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Laminin associated with BMP7 as potential secondary astrocytic glioma fiber differentiation targets

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Abstract. – OBJECTIVE: To investigate the activation of the BMP7 and laminin pathway is associated with glioma cell proliferation and differentiation.

PATIENTS AND METHODS: We enrolled 65 patients with primary operable glioma. Laminin and BMP7 protein expression and its subcellular localization were studied by immunofluorescence.

RESULTS: We detected a higher level of BMP7 expression in glioma tissue in patients with a lower grade of glioma who had a lower eosinophil count. Compared to patients with a higher grade of glioma, we observed a lower level of laminin expression in patients with a lower grade of glioma.

CONCLUSIONS: Our data indicated a potential link between eosinophil counts and the expression levels of laminin and BMP7 in glioma differentiation.

Key Words:
Laminin, BMP7, Eosinophil, Glioma.

Introduction

Astrocytic glioma is the most common central nervous system tumor in adults[1]. Despite all advances in cancer treatment, astrocytic glioma remains one of the deadliest human cancers. Tumor initiating cells (TICs) are referred to glioma-initiating cells (GICs)[2,3], and they have the capacity to self-renew and to generate new tumors, which consist entirely of clonally derived cell types present in the parental tumor. GICs are considered the most chemoresistant cells in the tumor bulk, which are responsible for the tumor relapse[4,5]. A growing number of studies on cancer microenvironment are focusing on extracellular matrices (ECMs), which perturb cell regulatory networks[6]. ECMs are prominent and influential components of cellular microenvironment and changes in ECM components were shown to have implications for cancer progression[7-11]. Laminin is one of the ECM molecules, which is involved in modulation of the architecture of the matrices[12,13]. Changes in laminin expression can modulate the behavior of cancer cell. GICs are sensitive to developmental bone morphogenetic protein (BMP) signaling pathways[1]. In this study, we investigated the prevalence of BMP7 expression on astrocytoma. Also, we verified whether the activation of BMP7 and laminin pathways are associated with glioma cell proliferation and differentiation.

Patients and Methods

Patients

Paraffin-Embedded specimens (stages I-IV)[14] from 82 astrocytoma patients were used in this study. Specimens belonged to patients who underwent surgery at the Department of Neurosurgery at Chinese People's Armed Police Force General Hospital between January 2001 and December 2014. Patients were divided into 4 groups according to their differentiation grade. The first group consisted of 8 patients with astrocytoma grade I. In the second group we had 32 patients with low-grade astrocytoma (A, grade II). The third group consisted of 21 patients with anaplastic astrocytoma (AA, grade III). Finally, in the fourth group, there were 21 patients with glioblastoma (GBM, grade IV). Additional pathological data were collected from reports issued at the time of resection. Clinic-pathological characteristics are summarized in Table I. This study was approved by our Research Ethical Committee at

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Table I. Characteristics of patients.

	Group 1 (n=8)	Group 2 (n=32)	Group 3 (n=21)	Group 4 (n=21)
Median Age (range), years	37.75±13.23	36.47±14.47	49.38±15.53	42.36±21.40
Gender, No. (%)				
Female	80.00%	50.00%	48.28%	36.36%
Male	20.00%	50.00%	51.72%	63.64%
Largest median diameter, cm (range)	3.42±1.14 (1.93-4.45)	4.87±1.51 (2.26-7.51)	5.62±2.30 (2.11-8.74)	4.70±1.37 (3.86-6.55)

Armed Police General Hospital. Informed consent was waived by the Institutional Review Board due to the retrospective nature of the study.

Histological Evaluation

Specimens were fixed in 4% formaldehyde and embedded in paraffin, according to routine procedures. A 4 mm section from each sample was cut, dried, de-waxed and rehydrated. The sample was then stained with hematoxylin and eosin (HE) for histological observation under the light microscope.

Immunohistochemistry

Formalin-fixed and paraffin-embedded melanoma tissue sections were obtained from the Archive of the Department of Pathology at the Chinese People's Armed Police Force General Hospital. Sections were dewaxed, quenched and incubated with mouse monoclonal antibody (25 μg/ml) raised against human BMP7 (MAB3541, R&D Systems, Minneapolis, MN, USA) overnight at 4°C. Sections were then washed and incubated with anti-mouse or anti-rabbit Ig secondary antibodies conjugated to Alexa dyes 488 or 568 (Invitrogen, Paisley, UK). Nuclear counterstaining was performed with DAPI (Roche, Mannheim, Germany). All counts were performed under 400X magnification. The evaluation of stained cells was conducted by experts who had no prior knowledge about the clinical condition of each patient. All positive cells were evaluated in the nuclei of at least 1000 tumor cells. Labeling index (LI) (15) was calculated as the percentage of each kind of positive cells per 1000 tumor cells counted at random in each section. All cells were counted by three independent experts.

