Short Communication

ISSN 0233-7657
Biopolymers and Cell. 2015. Vol. 31. N 4. P. 316–320
doi: http://dx.doi.org/10.7124/bc.0008F1

UDC 576.322 577.22

Glioma-associated protein CHI3L2 suppresses cells viability and induces G1/S transition arrest

S. S. Avdieiev[1], L. Gera [2], R. Hodges[2] , V. V. Dmytrenko[1]

[1] Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03680

[2] Department of Biochemistry and Molecular Genetics, University of Colorado Denver,
Anschutz Medical Campus, Aurora CO 80045, USA

stasavdieiev@gmail.com

Aim. To analyze the effect of the CHI3L2 protein on malignant and non-malignant cell viability, and determined the CHI3L2 impact on the cell cycle and signaling pathways involved in the cell cycle regulation. **Methods.** MTT-based cell proliferation assay, FACS, western blot analysis. **Results.** The CHI3L2 protein inhibits the glioma cells viability and potentiates the effect of anti-cancer cytotoxic agents. The CHI3L2 treatment results in the G1/S transition arrest. CHI3L2 provoked a dramatic reduction of pRB phosphorylation and a significant decrease in the cyclin D1 expression, whereas the p53 and p21 expression levels were substantially increased. **Conclusions.** The CHI3L2 protein, which is overexpressed in human gliomas, is a negative regulator of the glioma cells viability. The reduced cell viability after the CHI3L2 treatment could be due to the activation of pRB and p53 and the downregulation of cyclin D.

Keywords: chitinase-like proteins, glioma, bradykinin antagonists, cell cycle regulation signaling cascades.

Introduction

Almost half of the intracranial tumors are gliomas, the majority of which are astrocytic gliomas. They are considered to be the most lethal among all types of tumors. We have previously compared the gene expression in glioblastoma, the most common and aggressive malignant brain tumor, and normal adult human brain by Serial Analysis of Gene Expression (SAGE) to identify those involved in gliomagenesis and found that that 44 genes were expressed at a significantly higher level in the tumors compared to the normal brain cells [1]. *Chitinase 3-like 2* (*CHI3L2*) is one of the genes expressed in glyoblastoma; it encodes a 39 kDa secreted chitinase-like protein. CHI3L2 is a member of the 18 glycosyl hydrolase family [1]. It is closely related to another mammalian chitinase-like protein (CLP) CHI3L1. *CHI3L1* is overexpressed in glioblastoma and possesses oncogenic properties [2]. CHI2L1 and 2 may have different functions since the molecular structures of these homologous proteins have significant differences [3]. We have previously demonstrated that the activation of ERK1/2 phosphorylation by CHI3L2 inhibits the cell mitogenesis and proliferation. In contrast, the activation of ERK1/2 phosphorylation by CHI3L1 leads to the proliferation [4]. Here we have reported that CHI3L2 downregulates the glioma cells viability and potentiates the effect of cytotoxic agents; the CHI3L2-mediated growth suppression is mediated by G1/S transition arrest.

Materials and Methods

Cell proliferation assay (MTT-assay) was performed as described in [4]. The CHI3L2 protein was obtained using Bac-toBac expression system (Invitro-

© 2015 Avdieiev S . S. *et al.*; Published by the Institute of Molecular Biology and Genetics, NAS of Ukraine on behalf of Biopolymers and Cell.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited

gen, USA). Bradykinin (BK) antagonist BKM-570 was kindly provided by Dr. Lajos Gera (University of Colorado Denver). Temozolomide was purchased from Abcam (UK).

Cell cycle analysis. 293 cells were incubated with the CHI3L2 protein or transfected with pcDNA4/TO_*CHI3L2*. After 48 hours, the cells were fixed with cold 70 % ethanol, and resuspended in 1 ml of hypotonic buffer (0.1 % sodium citrate, 0.1 % Triton X-100, 5 mg/ml PI (Sigma, USA), 20 mg/ml RNase A (Thermo Scientific, USA) and 40 mg/ml propidium iodide (Sigma) for 1 hour. The cells were then analyzed using the BD Accuri C6 machine (Becton Dickinson, USA) according to the manufacturer's instructions. The data were analyzed using the software package CFlow (Becton Dickinson) and FlowJo (De Novo Software, USA).

