The TF-antigen binding lectin from Sclerotium rolfsii inhibits growth of human colon cancer cells by inducing apoptosis in vitro and suppresses tumor growth in vivo

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Glycan array analysis of Sclerotium rolfsii lectin (SRL) revealed its exquisite binding specificity to the oncofetal Thomsen-Friedenreich (Galβ1-3GalNAcα-O-Ser/Thr, T or TF) antigen and its derivatives. This study shows that SRL strongly inhibits the growth of human colon cancer HT29 and DLD-1 cells by binding to cell surface glycans and induction of apoptosis through both the caspase-8 and -9 mediated signaling. SRL showed no or very weak binding to normal human colon tissue cells but strong binding to cancerous and metastatic tissues. Intratumor injection of SRL at subtoxic concentrations in NOD-SCID mice bearing HT29 xenografts resulted in total tumor regression in 9 days and no subsequent tumor recurrence. As the increased expression of TF-associated glycans is commonly seen in human cancers, SRL has the potential to be developed as a therapeutic agent for cancer.

Keywords: antitumor effect / apoptosis / human colon cancer / Sclerotium rolfsii lectin / TF antigen

Introduction

Alterations of cell surface glycosylation are common in cancerous and precancerous tissues (Kim and Varki 1997; Ono and Hakomori 2004). These changes are observed in glycolipids, glycosphingolipids and glycoproteins (Ghazarian et al. 2011), which are associated with tumor progression and metastasis. Many of these glycosylation changes result in the exposure of oncofetal carbohydrate structures (Campbell et al. 1995; Springer 1997) which are recognized by specific carbohydrate-binding proteins (lectins) or antibodies. Lectins are ubiquitous in nature (Sharon and Lis 2004), and are useful tools in detecting cell surface-associated glycosylation changes (Mody et al. 1995). One of the most common glycosylation changes in epithelial cancers including colon cancer is the increased expression of the oncofetal Thomsen-Friedenreich antigen (Galβ1-3GalNAcα-O-Ser/Thr, T or TF), whose expression is correlated with tumor progression and metastasis (Hanisch and Baldus 1997; Yu 2007; Yu et al. 2007). Anti-TF-Ab and TF-specific lectins are gaining clinical implications as they are known to inhibit the cancer cell proliferation and metastasis in TF expressing cells (Almogren et al. 2012).

Sclerotium rolfsii is a soil-borne plant pathogenic fungus capable of forming fruiting bodies referred to as sclerotial bodies. Sclerotium rolfsii secretes a cell wall-associated, developmental stage-specific lectin—SRL (Swamy et al. 2001), which facilitates aggregation of the mycelium to form sclerotal bodies by interacting with its endogenous glycosyl ceramide receptor(s) (Swamy et al. 2004). The crystal structure of SRL, both in its free form and in complex form with N-acetyl-α-galactosamine (GalNAc) and N-acetyl-α-glucosamine (GlcNAc) has been determined at 1.1, 2.0 and 1.7 Å resolutions, respectively (Leonidas et al. 2007), showing the presence of two carbohydrate binding sites per SRL monomer. The primary binding site of SRL recognizes the TF disaccharide and GalNAc and the secondary binding site recognizes GlcNAc. Glycan array analysis revealed the exquisite binding affinity of SRL not only to TF, but also towards TF-associated O-linked glycans (Chachadi et al. 2011).

This study shows that, the presence of SRL inhibits the growth of human colon cancer HT29 and DLD-1 cells in vitro by induction of cell apoptosis. SRL shows strong binding to cancerous but not to normal human colon tissues. Intratumor injection of SRL into NOD-SCID mice bearing HT29 xenografts resulted in total tumor regression and no subsequent tumor recurrence.

