%) and intensity (0 = null, 1 = low, 2 = moderate, 3 = strong). **B** Kaplan–Meier curves of metastases-free survival in 402 patients with soft tissue sarcomas according to GSK3 β expression (blue line: low expression, < 50% of tumor cells $n = 296$; green line: high expression: $\geq 50\%$ of tumor cells $n = 106$). **C** Kaplan–Meier curves of metastases-free survival in 268 patients with grade 3 soft tissue sarcomas according to GSK3 β expression (blue line: low expression, < 50% of tumor cells $n = 200$; green line: high expression: $\geq 50\%$ of tumor cells $n = 68$)

high GSK3 β gene expression was significantly associated with worse disease-free survival (log-rank test, $p = 0.0088$, Additional file 1: Fig. 1). To confirm the prognostic value of GSK3 β expression, we analyzed by immunohistochemistry a series of 402 patients with STS (Fig. 1A and Additional file 1: Tables 1 and 2). We observed positive expression of GSK3 β in 220 (54.7%) cases. High expression of GSK3 β (>50% of tumor cells) was significantly associated with poor prognosis (median metastases-free survival 139 months versus not reached, $p = 0.02$, Fig. 1B) supporting the hypothesis that GSK3 β may represent a potential therapeutic target. As shown in Fig. 1C, GSK3 β protein expression provided extra information to further refine the prognosis of patients besides histological grade which is considered the most significant predictor of outcome.

9-ING-41 is a first-in-class, maleimide-based small molecule potent selective GSK3 β inhibitor that has recently entered into clinical development. To examine its antitumor effect on STS, a panel of 20 STS cell lines were plated and treated with increasing concentrations of 9-ING-41 for 72 h. We showed that pharmacological inhibition of GSK3 β by 9-ING-41 suppressed the viability of all the 20 STS cell lines encompassing several histological subtypes with IC50 values ranging from 0.1 to 0.6 μM (Additional file 1: Table 3, Additional file 1: Fig. 2A).

Then, we demonstrated that 9-ING-41 suppresses cell viability through induction of apoptosis in a large panel of STS in vitro by using annexin V/PI, and immunoblots of apoptotic markers such as phospho- γH2AX , cleaved-Caspase-3 and cleaved-PARP confirming the phenotype of significant cell death in 9-ING-41-treated cells (Additional file 1: Figs. 2B and 3).

GSK3 β controls many cellular process including the survival NF-kappaB pathway. Therefore, we assessed by Western blot the activation of the I κ B kinase (IKK), a key protein involved in the translocation of NF-kappaB to the nucleus. As shown in Additional file 1: Fig. 4, the phosphorylation of IKK α/β at serine 176/177 is decreased after 24 h of 9-ING-41 treatment in different STS cell lines suggesting an inhibition of the NF- κ B pathway. To confirm these results, the expression of three NF- κ B target genes involved in anti-apoptotic effect, *Bcl-2*, *Bcl-XL* and *XIAP*, was examined. Although immunoblots revealed no significant changes in expression of Bcl-2

and Bcl-XL, XIAP expression was reduced in almost all cell lines tested (Additional file 1: Fig. 4). These data suggest that 9-ING-41 induces apoptosis in STS cell lines through the inhibition of NF- κ B pathway and a subsequent decreased expression of the anti-apoptotic gene *XIAP*.

We next evaluated the effect of 9-ING-41 in vivo by using the IB115 liposarcoma model. Mice were randomized in 4 groups and treated by intraperitoneal injection with vehicle, 9-ING-41, doxorubicin (the standard of care for STS) or combination of both drugs. As shown in Fig. 2, the tumors expanded aggressively in vehicle-treated animals, whereas in monotherapy a slight decrease of tumor growth was observed for 9-ING-41- or doxorubicin-treated mice. On the other hand, the combination treatment caused a significant decrease in tumor growth suggesting that 9-ING-41 can potentiate the effect of doxorubicin even with a low dose of chemotherapy without significant signs of toxicity as shown by the stable body weight of the mice (Fig. 2B).

