College of Light Industry and Food Sciences[1], South China University of Technology, Guangzhou; Institute of Biotechnology[2], College of Chemical Engineering, Xiangtan University, Xiangtan, China

Recombinant *Microcystis viridis* lectin as a potential anticancer agent

YUQIN LI[1,2], XUEWU ZHANG[1]

Received July 14, 2010, accepted August 13, 2010

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

Pharmazie 65: 922–923 (2010)
doi: 10.1691/ph.2010.0713

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 IC_{50} 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.

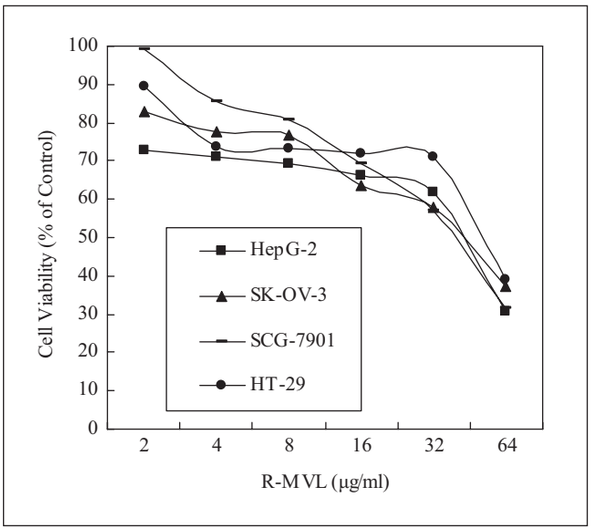

Fig.: The inhibition effect of R-MVL on the four tumor cell lines viability. The four cell lines (3×10^4/ml) were seeded in the absence or presence of R-MVL (2∼64 μg/ml). After 72 h, the cell viability was measured by MTT assay. Dose response curves of cell viability of recombinant MVL, presented as 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: IC_{50} values of R-MVL for $HepG_2$, 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

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 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, SCG-7901, and SK-OV-3. IC_{50} 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 IC_{50} 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 antiproliferative and apoptosis effect on cervical human tumor cells (HeLa cells) ($IC_{50} = 30 \pm 1.2$ mg/mL) (de Mejia et al. 2002); a mannose-binding lectin from *Sophora flavescens* was found to has a strong cytotoxicity against HeLa cells and induces apoptosis in a time- and dose-dependent manner ($IC_{50} = 1 \times 10^{-3}$ 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 IC_{50} 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% CO_2 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 in cubeted 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 IC_{50} 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.

References

Bains JS, Singh J, Kamboj SS, Nijjar KK, Agrewala JN, Kumar V, Kumar A, Saxena AK (2005) Mitogenic and anti-proliferative activity of a lectin from the tubers of Voodoo lily (*Sauromatum venosum*). Biochim Biophys Acta1723: 163–174.

Dall'olio F (1996) Protein glycosylation in cancer biology: an overview. Clin Mol Pathol 49: M126-M135.

de Mejía EG, Prisecaru V (2005) Lectins as bioactive plant proteins: a potential in cancer treatment. Crit Rev Food Sci Nutr 45: 425-445.

de Mejĭıa G, Rocha E, Winter N, Goldstein IJ (2002) Differential effect of a lectin from mesquite (Prosopis juliflora) on HeLa and normal human keratinocyte cells. FASEB J 15: C 128.

Dennis JW, Laferte S, Waghorne C, Breitman M, Kerbel RS (1987) Beta 1–6 branching of Asn-linked oligosaccharides is directly associated with metastasis. Science 236: 582–585.

Fik E, Wolun-Cholewa M, Kistowska M, Warchol JB, Gozdzicka-Jozefiak A (2001) Effect of lectin from Chelidonium majus L. on normal and cancer cells in culture. Folia Histochem Cytobiol 39: 215-216.

Ji YB, Gao SY, Ji CF, Zou X (2008) Induction of apoptosis in HepG2 cells by solanine and Bcl-2 protein. J Ethnopharmacol 115: 194–202.

Laidler P, Litynska A (1997) Tumor cell N-glycans in metastasis. Acta Biochim Pol 44: 343–357.

Li YQ, Zhang XW, Chen G, Wei D, Chen F (2008) Algal lectins for potential prevention of HIV transmission. Curr Med Chem15: 1096–1104.

Peumans WJ, Van Damme EJ (1995) Lectins as plant defense proteins. Plant Physiol 109: 347-352.

Wang H, Ng TB, Ooi VE, Liu WK (2000) Effects of lectins with different carbohydrate-binding specificities on hepatoma, choriocarcinoma, melanoma and osteosarcoma cell lines. Int J Biochem Cell Biol 32: 365–372.

Yamaguchi M, Ogawa T, Muramoto K, Kamio Y, Jimbo M, Kamiya H (1999) Isolation and characterization of a mannan-binding lectin from the freshwater Cyanobacterium (blue-green algae) *Microcystis viridis*. Biochem Bioph Res Comm 265: 703–708.

Zhen Liu, Bo Liu, Zi-Ting Zhang, Ting-Ting Zhou, He-Jiao Bian, Ming-Wei Min, Yan-Hong Liu, Jing Chen, Jin-Ku Bao (2008) A mannose-binding lectin from Sophora flavescens induces apoptosis in HeLa cells. Phytomedicine 15: 867–875.