Western-blot analysis was performed as described earlier [4]. 293 cells were incubated with the CHI3L2 protein or transfected with pcDNA4/TO_*CHI3L2*. After 48 hours, the cells were lyzed and probed with the following antibodies: anti-pRB (Abcam), anti-CyclinD1 (Cell Signaling Tech, USA) anti-p53 (Millipore, USA), anti-p21 (Santa Cruz Biotech., USA), and anti-beta-actin (Sigma). Secondary polyclonal HRP-conjugated anti-rabbit IgG or anti-mouse IgG Abs (AbD Serotec, UK) and the ECL Western blotting detection system (Amersham, USA) were used to reveal immunoreactivity.

Results and Discussion

Although the investigation of human CLPs was mainly focused on the expression patterns in a number of pathological conditions [5], CHI3L2 remains poorly characterized. To explore an effect of the CHI3L2 protein on the cells viability, we performed the MTT test with the human glioma cells U251 and non-transformed 293 cells. The CHI3L2 protein exhibits cytotoxic properties in both U251 and 293 cells: an addition of 100 ng/ml CHI3L2 to the cell culture medium led to ~ 40% reduction of the cell viability as compared to the control group (0 ng/ml) (Fig. 1 *a*). The ability of CHI3L2 to inhibit the viability of malignant and non-transformed cells lines may suggest that a mode of its action is cell-type independent.

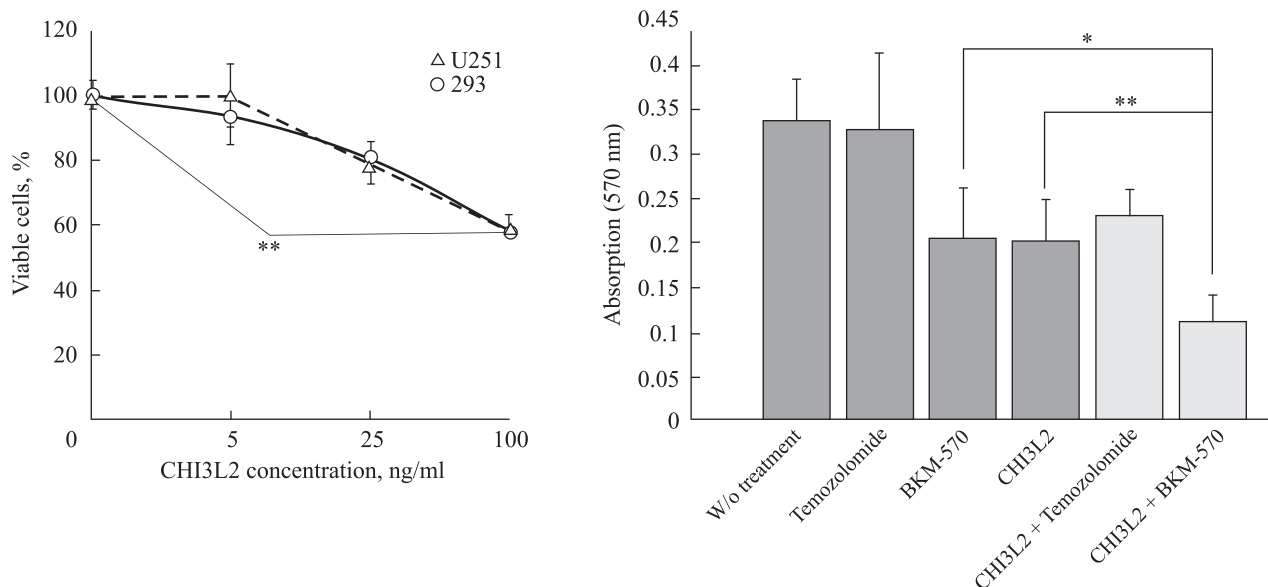

Fig. 1. Reduction of cells viability after CHI3L2 treatment. a) U251 or 293 cells were treated with CHI3L2 for 48 h in DMEM with 2,5 % FBS. b) U251 cells were treated with 100 μM temozolomide, 1 μM BKM-570, 100 ng/ml CHI3L2, or their combinations for 48 h in DMEM with 2,5 % FBS. Data are expressed as a percentage of growth compared to the vehicle control (100 %). Values are represented as means ± SD (n = 9). *p < 0.05 and **p < 0.01 *vs* control group

