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Short communication

BJcuL, a lectin purified from Bothrops jararacussu venom, induces apoptosis in human gastric carcinoma cells accompanied by inhibition of cell adhesion and actin cytoskeleton disassembly

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A B S T R A C T
We show that BJcul, a lectin purified from Bothrops jararacussu venom, exerts cytotoxic effects to gastric carcinoma cells MKN45 and AGS. This effect was due to the direct interaction with specific glycans on the cells surface and was observed by cell viability decrease, disorganization of actin filaments and apoptosis. In addition, BJcul was able to reduce tumor cell adhesion to matrigel, what was inhibited by specific carbohydrate or partially inhibited when cells were pre-incubated with matrigel. Our results suggest that BJcul was able to promote apoptosis in both tumor cells lines and therefore has a prospect for potential use in cancer therapy.

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The venom of Bothrops jararacussu contains a lectin, called BJcul, characterized as a C-type lectin with high affinity for β-D-galactosides (de Carvalho et al., 1998). When biologically assayed, BJcul has showed to promote edema and increased vascular permeability in the hind paws of mice (Panunto et al., 2006), which may induce a direct cellular infiltration by creating an adhesive surface for leukocyte rolling (Eliño-Esposito et al., 2007). Recently, we have shown that BJcul could recognize glycoligands on the neutrophil surface and induce potent human neutrophils activation observed by polarization, migration and adhesion to extracellular matrix (ECM). Also, BJcul induced neutrophil functional activation observed by phagocytic uptake, superoxide production and delay in cell apoptosis (Eliño-Esposito et al., 2011). Furthermore, like other venom lectins, BJcul was also particularly cytotoxic to different tumor cell lines (de Carvalho et al., 2001), but the initial mechanisms of this effect had not yet been described.

In the present work BJcul was purified from the crude venom by affinity chromatography on an agarose-β-galactose column as previously described (Eliño-Esposito et al., 2007) and biotinylated according to the manufacturer’s recommendations. Using biotinyl-BJcul, we observed that the lectin was evenly bound on the human gastric
carcinoma MKN45 and AGS cells surface (Fig. 1A and B, respectively) and this interaction was abolished in the presence of the Bjcul specific ligand (α-Gal) (Fig. 1C), while a non-specific monosaccharide (GlcNAc) did not affect the lectin binding (Fig. 1C). No labeling was detected when the cells were submitted to a similar process in which the lectin was absent (control cells) (Fig. 1C). Complex carbohydrate structures expressed by cells have potential for encoding information in recognition processes. Carbohydrates are differentiation markers and antigenic determinants (Feizi and Childs, 1987) and lectins are proteins that decode this specificity. It appears that the binding due to carbohydrate recognition domain (CRD) is a prerequisite for Bjcul cytotoxic effect (Fig. 1E), since the

![Image](image_url)