Results

SRL inhibits the growth of human colon cancer cells in vitro
The presence of SRL in the culture of human colon cancer HT29 and DLD-1 cells resulted in dose- and time-dependent
inhibition of the cell growth (Figure 1A–C). At 10 μg/mL SRL caused 84 ± 5% (n = 9; P < 0.0001) and 81 ± 4% (n = 9; P < 0.0001) inhibition after 72 h and 38.42 ± 8.1% (n = 9; P < 0.0162) and 55.27 ± 7% (n = 9; P < 0.0001) inhibition of HT29 and DLD-1 cell growth, respectively, after 48 h, whereas no significant influence on cell growth occurred before 24 h. The growth inhibitory effects of SRL in HT29 cells were blocked by the presence of TF-expressing Bovine asialo mucin (BAM) (100 μg/mL) (78 ± 14%; n = 9; P < 0.0001) and to a lesser extent by bovine submaxillary mucin (BM) (33 ± 7%; n = 9; P < 0.17) (Figure 1D), suggesting that the growth inhibitory effect of SRL is mediated by its carbohydrate binding sites.

**SRL-mediated growth inhibition is a direct consequence of cell surface binding and does not require lectin internalization**

SRL showed strong binding to both HT29 and DLD-1 cells (Figure 2A and B). Sepharose-conjugated, hence non-internalized SRL showed similar growth inhibitory effect on HT29 as native SRL (Figure 2C). This indicates that SRL cell surface binding is sufficient to trigger its growth inhibitory effect and lectin internalization is not required.

**SRL-mediated cell growth inhibition is associated with induction of cell apoptosis**

To assess whether the growth inhibitory effects of SRL were related to induction of cell apoptosis, we assessed the cellular caspase-3 activity in response to SRL treatment. After treatment with SRL (10 μg/mL) for 72 h, both HT29 (Figure 3A) and DLD-1 (Figure 3B) cells showed increased expression of the active caspase-3. An increase in caspase-3/7 activity by 2.79-fold (P < 0.001) was found in HT29 cells after treatment with SRL (20 μg/mL) for 48 h when compared with the untreated cells (Figure 3C). The presence of inhibitors to caspase-8, caspase-9 or pan-caspase could all largely prevent SRL-induced caspase 3/7 activation (Figure 3C), indicating the possible involvements of both the extrinsic and intrinsic apoptosis signaling pathways. The presence of pan-caspase inhibitor largely diminished the growth inhibitory effect of SRL on HT29 (Figure 3D) cells, indicating that induction of cell apoptosis is directly linked with SRL-mediated cell growth inhibition.

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**Fig. 1.** SRL inhibits human colon cancer cell growth. (A). SRL causes dose-dependent inhibition of human colon cancer HT29 and DLD-1 cell growth. The cells incubated with or without different concentrations of SRL for 72 h before the numbers of the cell were counted (B and C). The growth inhibitory effect of SRL is time-dependent. HT29 (B) or DLD-1 (C) cells were incubated with SRL (10 μg/mL) for various times before the numbers of the viable cells were assessed by Calcein AM method. (D). SRL-mediated cell growth inhibition is prevented by the presence of TF-expressing glycoprotein. HT29 cells were incubated with 10 μg/mL SRL in the presence or absence of 100 μg/mL of BAM or bovine mucin (BM) for 72 h before numbers of the cells were counted. Data represent Mean ± SD of triplicate determinations from three different assessments. *P < 0.05; **P < 0.001.
SRL-induced apoptosis potentially involves both extrinsic and intrinsic pathways
To delineate the mechanism(s) of SRL-induced apoptosis in HT29 cells, the effect of SRL on activation of initiator caspases-8 and -9 and effector caspase-3 and cleavage of PARP was measured. The presence of SRL was found to cause a time-dependent increase of active caspase-8 and -9 and time-dependent decrease of their inactive forms and PARP cleavage (Figure 3E). SRL also showed to induce a time-dependent increase of the expressions of FasL, FADD, and truncation of BID.

SRL shows preferential binding to cancer tissues
SRL showed strong binding to cancerous and metastatic tissues and no or very weak binding to the adjacent normal tissues of human colon (Figure 4, Table I). The preferential binding of SRL to cancer and metastatic tissues is in keeping with its binding specificity to the TF-related glycans, which are known to be over-expressed in cancerous especially in metastatic tissues.