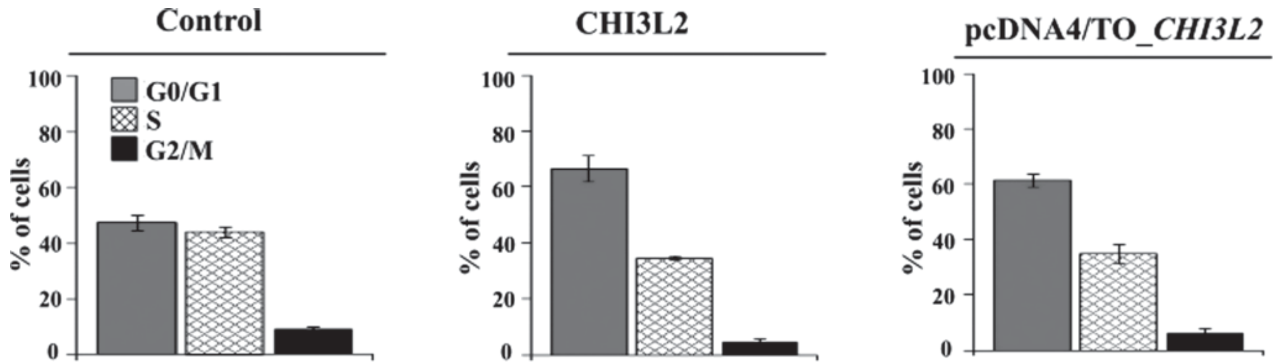

Fig. 2. Impact of CHI3L2 on cell cycle. 293 cells were treated with 100 ng/ml CHI3L2 or transfected with pcDNA4/TO_*CHI3L2* in DMEM with 2,5 % FBS. Cell cycle analysis was performed 48h after tretment