**Fig. 1.** Cell-bound Bjcul is dependent on the carbohydrate recognition and promotes cytotoxicity and adhesion inhibition. (A–C) Cells adhering to coverslips were fixed with 2% paraformaldehyde, washed and then incubated with 1% H₂O₂/methanol for 10 min at room temperature (RT). After rinsing, cells were incubated with 5 μg/ml biotinyl-Bjcul, for 2 h at RT and this binding was revealed by incubation with avidin-FITC conjugate for 45 min. Images were provided by a Leica DMLB microscope (Leica Microsystems), captured by a Digital Microscope Camera (Polaroid). Quantification of bound-Bjcul on the cell surface was obtained with the Image-Pro Plus Software (Media Cybernetics). (D–E) The cytotoxic effect of Bjcul was evaluated by MTT assay. Cells (1 × 10⁶ cells/well) were incubated for 24 h at 37 °C with different concentrations of Bjcul (0.625; 1.25; 1.8 and 2.5 μg/ml in 5% RPMI). After that, 10 μl of the MTT solution (5 mg/ml) were added for more 3 h-incubation and reaction was stopped by the addition of DMSO. The optical density (OD) was measured at 570 nm. (F) Tumor cells suspension (1 × 10⁴ cells/well) in 5% RPMI, containing or not Bjcul (2.5 μg/ml), was distributed in matrigel-coated wells, and incubated for 24 h at 37 °C. (G) Tumor cells were distributed in the matrigel-coated wells and incubated for 1 h at 37 °C to promote cell adherence. After that, Bjcul was added for further 24 h incubation. For both protocols (F–G), the cells supernatants were carefully transferred to a fresh plate and submitted to additional 24 h incubation at 37 °C for cell viability analysis. Cells that remained adhered to the matrigel-coated wells were analyzed by MTT assay. For all experiment, negative control cells were incubated only with 5% RPMI and for inhibition assays the lectin was pre-incubated for 45 min at 37 °C with 2 mM Gal or 2 mM GlcNAc. Data represent means ± SD. Statistical differences were determined by Student t-test (** p < 0.01 and * p < 0.05).
lectin showed a dose-dependent cytotoxic activity in both tumor cells (Fig. 1D) after 24 h incubation. The lowest dose evaluated (0.625 μg/ml) promoted a 50% decrease in cell viability, which was progressively reduced to 80% in the highest doses (2.5 and 5 μg/ml). Because of this result we chose the concentration of 2.5 μg/ml for the other biological assays. Furthermore, carcinoma cells were highly adherent to the matrigel-coated wells, but the incubation with BjcuL inhibited by 74% and 83% the adhesion of MKN45 and AGS cells, respectively (Fig. 1F). Non-adhered cells, collected in the supernatant of cultures, were not viable, as revealed by microscopic analysis (not shown). When cells were incubated with matrigel-coated wells 1 h before the lectin was added, the strong inhibition showed in Fig. 1F was decreased to 42% and 55%, respectively (Fig. 1G). Again, inhibition of cells adherence determined by BjcuL was selectively reverted in the specific carbohydrate presence (Fig. 1G). These results suggests that glycosylated molecules that abound in the ECM may contain targets for BjcuL recognition and so interfere in the lectin activity or that lectin binding sites on the cancer cells may be blocked sterically in the already adherent cells. The effect of BjcuL on cell adhesion was not observed by de Carvalho et al. (2001), who demonstrated that BjcuL was not able to inhibit human metastatic breast cell (MDA-MB-435 cell line) and human ovarian carcinoma (OVCAR-5) adhesion to ECM proteins such as fibronectin, laminin and collagen type I.

In order to characterize the effects of BjcuL on the cell morphology, the cytoskeleton was examined by fluorescence microscopy. We observed that MKN45 (Fig. 2A) and AGS (Fig. 2C) control cells showed a symmetrical distribution of F-actin around cells with dense meshwork of polarized actin filaments in the limit of adjacent cells. BjcuL caused a significant decrease in F-actin in both MKN45 (Fig. 2B) and AGS (Fig. 2D) cells, in comparison to control cells. Actin filaments of both tumor cells began to shrink into the center at cellular focal contacts and were completely disassembled, resulting in decreased fluorescence by FITC-phalloidin conjugate (arrows, Fig. 2B and D, respectively). The oncogenic transformation is often associated with changes in the cytoskeleton organization that may influence cell migration, adhesion and invasiveness. The actin cytoskeleton plays an important role in tumor cell locomotion through recognition and binding to the ECM (Hall, 1998; Adams, 2001). In this study, treatment with BjcuL resulted in complete actin filament disorganization and disassembly in MKN45 and AGS tumor cells, as well as detachment from extracellular matrix. The cytoskeleton is also involved in apoptotic signaling such as sensitization to Fas-induced apoptosis by polymerized actin, triggering of membrane blebbing by acto-myosin contraction, and

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**Fig. 2.** Actin cytoskeleton is disassembled by BjcuL. Glass coverslips were placed in a 24-well plate and MKN45 (A and B) and AGS (C and D) cells (5 × 10⁴) were allowed to adhere for 24 h at 37 °C. Subsequently, cells were incubated with only 5% RPMI (A and C) or with BjcuL (2.5 μg/ml) (B and D) for 24 h. After washing twice with PBS, cells were fixed with 4% paraformaldehyde for 20 min at RT and permeabilized with 0.1% Triton X-100 for 5 min. Actin cytoskeleton was stained with FITC-conjugated phalloidin and the nuclei was stained with DAPI for 30 min at RT, in the dark. Areas with cytoskeleton disassembly are indicated with arrows. Cells were washed with PBS and then coverslips were inverted on microscope slides and mounted with Vecta-shield medium. The images were obtained using an Olympus IX70 microscope.
uptake of apoptotic cells by phagocytes through the activation of Rac. The cleavage of several cytoskeleton components by caspases ensures the orderly dismantlement of the dying cell (Ndozangue-Touriguine et al., 2008).