SRL suppresses tumor growth in mice
Intra-tumor injection of SRL in NOD-SCID mice bearing HT29 xenografts at 350 and 500 μg/mouse or 300 μg/mouse of Taxol, a known anticancer drug used as positive control on every alternate day resulted in total regression of the tumors after 9 days (Figure 5A). SRL at 250 μg/animal also caused significant reduction of the tumor size when compared with the control groups after 14 days following first dose (Figure 5A and B). The animals in group I (250 μg/mice) showed an average tumor weight of 0.113 ± 0.01 g (P < 0.05) and tumor volume of 256.2 ± 47 mm³ (P < 0.05) (Figure 5C and D) in contrast to group V [25 mM Tris-buffered saline (TBS), pH 7.5] with tumor weight 0.273 ± 0.04 g and volume of 628.6 ± 115 mm³. To check whether the increase in the number of SRL injections could further improve the antitumor effect of SRL, another two groups of mice (six mice each) with HT29 transplant were injected with TBS (control group) or SRL (250 μg/mice) on every alternate day for 17 days and observed for 40 days. It was found that the animals in SRL-treated group showed total tumor regression after 17 days and no signs of tumor recurrence within the remaining days. All those animals were healthy without even a scar left at the site of tumor as revealed and confirmed by histology of colon xenografts in SRL-treated and control groups (Figure 5E and F).

Toxicity studies of SRL at different concentrations (1000, 500 and 250 μg/animal) showed no difference in body weights, total blood cell counts and serum biochemical...
parameters in group III (250 μg/mouse) when compared with group IV (control). In group II (500 μg/mouse), the animal body weight and the hematological parameters were slightly decreased while the serum biochemical parameters (aspartate transaminase and alanine transaminase) were unaltered. SRL at a higher dose (1000 μg/mouse) was found to be lethal and the mortality occurred after 4th dose (7–8th day, Table II). These results suggest that although SRL showed toxicity to the animals at a higher dose, it is sustainable in vivo by the mice at lower doses, which show effective anti-tumor activity.

**Discussion**

The present study shows that SRL, a lectin isolated from the phytopathogenic fungus *S. rolfsii* that has exquisite binding specificity toward the TF-related O-glycans, inhibits growth of human colon cancer cells in vitro as a result of its cell surface binding and subsequent induction of cellular apoptosis, a process that involves likely the activation of both extrinsic and intrinsic apoptotic signaling pathways. SRL shows strong binding to cancer/metastatic tissues but no or weak binding to adjacent normal tissue in the human colon.
Patients → colon tissues lectins (Presant and Kornfeld 1972; Lotan et al. 1975; glycans. It is recognized by a group of plant and fungal xenografts in mice resulted in total tumor regression. Direct injection of SRL at subtoxic doses to colon cancer SRL binding to normal, primary and metastatic cancerous human colon tissues. Table I. SRL binding to normal, primary and metastatic cancerous human colon tissues.  

<table>
<thead>
<tr>
<th>Patients →</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal tissue</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Primary tumor</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Metastasis</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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</table>

Human colon tissue samples (normal, primary cancer and metastatic) were processed for lectin histochemistry. SRL binding was then evaluated through optical analysis by measuring the mean area of stained cells scored arbitrarily as intense (+++), moderate (++), weak (+) and no binding (−).

Fig. 4. SRL binding to normal and cancerous human colon tissues. SRL histochemistry shows no or very weak binding to normal human colon tissues but strong binding to primary cancer and metastatic colon tissues. All the images were obtained with ×400 magnification except the primary colon cancer with adjacent normal tissues which were taken with ×100 magnification. “Arrows” point to SRL binding to apical surface of the secretory gland epithelia. Representative images are shown.

