

College of Light Industry and Food Sciences¹, South China University of Technology, Guangzhou; Institute of Biotechnology², College of Chemical Engineering, Xiangtan University, Xiangtan, China

Recombinant *Microcystis viridis* lectin as a potential anticancer agent

YUQIN LI^{1,2}, XUEWU ZHANG¹

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Dr. X.W. Zhang, College of Light Industry and Food Sciences, South China University of Technology, 381 Wushan Road, Guangzhou 510640, China
snow_dance@sina.com

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MVL (*Microcystis viridis* lectin), a glycoprotein originally isolated from freshwater blue-green algae *Microcystis viridis*, has been reported to have potent anti-HIV activity. However, the objective of this study was to evaluate the anticancer activity of recombinant MVL (R-MVL). MTT assay was used to evaluate the *in vitro* anticancer activity of R-MVL, and the results show that the IC₅₀ for HT-29, HepG2, SGC-7901 and SK-OV-3 were 40.20, 42.67, 49.87, and 53.40 µg/ml, respectively. Our data, for the first time, demonstrated that R-MVL exhibits potential anticancer activity, and open new therapeutic possibilities for the use of R-MVL as anti-cancer agent.

Microcystis viridis lectin (MVL) is a mannose-binding protein, originally isolated from aqueous extracts of the unicellular freshwater bloom-forming cyanobacterium (blue-green algae) *Microcystis viridis* NIES-102 (Yamaguchi et al. 1999). It is a 13-kDa single polypeptide containing 113 amino acid residues and possesses two highly homologous domains, each domain involved 54 amino acid and the two domains are connected by a linker consisting of 5 amino acid residues. Similar to other algal lectins, such as cyanovirin-N (CV-N), scytovirin (SVN), griffithsin (GRFT) and *Oscillatoria agardhii* agglutinin (OAA), MVL displays significant inhibition activity to laboratory and clinical isolates of HIV and other enveloped viruses by binding oligosaccharides on the surface of the viral envelopes (Li et al. 2008).

Lectins are present in a diversity of organisms, such as plants, animals, fungi, cycads, ferns and algae (Peumans and Van Damme 1995) and have been shown to have anti-tumor proliferation activities and to induce apoptosis in a series of tumor cell lines (de Mejía and Prisecaru 2005). Thus, the question arises whether MVL, as a member of lectins family, also possesses some anti-cancer activities. The purpose of this study was to answer this question.

Recently, due to very low contents of MVL in the corresponding algal species, we have successfully cloned and expressed R-MVL in *E. coli* and obtained biologically active MVL. In this study, several common cell lines, human colon cancer (HT-29), human hepatocellular liver carcinoma (HepG2), human ovarian

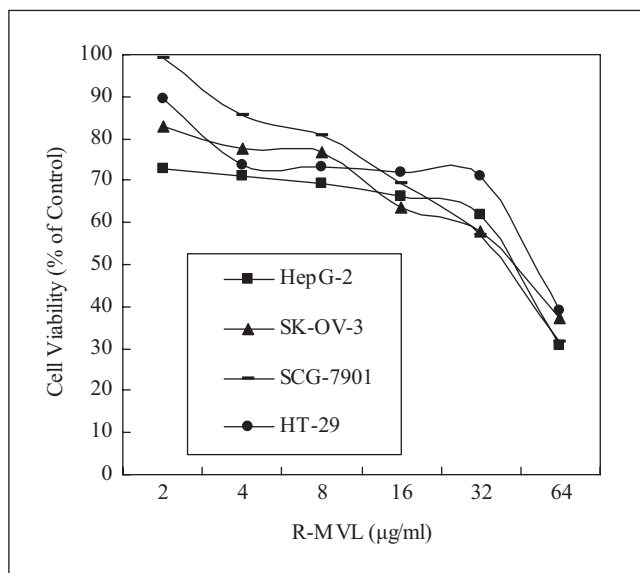


Fig.: The inhibition effect of R-MVL on the four tumor cell lines viability. The four cell lines (3×10^4 /ml) were seeded in the absence or presence of R-MVL (2~64 µg/ml). After 72 h, the cell viability was measured by MTT assay. Dose response curves of cell viability of recombinant MVL, presented as viability ratio compared with the control group (untreated cells). Each of cell lines was administrated to various concentration of MVL in triplicate, and each experiment was repeated three times