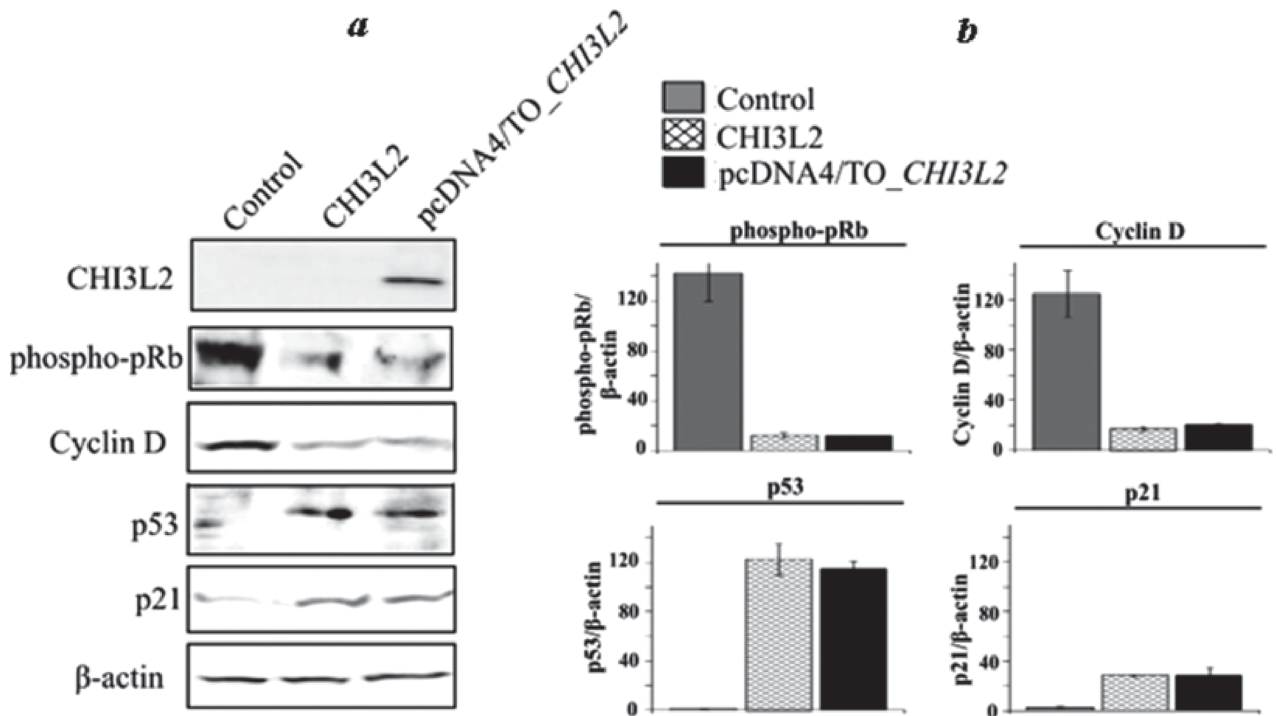

Fig. 3. CHI3L2 affects cell cycle regulation cascades. 293 cells were treated with 100 ng/ml CHI3L2 or transfected with pcDNA4/TO_*CHI3L2* in DMEM with 2,5 % FBS and lyzed after 48 h. Western blot analysis was performed with appropriate antibodies (a) and immunoreactive bands were analyzed by densitometry (b)

The proteins with cytotoxic properties could increase the efficacy of anticancer chemotherapy when applied in combinations [6]. Taking into account that CHI3L2 is overexpressed in glioblastoma, we aimed to see the outcome of the combination of CHI3L2 with the first-line anti-gliomic drug temozolomide, as well as with a highly potent anti-cancer agent, bradykinin antagonist BKM-570, possessing a significant cytotoxic activity against the glioma cell lines [7]. We did not observe any potentiation of the temozolomide activity but the combination of CHI3L2 with BKM-570 led to an increased cytotoxic effect (Fig. 1 *b*), suggesting that CHI3L2 could potentially be used as a component of a combo approach for the glioma treatment.

To elucidate the molecular mechanisms of the observed cell viability reduction, we studied the impact of CHI3L2 on cell cycle. While no apoptotic action of CHI3L2 was observed, CHI3L2 induced the G1/S transition arrest. The proportion of cells in G1 phase increased after treatment by exogenous CHI3L2 as well as after its ectopic expression by 19 % and 13,7 %, correspondingly (Fig. 2). To explore how CHI3L2 affects the specific signal cascades involved in the cell cycle regulation, we have studied the state of some proteins essential for the G1/S cell cycle transition. The CHI2L2 treatment or ectopic expression leads to a reduction of pRB phosphorylation, as well as to a decrease of the cyclin D1 expression level (Fig. 3). Furthermore, the expression levels of p53 and its transcriptional target p21 were significantly upregulated. Thus, the G1/S transition arrest through changes in the cell cycle regulation cascades is a putative component of the molecular mechanism underlying the cytotoxic effect of CHI3L2 protein. The blockage of cell cycle in a certain phase is the mechanism of action described for a set of cytotoxic agents [8], however, it has never been reported for the chitinase-like proteins. CHI3L1, the most investigated protein in this family, is also overexpressed in gliomas and was shown to be an oncogen [2]. CHI3L2 has a high level of homology to CHI3L1, however, a set of structural differences exists between them giving a ground to distinct functions [3]. CHI3L2 is not a glycoprotein and does not bind heparin, a component of the important receptor complexes [3]. Nevertheless nothing is know about a function of CHI3L2 in brain issue, the results described in this paper is a step forward shedding a new light on molecular pathways involved by these similar proteins to realize their function.

The complex application of chemotherapeutic agents and cytotoxic proteins is a promising approach to treat cancer [6]. Taking into account the cytotoxic properties of CHI3L2 and its overexpression in glioblastomas, one could speculate that at least in a certain part of patients the CHI3L2 protein could potentiates the action of chemotherapeutics. Further investigations are needed to clarify a role of the chitinase-like proteins in the human gliomas.

Conclusions

In summary, we have demonstrated that CHI3L2 acts as a negative regulator of the cell growth and affects the cell cycle regulation machinery, resulting in the G1/S transition blockage.

Acknowledgements

This work was supported in part by a travel grant of the Boehringer Ingelheim Fonds ‘Complex approaches for the treatment of chemoresistant gliomas and lymphomas’ and The ERA-WIDE Project 294932 ‘COMBIOM’.