Immunofluorescence

Tibias were embedded in paraffin wax after decalcification in buffered EDTA (14.5%; pH 7.2) for 2 weeks and were sliced into 3-mm sections following the standard method. Slides were rinsed twice in phosphate buffered saline (PBS), followed by a wash in PBS containing 0.25% Triton X-100 (PBS-TX). Sections were then incubated overnight in a dark humid chamber at room temperature with rabbit anti-human ANA (US Biological C7150-13B), rabbit anti-human OPG, rabbit anti-rat BMP-2, rabbit anti-human BGP or rabbit anti- human BSP (Cell Signaling Technology, Inc., Danvers, MA, USA) diluted 1:200 in PBX-TX containing 1% bovine serum albumin. After several washes in PBS, sections were incubated for 1 h in a dark humid chamber at room temperature with goat anti-rabbit IgG conjugated with Alexa488 (Molecular Probes, Grand Island, NY, USA) Invitrogen (Carlsbad, CA, USA) or anti-rabbit IgG conjugated to Dylight594 (Molecular Probes, Grand Island, NY, USA) Invitrogen (Carlsbad, CA, USA) diluted 1:200 in PBS containing 1% bovine serum albumin (BSA). Sections were rinsed several times in PBS, mounted on cover slips in FluoroSave mounting medium and visualized under a Nikon Eclipse 800 fluorescent microscope (Nikon Instruments, Melville, NY, USA). Stained cells were counted in each slice by three blinded independent experts to assess the proliferation, localization, and differentiation potential of the hUC-MSCs among the groups. DAPI (Molecular Probes, Grand Island, NY, USA) were used as a nuclear counterstain.

Statistical Analysis

Stained cells were counted per 0.018 mm^2. Statistical analyses were performed using SPSS version 19.0 (IBM SPSS, Chicago, IL, USA). Cross-tabulations variables were analyzed with the Fischer IgG (C7150-13B), rabbit anti-human OPG, rabbit anti-rat BMP-2, smaller than 0.05.

Results

Patient Characteristics

Patients' clinical characteristics entered into our study are summarized in Table I. Patients were 3 females and 1 male with the median age of 44.75

Table II. The relationship between individual eosinophils and the differentiation of astrocytoma. (Data shown as Mean ±SD).

	G1	G2	G3	G4
% of individual eosinophils	0.95±0.96	1.72±1.23*	1.60±1.68*	0.72±0.63Δ

Compare to G1, parameters in G2, G3 and G4: Δp<0.05; *p<0.01.

Table III. The relationship between BMP7/Laminin and differentiation of CRC.

	G1	G2	G3	G4
BMP7	70.38±5.76	45.91±11.32*	20.90±5.99*Δ	24.26±3.91*Δ
Laminin	44.13±5.49	33.81±9.25*	12.29±5.23*Δ	16.28±4.06*Δ

(Data are shown as Mean±SD). Compare to G1, parameters in G2 and G3: *p<0.05; Δp<0.01. Compare to G2, parameters in G3: Δp<0.05; *p<0.01. (Data shown as Mean ±SD)

years (range=26 to 54 years). The median duration of ITP before HUC-MSCs transplantation was 74 months (range=13 to 120 months), and the median duration of prior treatments was 2 months (range=1 to 3 months). These treatments included splenectomy, prednisone, intravenous immune globulin, cyclosporine, and vincristine. All patients had a history of major bleeding, and these episodes were often transient but recurrent. Major hemorrhagic events included genitourinary bleeding, diffuse ecchymosis, and prolonged epistaxis.

Patient-Specific Extent and Eosinophils

The distribution of eosinophils in patients is summarized in Table II and is illustrated in Figure 1. A significant link between astrocytoma and low counts of eosinophils was detected. We also investigated the percentage of other leukocytes and their subtypes.