Our earlier studies on glycan array analysis have shown that, although SRL binds to the TF antigen, its binding specificity toward the TF antigen is not the same as those of dietary TF-binding lectins. SRL binds with high specificity to “α-anomers” but not the “β-anomers” of the TF structure. The axial C4-OH group of GalNAc and C2-OH group of Gal are both essential for SRL interaction with TF disaccharide and substitution on C3 of galactose by either sialic or sialic acid or N-acetyl glucosamine, significantly enhances the avidity of the lectin binding to these TF derivatives (Chachadi et al. 2011). It was found that the growth inhibitory effect of SRL is not linked to lectin internalization and its surface binding is enough to trigger its growth inhibitory effect. The growth inhibitory effect of SRL was seen to be linked with induction of cell apoptosis. Cell apoptosis can be activated by both the extrinsic and intrinsic apoptosis pathways. The extrinsic pathway is convened by the activation of initiator caspase-8, initiated through ligation of the death receptors to their ligands [e.g. Fas L (FAS ligand) to Fas] followed by recruitment of FADD (Fas-associated death domain adaptor protein) to the death receptor cytoplasmic domain. The intrinsic apoptosis pathway is associated with cytochrome-c release from the mitochondria and subsequent activation of caspase-9 and apoptosome formation (Boatright and Salvesen 2003; Pop and Salvesen 2009). Although Caspase-8 is the key initiator caspase in the death-receptor pathway, its activation can also result in the truncation of the proapoptotic protein BID and lead to the depolarization of mitochondria and activation of caspase-9 of the intrinsic apoptotic signaling (Khosravi-Far and Esposti 2004; Sayers 2011). It was found that the presence of SRL resulted in increased expression of FasL (that binds to FAS), FADD, active Caspase-8, as well as activation of BID to t-BID, activation of caspase-9 and -3 and also downstream PARP cleavage suggesting the likely involvement of both the extrinsic and intrinsic apoptosis signaling in SRL-mediated apoptosis.

Direct injection of SRL at subtoxic doses to colon cancer xenografts in mice resulted in total tumor regression.

TF antigen is the core 1 structure of O-linked mucin type glycans. It is recognized by a group of plant and fungal lectins (Presant and Kornfeld 1972; Lotan et al. 1975; Rinderle et al. 1989; Kabir 1998; Lei et al 2010) and is implicated in cancer progression and metastasis (Yu et al. 2007; Zhao et al. 2010). TF-binding lectins isolated from dietary sources have been shown previously to affect the growth of cancer cells in vitro in diverse manners. The TF-binding lectins from Agaricus bisporus (ABL) (Yu et al. 1993, 1999) and Artocarpus integrifolia (jacalin) have shown to inhibit proliferation of human colon cancer cells in reversible ways (Kabir 1998; Yu et al. 2001), whereas the TF-binding lectins from Amarantus caudatus and Arachis hypogea (Peanut agglutinin) stimulate cell proliferation (Ryder et al. 1992; Singh et al. 2001, 2006; Yu et al. 2001). The growth inhibitory effect of ABL is related to lectin internalization and inhibition of NLS-dependent nuclear protein import (Yu et al. 1999) whereas jacalin-mediated cell growth inhibition is associated with activation of the tumor suppressor PHAP1 and subsequent activation of protein phosphatase 2A and suppression of ERK signaling (Yu et al. 2004). Apoptosis induction by AAL, a TF-specific lectin from edible mushroom Agrocybe aegerita is due to its internalization into the nucleus and binding to nuclear ligand MRG15 (Zhao et al 2003; Liang et al 2009, 2010; Feng et al. 2010). It is believed that the differential effects of these TF-binding lectins on cell growth are attributed to the subtle differences in their fine sugar-binding specificity towards alpha or beta anomers of TF (Chachadi et al. 2011; Almogren et al. 2012).
results suggest that SRL at subtoxic concentrations maintains an effective anti-tumor activity in vivo.

SRL thus inhibits human colon cancer cell growth by induction of apoptosis in vitro and suppresses tumor growth in vivo. As a naturally occurring lectin that binds specifically to cancer-associated glycans, SRL has the potential to be developed as a useful therapeutic agent for cancer treatment.