By analyzing two large independent data sets, we show here that GSK3 β expression both at the gene and protein expression level is associated with increased risk of metastatic relapse and adverse outcome in STS patients suggesting an important role in sarcoma tumorigenesis and a potential role as a therapeutic target. Moreover, instead of using the commercially available toolkit GSK-3 β inhibitors, which are not amenable for clinical studies, we decided here to investigate 9-ING-41, a first-in-class GSK-3 β inhibitor which has recently entered clinical development in cancer patients. Preclinical studies have shown that 9-ING-41 induces significant apoptosis of cancer cell survival via suppression of NF- κ B-mediated B cell lymphoma 2 (Bcl-2) and XIAP expression in leukemia and solid tumors [10, 11]. The NF κ B transcription factor family is a highly conserved group of proteins playing an important role in the regulation of the cell physiology such as differentiation, apoptosis and survival. Pharmacological NF κ B inhibition has been shown to reduce cell growth in a spectrum of soft tissue sarcomas [12]. Here, we have shown that 9-ING-41 led to a decreased expression of the antiapoptotic molecule, XIAP, and resulted in an increased apoptosis as shown by PARP cleavage and caspase activation assay in STS cells. This suggests that 9-ING-41 may represent a candidate for the targeted therapy of STS. 9-ING-41 is currently

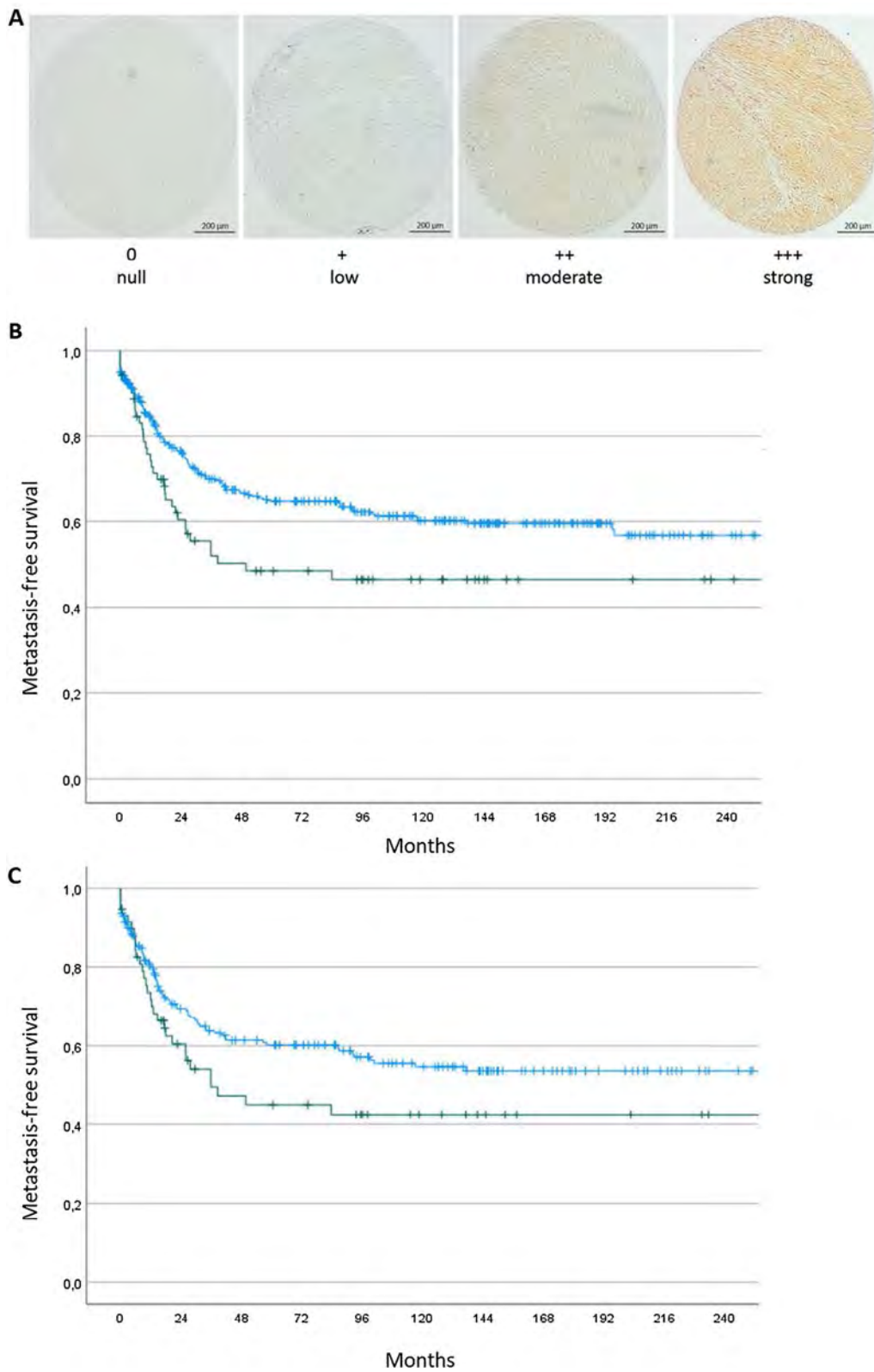


Fig. 1 (See legend on previous page.)