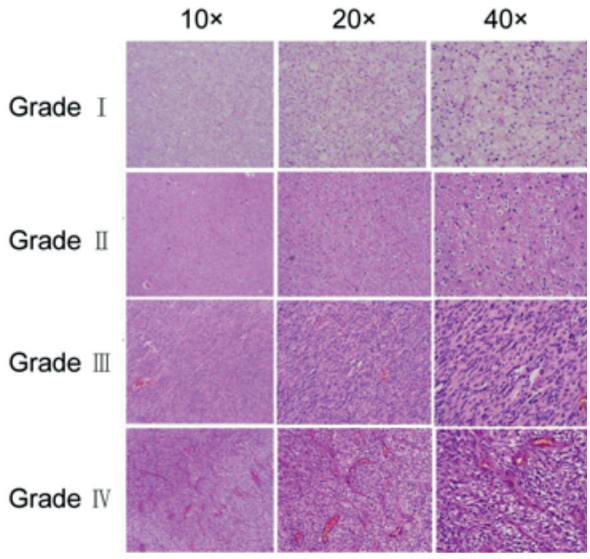

Figure 1. Patient-specific extent and eosinophils.

Expression Levels of BMP7 and Laminin in Astrocytoma Tissue

The expression patterns and cellular localization of BMP7 and laminin in 65 astrocytoma tissues in four differentiation levels were assessed by immunofluorescence analysis. The BMP7 immunoreactivity was predominantly localized in the nuclei of astrocytoma cells, while laminin immunoreactivity was predominantly localized in the member of astrocytoma cells (Table III and Figure 2A-B). The BMP7 level in the poor differentiation group was significantly higher than that in the high differentiation group (p<0.01) (Table III and Figure 2). Laminin expression level was significantly lower in the poor differentiation group compared with that in the high differentiation group (p<0.01) (Table III and Figure 3). Our results showed that BMP7 and laminin the expression levels were linked to the astrocytoma differentiation (p<0.05).

Discussion

Compared with higher-grade astrocytoma patients, BMP7 expression level in lower grade astrocytoma patients was higher. Lower level of laminin protein expression was detected in astrocytoma cells. It appears that eosinophils may play a protective role in astrocytoma differentiation. Our data suggested that circulating eosinophils might contribute to the progress of astrocytoma. To our knowledge, this is the first

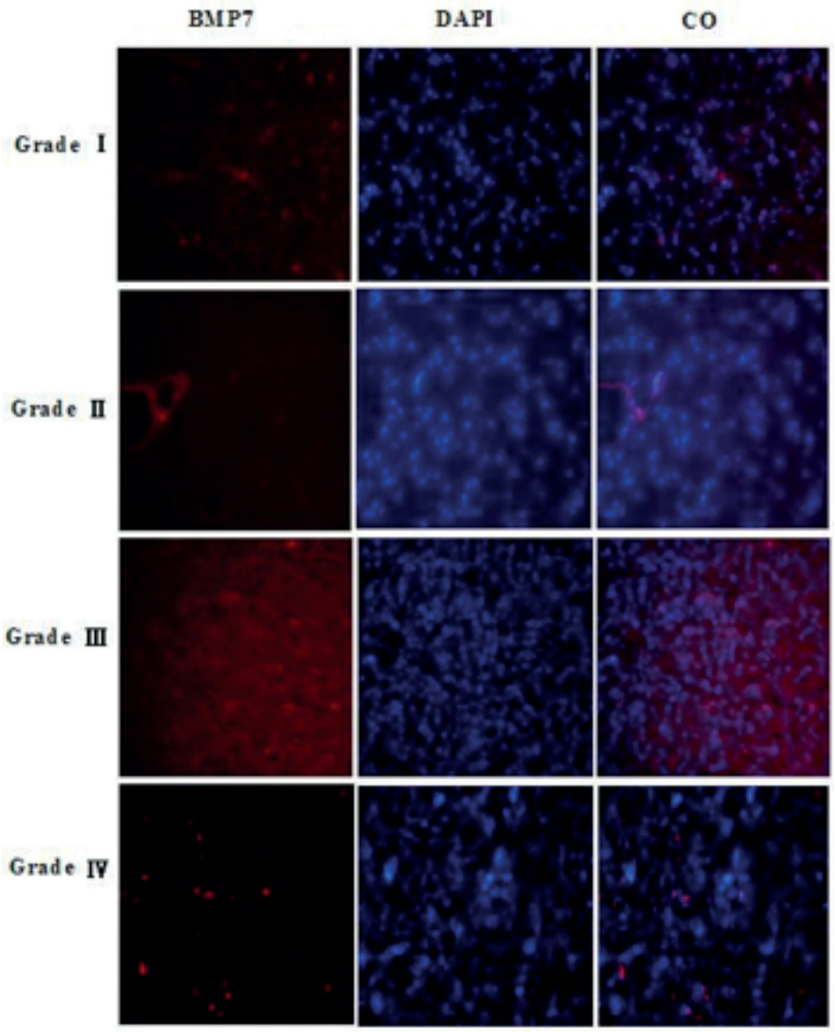

Figure 2. Expression of BMP7 in astrocytoma tissue.