**Materials and methods**

**Materials**

Bovine serum albumin (BSA), Bovine mucin (BM) and Taxol (Paclitaxel) from *Taxus brevifolia* were obtained from Sigma Chemical Co. (St. Louis, USA). Fetal calf serum (FCS) was from Gibco Invitrogen (Paisley, UK), FITC-active caspase-3 antibody was from R&D Systems Europe Ltd. (Abingdon, UK), 3-3′diaminobenzidine chromogen/H2O2 substrate in buffered solution (pH 7.5) (DAB kit) was obtained from Bangalore Genei, India. Calcein AM fluorescent dye was from Invitrogen Eugene, Oregon, Caspase Glo3/7 Assay kit was from Promega, Madison,USA and caspase inhibitors, Caspase-3 [z-VAD(OMe)], caspase-8 (z-IETD), caspase-9 (Z-LEHD), were from Calbiochem, Nottingham (UK). Polyclonal mouse antibodies to FasL, FADD, Caspase-8, -9, β-actin and BID were procured from Santa Cruz Biotechnology, CA, USA. Rabbit polyclonal anti active

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**Table II. SRL toxicity in mice**

<table>
<thead>
<tr>
<th>SRL</th>
<th>Control 250 µg/mice</th>
<th>500 µg/mice</th>
<th>1000 µg/mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>23.33</td>
<td>22.28</td>
<td>22.7</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>23.45</td>
<td>23.08</td>
<td>19.88</td>
</tr>
<tr>
<td>Mortality</td>
<td>None</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>RBC (×10^6/cm³)</td>
<td>6.5</td>
<td>6.7</td>
<td>5.1</td>
</tr>
<tr>
<td>WBC (×10^3/cm³)</td>
<td>4.5</td>
<td>5.2</td>
<td>3.8</td>
</tr>
<tr>
<td>Platelet count (lakhs/cm³)</td>
<td>7.3</td>
<td>3.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
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<td>11.0</td>
<td>8.4</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>87.6</td>
<td>98</td>
<td>117.8</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>31</td>
<td>34.1</td>
<td>19.8</td>
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</table>

NOD-SCID mice were injected intraperitoneally with SRL (250, 500 and 1000 µg/mice) and TBS on alternate days for 9 days containing 6 animals in each treatment. The average body weight, total blood cell count and biochemical parameters in serum were determined after 14th day.
caspase-3, and mouse polyclonal PARP antibodies were from PIERCE, Barrington, USA. Species-specific HRP-labeled secondary antibodies were procured from BioRad, Hercules, USA.

BAM was prepared by acid hydrolysis of BM as described by Spiro (1960).

Cell culture
The human colon cancer HT29 and DLD-1 cells were obtained from the European Cell Culture Collection via the Public Health Laboratory Service (Porton Down, Wiltshire, UK). HT29 cells were cultured in DMEM supplemented with 10% FCS, 100 units/mL penicillin and100 μg/mL streptomycin (complete DMEM) at 37°C in 5% CO2 and DLD-1 cells were cultured in RPMI-1640 supplemented with 10% FCS, 100 units/mL penicillin and 100 μg/mL streptomycin at 37°C in 5% CO2 (complete RPMI).

SRL conjugation
Purification of SRL from the sclerotial bodies of the fungus was carried out as described previously (Swamy et al. 2001). Conjugation of SRL with FITC was conducted as described by Goldman (1968). Biotinylation of SRL was carried out according to the method of Duk et al. (1994). Conjugation of SRL to Sepharose-4B was as described by March et al. (1974). SRL-coupled Sepharose-4B was suspended in 25 mM TBS (pH 7.5, TBS) and stored at 4°C.