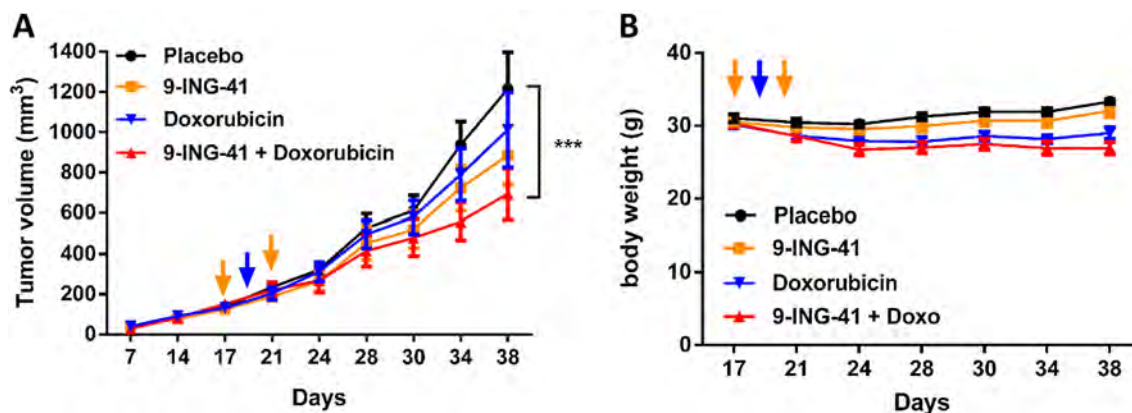


Fig. 2 In vivo effect of GSK3 β inhibitor 9-ING-41 in soft tissue sarcoma. IB115 liposarcoma cells were xenografted in NSG mice. Once tumors reached 100mm³ (day 17), mice were treated either with placebo (9 mice) or with two injections of 9-ING-41 at 70 mg/kg (orange arrow, 7 mice) or one injection of Doxorubicin at 1 mg/kg (blue arrow, 6 mice) or both drugs (6 mice). Tumor growth (**A**) and mice body weight (**B**) were monitored until day 38 and analyzed with GraphPad prism software using two-way ANOVA test and Bonferroni post-hoc test (***p* < 0.001). Combination treatment of 9-ING-41 and doxorubicin reduces significantly tumors volume

under investigation as a single agent and in combination with chemotherapy in cancer patients with advanced disease [13]. Preliminary results have shown a good safety profile and promising early signs of clinical activity in several solid tumors including melanomas and pancreatic cancers. Based on these results, a clinical trial investigating 9-ING-41 in sarcoma patients will be conducted.

Abbreviations

GEPIA	Gene Expression Profiling Interactive Analysis
GSK-3 β	Glycogen synthase kinase 3 β
GTex	Genotype-Tissue Expression
IKK	The I κ B kinase
TCGA	The Cancer Genome Atlas
TRAIL	TNF-related apoptosis-inducing ligand
STS	Soft tissue sarcomas
XIAP	X-linked inhibitor of apoptosis protein

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13045-021-01215-x>.

Additional file 1: Supplementary methods and results.

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Authors' contributions

AI, FG, LC and SV contributed to conceptualization; SV, VC, ER, VV and RP provided methodology; AI, SV and RP performed validation; SV carried out formal analysis and visualization; SV, VC, ER and VV performed investigation; AI, DD and FG provided resources; SV and AI performed writing—original draft preparation; AI, FG and LC performed writing—review and editing; AI contributed to supervision and project administration. All authors read and approved the final manuscript.

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Availability of data and materials

Source data are available on request to the corresponding author.

Declarations

Ethics approval and consent to participate

This study has been approved by the ethic committee for animal studies at the University of Bordeaux. The animal study was conducted according to the French and European Union guidelines for animal experimentation (RD 1201/05, RD 53/2013 and 86/609/CEE, respectively) and approved by the institutional animal use and care committee under project license APAFIS #17900-2018112722234037. The study was also approved by the Institutional Review Board of Institut Bergonié.

Consent for publication

Not applicable.

Competing interests

FG and LC are consultants to Actuate Therapeutics.

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