Table: IC₅₀ values of R-MVL for HepG₂, HT-29, SGC-7901, and SK-OV-3

	IC ₅₀ (µg/ml)			
Recombinant MVL	HepG ₂	HT-29	SGC-7901	SK-OV-3
	42.67	49.87	40.20	53.40

cancer (SK-OV-3), and stomach cancer cells (SCG-7901) were used to investigate the *in vitro* anticancer activity of R-MVL. The MTT measurement results showed that R-MVL (2~64 µg/ml) can exert a remarkable inhibitory effect on the growth of HT-29, HepG2, SGC-7901, and SK-OV-3. IC₅₀ values have been calculated from the curves in the Fig. and are summarized in the Table. Treating the cells with R-MVL (2~64 µg/ml) resulted in a dose-dependent inhibition of cellular proliferation. The IC₅₀ values of R-MVL in the four cell lines tested ranged from 40.20 µg/ml to 53.4 µg/ml.

During the last decade, there is a growing interest in lectins, many of them exhibit anticancer activities (Bains et al. 2005). For example, a lectin purified from mesquite seed had an anti-proliferative and apoptosis effect on cervical human tumor cells (HeLa cells) (IC₅₀ = 30 ± 1.2 mg/mL) (de Mejía et al. 2002); a mannose-binding lectin from *Sophora flavescens* was found to have a strong cytotoxicity against HeLa cells and induces apoptosis in a time- and dose-dependent manner (IC₅₀ = 1×10^{-3} mg/mL, 24 h) (Zhen et al. 2008).

In this paper, for the first time, we reported that the algal lectin MVL exhibits significant inhibition effect on cell proliferation of some cancer cells in a dose-dependent manner, with IC₅₀ values of 40.20, 42.67, 49.87, and 53.40 µg/ml, for HT-29, HepG2, and SGC-7901 respectively. Previous studies have indicated that tumorigenesis and metastasis always accompany the expression of high mannose type oligosaccharides on the tumor cell membrane surface (Dallolio 1996; Dennis et al. 1987; Laidler and

Litynska 1997). Some other studies have suggested a direct correlation between certain lectin-binding patterns and apoptotic mechanisms in various tumors (Fik et al. 2001; Wang et al. 2000). While MVL can bind to mannose oligosaccharides with high affinity, it is completely possible that the anticancer activity of MVL might be correlated to its mannose-binding capacity. This calls for further study of such a potential anti-cancer candidate agent.

Experimental

HT-29, HepG2, SK-OV-3, and SCG-7901 cell lines were obtained from the people hospital of Guangdong province (Guangzhou, China). All other chemicals were of analytical reagent grade and were obtained from Sigma. For routine maintenance, cells were cultured in suspension in RPMI-1640 media (GIBCO BRL, Grand Island, NY) with 20% fetal bovine serum (GIBCO), 100 µg/mL penicillin and 10 µg/mL streptomycin (GIBCO) in a water-jacketed 5% CO₂ incubator at 37 °C (Forma Scientific, Marietta, OH). Drug sensitivity was evaluated using a standard colorimetric MTT assay.

Briefly, cells in the logarithmic growth phase were plated in 96-well microtitre plates at a density of 3×10^4 cells/100 µl/well and allowed to incubate 24 h at 37 °C for attachment. Subsequently, the purified R-MVL protein sample was diluted with complete medium to the desired initial concentration and then added to cells of the logarithmic phase. After incubation for another 72 h, MTT solution (Bornem, Belgium) (20 µl of 5 mg/ml) was added to each well, which were then incubated for additional 4 h at 37 °C. MTT-containing medium were removed and the formazan crystals, formed within the cells, were solubilized by the addition of DMSO (Bornem, Belgium) (150 µl/well) and agitation. The absorbance of the samples and control cultures were read at 570 nm with a spectrophotometer (Model 3550 Microplate Reader).

The inhibition rate was calculated according to inhibition rate formula (Ji et al. 2008) and the experiment data were processed using Excell to yield IC₅₀ values.

All experiments were performed at least three times. All values were expressed as mean ± S.D. Statistical analysis was performed using Student's t-test and $p < 0.05$ were considered to be significant.

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