Assessment of cell proliferation
Subconfluent HT29 or DLD-1 cells were seeded at 2 × 10^4 cells/mL in 96-well plates in DMEM or RPMI-1640 complete medium for 72 h. The medium was replaced with serum-free DMEM or RPMI-1640 containing 0.5% BSA (w/v) and the cells were incubated with various concentrations of SRL for 72 h and at 10 μg/mL of SRL in presence or absence of TF-expressing glycoproteins for different time intervals at 37°C. HT29 cells were preincubated with Pan caspase-inhibitor [Z-VAD(Om)e] before treating with SRL and incubated for 48 h. HT29 cells were treated with Sepharose-conjugated SRL and SRL, and incubated at 37°C for 72 h. The cells were either trypsinized and counted in a hemocytometer or labeled with 10 μM Calcein AM at 37°C for 30 min in a CO2 incubator, lysed with 100 μL lysis buffer (50 mM Tris–HCl, pH 6.8, with 5% SDS, and 2% mercaptoethanol). The fluorescent intensity was read using Tecan i-200 microplate reader with an excitation wave length of 485 nm and an emission wave length of 535 nm.

Assessment of SRL cell surface binding
HT29 and DLD-1 cells (1 × 10^6 cells/mL) were incubated with 1% BSA in PBS (200 μL) in ice for 1 h. FITC-conjugated SRL (10 μg/mL) was added to the cells and incubated for 1 h on ice in dark. The cells were washed with PBS and fixed in 2% paraformaldehyde. The cells were resuspended in PBS and analysed using FACScan flow cytometer (FACS VANTAGE-SE, Becton Dickinson).

Assessment of cellular apoptosis
HT29 or DLD-1 cells were cultured at 3 × 10^5 cells/mL in 6-well plates were incubated with or without SRL (10 μg/mL) in serum-free media for 48 h. The cells were harvested by gentle trypsinization and fixed in 2% paraformaldehyde. After two washes with PBS, the cells were resuspended in 400 μL of permeabilization buffer (0.1% Triton X-100 in PBS) for 10 min at room temperature (RT). Nonspecific binding sites were blocked by incubation with 10% goat serum for 20 min at RT. The cells were stained with 500 μL of staining buffer (2% FCS in PBS) containing 1 μL of FITC-anti-active-caspase-3 antibody at RT for 30 min and FITC positive cells were analysed using FACScan flow cytometer.

In some experiments, HT29 cells were cultured at 4 × 10^4 cells/mL in DMEM media for 48 h before introduction of inhibitors to caspase-3, -8, and -9 in serum-free media followed by addition of SRL (20 μg/mL) and incubated for 72 h. Cell apoptosis was then determined by Caspase Glo3/7 assay.

Western blotting
HT29 cells were treated with SRL (20 μg/mL) in serum-free media and incubated at 37°C and cells were harvested by gentle trypsinization at different time intervals. Cells were lysed using RIPA lysis buffer (120 mM NaCl, 1.0% Triton X-100, 20 mM Tris–HCl, pH 7.5, 100% glycerol, 2 mM EDTA, protease inhibitor cocktail, Roche, Germany). Total protein was fractionated on SDS-PAGE and blotted onto an Immobilon polyvinyli dine difluoride membrane. After blocking with 5% BSA, blots were incubated with antibodies for FasL, FADD, Caspase-8, -9, active caspase-3, tBID and PARP for 2 h and were followed by incubation for 1 h with the species-specific HRP-conjugated secondary antibodies. The blots were developed and visualized by chemiluminescence using Super signal West Femto maximum sensitivity substrate (Pierce, USA). The blots were stripped and reprobed for the β-actin which was used as the loading control.

Lectin histochemistry
Human colon tissue samples (normal, primary and metastatic cancer tissues) were procured from S. L. Raheja Hospital, Mumbai, India, with the approval of the ethical committee (IRB No.08/2009). Tissues were obtained during surgery or colonoscopic polypectomy, fixed in buffered formalin and embedded in paraffin for routine pathological examination. Additional 5 μm sections were prepared for lectin histochemistry after the pathological diagnosis was confirmed.

Lectin histochemistry of biotinylated SRL was carried out as described by Boland et al. (1991). SRL binding was then evaluated through optical analysis by measuring the mean area of stained cells scored arbitrarily as intense (++++), moderate (+++), weak (+) and no binding (−).