report to show that eosinophil counts can be an independent prediction factor in astrocytoma patients. Why did eosinophils counts decrease in astrocytoma patients? We argued that it might be due to apoptosis or migration into tissues during the progress of astrocytoma. Our hypothesis was supported by developing evidence from both human and animal studies. Results obtained from such studies suggested that eosinophils are participating in tissue remodeling[16]. Our results suggested that the absence of laminin's anchoring property might lead to astrocytoma proliferation and differentiation. Our data supported those results obtained from prior studies[7-11]. ECMs and microenvironment can alter cell-to-cell interaction and lead to GICs invasion and metastasis[17]. GICs are sensitive to developmental signaling pathways such as the Bone Morphogenetic Protein (BMP) pathways[1]. Our data showed that in lower grade astrocytoma patients, BMP7 protein expression was higher compared to that in higher-grade astrocytoma patients. More recent studies[18,19] showed that BMP7 expression was correlated with tumor progression and disease recurrence. Paradoxically, overexpression of BMP7 inhibited cell growth through G0 to G1 cell cycle arrest and induction of apoptosis[20].

Conclusions

We demonstrated that eosinophil counts were associated with the expression of laminin and BMP7 in astrocytoma differentiation. As it was reported previously[21-23], our results suggested that

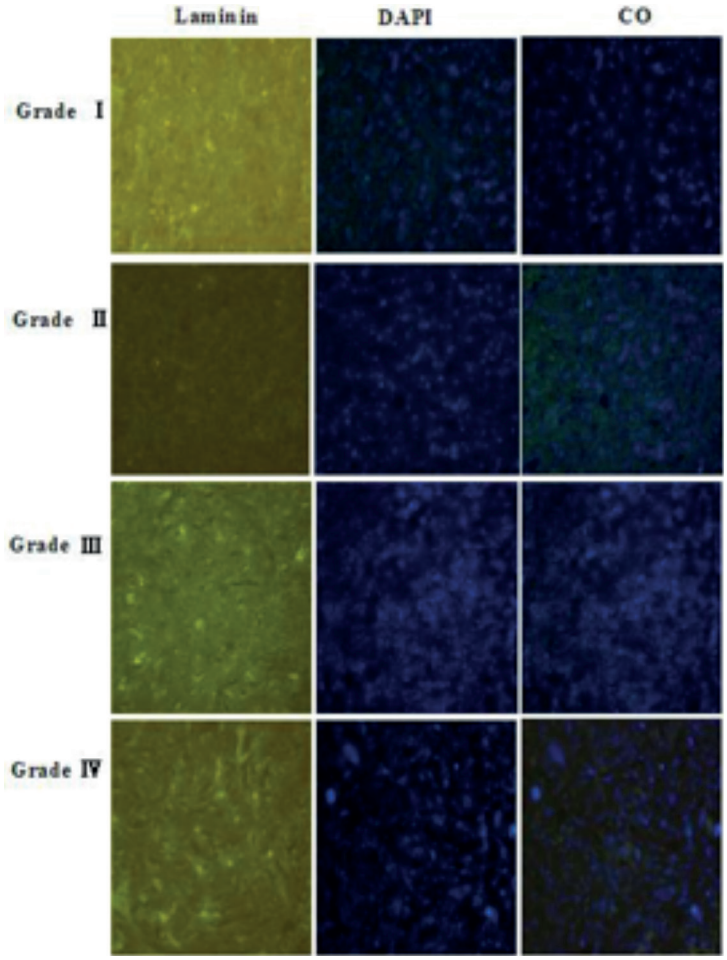

Figure 3. Expression of laminin on astrocytoma tissue.

biological treatment using targets such as BMP7 and laminin might induce secondary fibrosis in GICs. Also, eosinophil counts in peripheral blood might be a valuable index for predicting the progression of the disease. Further investigation is needed to bridge the gap between our current knowledge about laminin/BMP7 signaling in astrocytoma and its potential as a therapeutic target.

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Conflict of interest

The authors declare no conflicts of interest.