REFERENCES

1. *Kavsan V, Shostak K, Dmitrenko V, Zozulya Y, Rozumenko V, Demotes-Mainard J.* Characterization of genes with increased expression in human glioblastomas. *Tsitol Genet.* 2005;**39**(6):37–49.
2. *Kavsan VM, Baklaushev VP, Balynska OV, Iershov AV, Areshkov PO, Yusubalieva GM, Grinenko NP, Victorov IV, Rymar VI, Sanson M, Chekhonin VP*. Gene encoding Chitinase 3-Like 1 Protein (CHI3L1) is a putative oncogene. *Int J Biomed Sci.* 2011;**7**(3):230–7.
3. *Iershov A, Odynets K, Kornelyuk A, Kavsan V.* Homology modeling of 3D structure of human chitinase-like protein CHI3L2. *Cent Eur J Biol*. 2010; **5**(4):407–20.
4. *Areshkov PO, Avdieiev SS, Balynska OV, Leroith D, Kavsan VM.* Two closely related human members of chitinase-like family, CHI3L1 and CHI3L2, activate ERK1/2 in 293 and U373 cells but have the different influence on cell proliferation. *Int J Biol Sci.* 2012;**8**(1):39–48.
5. *Johansen JS.* Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer. *Dan Med Bull.* 2006;**53**(2):172–209.
6. *Asmana Ningrum R.* Human interferon alpha-2b: a therapeutic protein for cancer treatment. *Scientifica (Cairo).* 2014;**2014**:970315.
7. *Avdieiev S, Gera L, Havrylyuk D, Hodges RS, Lesyk R, Ribrag V, Vassetzky Y, Kavsan V.* Bradykinin antagonists and thiazolidinone derivatives as new potential anti-cancer compounds. *Bioorg Med Chem*. 2014;**22**(15):3815–23.
8. *Shapiro GI, Harper JW*. Anticancer drug targets: cell cycle and checkpoint control. *J Clin Invest.* 1999;**104**(12): 1645–53.

Гліома-асоційований протеїн CHI3L2 пригнічує життєздатність клітин та блокує G1/S перехід

С. С. Авдєєв, Л. Гера, Р. Ходжес, В. В. Дмитренко

Мета. Проаналізувати життєздатність злоякісних та незлоякісних клітин за дії протеїна CHI3L2, а також визначити вплив CHI3L2 на клітинний цикл і сигнальні шляхи, залучені до його регуляції. **Методи**. МТТ–тест, проточна цитофлуориметрія, вестерн блот аналіз. **Результати**. CHI3L2 пригнічує життєздатність клітин гліоми людини і підсилює дію протиракових цитотоксичних агентів. CHI3L2 інгібує перехід клітин із G1- до S-фази клітинного циклу. CHI3L2 зумовлює зниження кількості фосфорильованої форми pRB, зменшення вмісту цикліну D1 та зростання вмісту p53 і p21. **Висновки**. CHI3L2, що надекспресується в гліомах людини, є негативним регулятором життєздатності клітин гліоми. Цитотоксичний вплив CHI3L2 може бути пов'язаним з активацією pRB та p53, а також зниженням вмісту цикліну D.

Ключові слова: хітиназо-подібні білки, гліоми, антагоністи брадикініну, сигнальні каскади, що регулюють клітинний цикл.

Глиома-ассоциированный протеин CHI3L2 подавляет жизнеспособность клеток и блокирует G1 / S переход

С. С. Авдеев, Л. Гера, Р. Ходжес, В. В. Дмитренко

Цель. Проанализировать жизнеспособность злокачественных и незлокачественных клеток при воздействии протеина CHI3L2, а также определить влияние CHI3L2 на клеточный цикл и сигнальные пути, вовлеченные в его регуляцию. **Методы**. МТТ-тест, проточная цитофлуориметрия, вестерн блот анализ. **Результаты**. CHI3L2 подавляет жизнеспособность клеток глиомы человека и усиливает действие противораковых цитотоксических агентов. CHI3L2 ингибирует переход клеток с G1- в S-фазу клеточного цикла. Влияние CHI3L2 приводит к снижению количества фосфорилированной формы pRB, уменьшению содержания циклина D1 и увеличению содержания p53 и p21. **Выводы**. CHI3L2, оверэкспрессия которого характерна для глиом человека, является негативным регулятором жизнеспособности клеток глиомы. Цитотоксическое влияние CHI3L2 может быть связано с активацией pRB и p53, а также снижением содержания циклина D.

Ключевые слова: хитиназа-подобные белки, глиомы, антагонисты брадикинина, сигнальные каскады, регулирующие клеточный цикл.

Received 03.06.2015