The apoptosis of tumor cells was analyzed by degradation of nuclear DNA. Mostly MKN45 or AGS tumor cells incubated with BJcuL (2.5 μg/ml) have incorporated fluorescein-12-dUTP (light blue fluorescence) which labels DNA fragments in apoptotic cells (arrows, Fig. 3B and D, respectively), whereas only few dark blue staining cells, counterstained with DAPI, were detected. On the other hand, untreated MKN45 and AGS cells were mainly dark blue indicating non-apoptotic cells (arrowheads, Fig. 3A and C). Apoptosis is an intrinsic cell death mechanism that plays an important role in the maintenance of healthy tissue and is characterized by cell contraction, chromatin condensation, DNA fragmentation, and activation of caspases (Salvesen and Dixit, 1997). Therefore, searching for agents that trigger apoptosis of tumor cells has become an attractive strategy in anticancer drug discovery (Reed, 2003; Liu et al., 2009a, 2009b). One of the major findings in this study is that BJcuL induced apoptotic cell death in gastric carcinoma MKN45 and AGS cell lines, as reported by other lectins. The *Viscum album* agglutinin-I (VAA-I) and WGA were able to induce apoptosis in various tumor cells (Gastman et al., 2004). ConA and PCL (*Phaseolus coccineus* lectin) induced apoptosis in tumor cells in a caspase-dependent manner (Liu et al., 2009a) like as the *Polygonatum odoratum* lectin that increased the activity of caspase-3, -8 and -9 in a time-dependent manner (Liu et al., 2009b).

The antitumoral properties of lectins have been demonstrated *in vivo* and *in vitro* and suggests a potential clinical use in slowing tumor progression by cytotoxicity, apoptosis, and inhibition of tumor growth (De Mejia and Prisecaru, 2005). VAA-I was introduced in the treatment of cancer in 1917, and today has still been used in adjuvant cancer therapy in several European countries. It seems that mistletoe extracts stimulate the immune system, enhance the cytotoxic effect of chemotherapeutic drugs, improve survival and quality of life, and reduce adverse effects of chemo- and radiotherapy in cancer patients (Salzer, 1981; Olsnes et al., 1982; Horneber et al., 2008). BJcul. (de Carvalho et al., 2001), CEL-I and CEL-III (lectins from marine invertebrate *Cucumis echinata*) (Kuramoto et al., 2005), WGA (Ohba et al., 2003), Con A (Kim et al., 1993) and others, are known to bind on tumor cells membranes, affecting cell viability. We have recently reported that BJcul. is capable of inducing potent neutrophil activation without exerting cytotoxicity (Elifio-Esposito et al., 2011). Other authors have proposed that lectins have the ability to distinguish normal from malignant cells (Sharon and Lis, 1995; Sabova et al., 2010) and for that have different toxic abilities that can be due to different glycans expressed on the surface of these cells (Miller et al., 1973; Matsuda et al., 2010). There are lectins that interact strongly with leukemia cells, triggering agglutination and

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**Fig. 3.** BJcul. promote tumor cells apoptosis. Representative photomicrographs showing labeling of DNA strand breaks associated with apoptosis assayed by terminal deoxynucleotidyl tranferase-mediated dUTP nick end-labeling (TUNEL) method. Tumor cells were seeded onto coverslips and (A) MKN45 and (C) AGS cells were incubated only with 5% RPMI and (B) MKN45 or (D) AGS cells were incubated with BJcul. (2.5 μg/ml) for 24 h at 37 °C. For apoptosis evaluation, after incubation, adherent cells on coverslips were processed using a TdT FragEL-DNA fragmentation detection kit (Calbiochem-Oncogene). Arrows show apoptotic cells and arrowheads, the DAPI staining.
cytotoxicity, but exert no effect on normal lymphocytes (Ohba and Bakalova, 2003).

This study confirmed the cytotoxicity of Bjcul to tumor cells mainly by altering cell adhesion and inducing apoptosis. Therefore, we suggest that Bjcul may compete for binding to the cell surface with ECM glycoproteins and promote the actin disassemblies and could possibly accelerate cellular detachment from the ECM. Consequently Bjcul interaction with glycoligands on the tumor cell surface may induce the activation of intracellular signals capable to inhibit cell proliferation, leading to apoptosis.

**Ethical statement**

This study was performed with cell lines and did not request approval for the ethical committee.

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**Conflict of interest statement**

All authors declare that there is no potential conflict of interests in manuscript entitled Bjcul, a lectin purified from *Bothrops jararacussu* venom, induces apoptosis in human gastric carcinoma cells accompanied by inhibition of cell adhesion and actin cytoskeleton disassembly.

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