Assessment of the effect of SRL on tumor growth in NOD-SCID mice
In-bred NOD-SCID mice were procured from Advanced Centre for Treatment, Research and Education in Cancer, India, and maintained on synthetic diet and used for the toxicity and anticancer studies with clearance from the ethical
committee (IRB approval No.11/2009). HT29 cells were suspended at $1 \times 10^7$ cells/mL in DMEM media and cell suspension (0.1 mL) was injected subcutaneously into two mice. After 4 weeks, the tumors from these donor mice were excised, chopped into 2–3 mm fragments and a single piece of tumor was transplanted subcutaneously into each of 30 NOD-SCID mice. First, tumors were allowed to grow 5 of tumor was transplanted subcutaneously into each of 30 excised, chopped into 2

After 4 weeks, the tumors from these donor mice were suspension (0.1 mL) was injected subcutaneously into two mice.

The animals in group IV and V were injected with the anticancer drug Taxol (300 μg/mouse) and with TBS and used as a positive and negative control, respectively. The animals in groups I and V were sacrificed on the 14th day following the first dose with tumor weight and size being measured and photographed. Animals in groups II, III and IV were maintained for 40 days after the last injection for the observation of tumor recurrence.

In a separate experiment, 12 mice were each transplanted with a single HT29 tumor fragment as described above. The mice were then divided randomly into two groups with six animals in each group. Mice in group A were injected with SRL (250 μg/mouse) and the mice in group B with TBS (negative control) on every alternate day for 17 days (9 injections) and were maintained for 40 days. The animals were sacrificed after 40 days following the last injection and histological sections of colon xenografts in both groups were analyzed for the presence of any tumor or tumor remnants.

**Assessment of SRL toxicity in mice**

Twenty four NOD-SCID mice were divided into four groups with six animals in each group. Groups I, II and III were injected intraperitoneally with a different concentration of SRL (1000, 500 and 250 μg/mouse), respectively, and group IV with TBS (control) on every alternate day for 9 days. The body weights of mice were measured on every alternate day. On the 11th day, mice were sacrificed and different parameters of toxicity including total blood cell count, hemoglobin content, serum liver enzymes (aspartate transaminase and alanine transaminase) were measured.

**Statistical analysis**

Each experiment was performed at least twice, each time in a triplicate. Results were analyzed by one-way ANOVA followed by ‘Newman–Keuls’ multiple comparisons using Stat Direct software and data were considered significant when $P < 0.05$.

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**Conflict of interest**

None declared.

**Abbreviations**

AAL, Agrocybe aegerita lectin; ABL, Agaricus bisporus; AM, Acetoxy Methyl; anti-TF-Ab, Anti-TF antibodies; BAM, bovine asialo mucin; BID, BH3 interacting-domain death agonist; tBID, Truncated BID; BM, bovine submaxillary mucin; BSA, bovine serum albumin; DLD-1, D L Dexter -1, human colorectal adenocarcinoma cell line; DMEM, Delbeco’s modified eagle medium; FasL, Fas Ligand, Tumor Necrosis Factor, Member 6; FADD, Fas associated death domain; FAS, Cell surface receptor protein, binds to FasL; FITC, Fluorescein isothiocyanate; FCS, fetal calf serum; GalNAc, N-acetyl-d-galactosamine; GlcNAc, N-acetyl-d-glucosamine; HRP, Horse radish peroxidase; HT-29, Human colorectal adenocarcinoma grade II cell line; MRG15; Chromodomain Protein encoded by M304F; NOD-SCID, Non-obese diabetic/severe combined immune deficient; NLS, Nuclear Localization Sequence; PARP, Poly ADP ribose polymerase; PBS, Phosphate buffered saline; PHAP1, Putative human HLA class II-associated protein 1; RT, room temperature; SRL, Sclerottium rolfsii lectin; SDS, Sodium dodecyl sulphate; TBS, tris-buffered saline; TF, Thomsen-Friedenreich antigen; z-VD(AO), N-Benzylxoycarbonyl-Val-Ala-Asp (O-Me); Z-LEHD, Leu-Glu-(OMe)-Thr-Asp-(O-Me).

**References**