References

1) Lu HC, Ma J, Zhuang Z, Qiu F, Cheng HL, Shi JX. Exploring the regulatory role of isocitrate dehydrogenase mutant protein on glioma stem cell proliferation. Eur Rev Med Pharmacol Sci 2016; 20: 3378-3384.
2) Li HG, Chen JX, Xiong JH, Zhu JW. Myricetin exhibits anti-glioma potential by inducing mitochondrial-mediated apoptosis, cell cycle arrest, inhibition of cell migration and ROS generation. J Buon 2016; 21: 182-190.
3) Xu H, Zhang K, Zong H, Shang M, Li K, He X. Exosomal communication in glioma - a review. J Buon 2016; 21: 1368-1373.
4) Lee J, Kotliarova S, Kotliarov Y, Li A, Su Q, Donin NM, Pastorino S, Purow BW, Christopher N, Zhang W, Park JK, Fine HA. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 2006; 9: 391-403.

5) Guo E, Wang Z, Wang S. MiR-200c and miR-141 inhibit ZEB1 synergistically and suppress glioma cell growth and migration. Eur Rev Med Pharmacol Sci 2016; 20: 3385-3391.
6) Hu M, Polyak K. Microenvironmental regulation of cancer development. Curr Opin Genet Dev 2008; 18: 27-34.
7) Anderson AR, Weaver AM, Cummings PT, Quaranta V. Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment. Cell 2006; 127: 905-915.
8) Schafer ZT, Grassian AR, Song L, Jiang Z, Gerhart-Hines Z, Irie HY, Gao S, Puigserver P, Brugge JS. Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment. Nature 2009; 461: 109-113.
9) Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W, Yamauchi M, Gasser DL, Weaver VM. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 2009; 139: 891-906.
10) Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002; 2: 161-174.
11) Bissell MJ, Radisky D. Putting tumours in context. Nat Rev Cancer 2001; 1: 46-54.
12) Yurchenco PD, Patton BL. Developmental and pathogenic mechanisms of basement membrane assembly. Curr Pharm Des 2009; 15: 1277-1294.
13) Kadler KE, Hill A, Canty-Laird EG. Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. Curr Opin Cell Biol 2008; 20: 495-501.
14) Kleihues P, Louis DN, Scheithauer BW, Rorke LB, Reifenberger G, Burger PC, Cavenee WK. The WHO classification of tumors of the nervous system. J Neuropathol Exp Neurol 2002; 61: 215-225.
15) Zhang T, Fu J, Li Y, Wang Y, Zhang L, Liu Y. Bone morphogenetic protein (BMP)-7 is associated with the nodal invasion of colon cancer. Oncol Lett 2016; 11: 1707-1712.
16) Jia WZ, Zhao JC, Sun XL, Yao ZG, Wu HL, Xi ZQ. Additive anticancer effects of chrysin and low dose cisplatin in human malignant glioma cell (U87) proliferation and evaluation of the mechanistic pathway. J Buon 2015; 20: 1327-1336.
17) Akhavan A, Griffith OL, Soroceanu L, Leonoudakis D, Gloria Luciani-Torres M, Daemen A, Gray JW, Muschler JL. Loss of cell surface laminin anchoring promotes tumor growth and is associated with poor clinical outcomes. Cancer Res 2012; 72: 2578-2588.
18) Klose A, Waerzeggers Y, Monfared P, Vukicevic S, Kaijzel EL, Winkeler A, Wickenhauser C, Löwik CW, Jacobs AH. Imaging bone morphogeneticprotein 7 induced cell cycle arrestin experimental gliomas. Neoplasia 2011; 13: 276-285.
19) Tate CM, Pallini R, Ricci-Vitiani L, Dowless M, Shiyanova T, D'Alessandris GQ, Morgante L, Giannetti S, Larocca LM, di Martino S, Rowlinson SW, De Maria R, Stancato L. A BMP7 variant inhibits the tumorigenic potential of glioblastoma stem-like cells. Cell Death Differ 2012; 19: 1644-1654.
20) Rothhammer T, Wild PJ, Meyer S, Bataille F, Pauer A, Klinkhammer-Schalke M, Hein R, Hofstaedter F, Bosserhoff AK. Bone morphogenetic protein 7 (BMP7) expression is a potential noval prognostic marker for recurrence in patients with primary melanoma. Cancer Biomark 2007; 3: 111-117.
21) Klion AD. Eosinophilia: a pragmatic approach to diagnosis and treatment. Hematology Am Soc Hematol Educ Program 2015; 2015: 92-97.
22) Rosa H, Parise ER. Is there a place for serum laminin determination in patients with liver disease and cancer? World J Gastroenterol 2008; 14: 3628-3632.
23) Boon MR, van der Horst G, van der Pluijm G, Tamsma JT, Smit JW, Rensen PC. Bone morphogenetic protein 7: a broad-spectrum growth factor with multiple target therapeutic potency. Cytokine Growth Factor Rev 2011; 22: 221-229.