Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2024;49(3):600-606
DOI: 10.17826/cumj.1455861

RESEARCH

Immunohistochemical analysis of the immune checkpoint molecule Galectin-9 in meningiomas

Meningiomlarda bağışıklık kontrol noktası molekülü Galektin-9'un immünohistokimyasal analizi

İsmail Saygın[1], Emel Çakır[2], Seher Nazlı Kazaz[3], Ali Rıza Güvercin[1], İlker Eyüboğlu[1], Müşerref Müge Ustaoğlu[1]

[1]Karadeniz Technical University, Trabzon, Türkiye
[2]Sancaktepe Şehit Prof.Dr. İlhan Varank Training and Research Hospital, İstanbul, Türkiye
[3]İstinye University, İstanbul, Türkiye

Abstract

Purpose: Most meningiomas can be treated by surgical resection. However, depending on the location of the lesion, incomplete resection or high-grade meningiomas may have a poor prognosis. The new methods such as immunotherapy may improve our options for effective, patient-specific treatment of meningiomas. We aim to contribute to the development of new personalized treatment strategies by investigating the status of Gal-9 in meningiomas.

Materials and Methods: Four hundred two cases diagnosed in our laboratory between 2007 and 2020 were used for the study. New blocks of multiple tissues were prepared for immunohistochemistry using the tissue microarray method. Immunohistochemical staining of Gal-9 antibody was evaluated using the H-score method.

Results: Of the 402 cases studied, 289 were female and 113 were male. Two hundred and seventy-one (67.4%) cases were WHO grade 1; 121 (30.1%) were grade 2 and 10 (2.5%) were grade 3. A high H-score was observed in grade 1 and 2 tumors (H-score: 93.38 and 93.91) and a low H-score in grade 3 tumors (H-score: 59.40). There was no significant correlation between brain invasion and Gal-9 expression. No significant correlation was found between Gal-9 expression and minor criteria used in tumor grading.

Conclusion: A statistically significant difference was found between Gal-9 H-score and tumor grade. Gal-9 had a lower H-score in high-grade meningiomas and its expression level decreased. Therefore, Gal-9 with different expression levels can be used as a prognostic and predictive biomarker as well as an important molecule for treatment.

Öz

Amaç: Meningiom vakalarının çoğu cerrahi rezeksiyonla tedavi edilebilir. Ancak lezyonun lokalizasyonuna bağlı olarak tam çıkarılamayan veya yüksek dereceli meningiomların prognozu kötü olabilir. İmmünoterapi gibi yeni yöntemler, meningiomun hastaya özel, etkili tedavisine yönelik kapasitemizi artırabilir. Bu çalışmayla meningiomlarda Gal-9'un durumunu araştırarak kişiye özel yeni tedavi seçeneklerinin geliştirilmesine katkıda bulunmayı amaçlıyoruz.

Gereç ve Yöntem: Çalışmada 2007-2020 yılları arasında laboratuvarımızda tanısı konulan 402 olgu kullanıldı. İmmünhistokimya için doku mikroarray yöntemiyle birden fazla doku içeren yeni bloklar hazırlandı. Gal-9 antikorunun immünohistokimyasal boyanması H skoru yöntemi ile değerlendirildi.

Bulgular: Çalışmaya alınan 402 olgunun 289'u kadın, 113'ü erkekti. İki yüz yetmiş bir (%67,4) vaka WHO derece 1 idi; 121'i (%30,1) derece 2, 10'u (%2,5) derece 3 idi. Derece 1 ve 2 tümörlerde yüksek H skoru (sırasıyla 93,38 - 93,91), derece 3 tümörlerde ise düşük H skoru (59,40) gözlendi. Beyin invazyonu ile Gal-9 ekspresyonu arasında anlamlı bir ilişki yoktu. Gal-9 ekspresyonu ile tümör derecelendirmesinde kullanılan minör kriterler arasında anlamlı bir korelasyon bulunamadı.

Sonuç: Gal-9 H skoru ile tümör derecesi arasında istatistiksel olarak anlamlı fark gözlendi. Gal-9, yüksek dereceli meningiomlarda daha düşük bir H skoru gösterdi ve ekspresyon seviyeleri azaldı. Bu nedenle farklı ekspresyon seviyelerine sahip Gal-9, prognostik ve prediktif bir biyobelirteç olarak kullanılabileceği gibi tedavi için de önemli bir molekül olarak kullanılabileceği öngörülebilir.

Address for Correspondence: İsmail Saygın, Karadeniz Technical University, Faculty of Medicine, Department of Pathology, Trabzon, Türkiye E-mail: ismailsaygin@ktu.edu.tr
Received: 21.03.2024 Accepted: 02.06.2024

Keywords:. Meningioma, Galectin-9, immunotherapy, immune checkpoint molecules, H score

Anahtar kelimeler: Meningiom, Galektin-9, immünoterapi, immün kontrol noktası molekülleri, H skoru

INTRODUCTION

Meningiomas are the most common primary tumors of the brain[1]. They occur in middle-aged adults and affect more women than men. Some cases of meningiomas are treated surgically. However, high-grade meningiomas are particularly grow rapidly and often recur despite treatment, leading to a poor prognosis[2,3].

It is necessary to determine the subtypes of meningiomas and tumor grade according to the WHO in order to develop disease management and new targeted therapies[2]. Subjective histological examinations used to determine tumor grade in meningiomas may prevent accurate diagnosis in some cases. Misclassification and the inability to accurately determine the risk of recurrence can also prevent optimal treatment[2]. Therefore, tumor grading is very important. In 2021, some changes were made to the tumor grading of meningiomas. Tumor grading has been expanded to include molecular changes[4].

Complete removal of the tumor is the most effective method of treating meningiomas[5]. Depending on the location of the lesion, however, adjuvant radiotherapy is given in addition to surgical treatment in the event of incomplete resection in order to reduce recurrence and mortality in grade 2 and 3 meningiomas. Chemotherapy has no effect on meningiomas[6].

The new methods, such as immunotherapy, which is used for some other tumors, may expand our options for effective therapy, especially person-specific therapy. Studying immune cells in the tumor not only helps in diagnosis, but also in identifying biomarkers of tumor progression, understanding response to treatment or sensitivity of targeted therapeutics[2].

Immune checkpoint molecules (ICMs) are cell membrane proteins that regulate the immune response. Immune checkpoints are activated when these molecules on the surface of immune cells recognize and bind to partner molecules on tumor cells (Figure 1). The binding of checkpoint proteins and partner proteins can prevent the immune system from killing the tumor. Immune checkpoint inhibitors (ICI) block this mechanism[7,8].

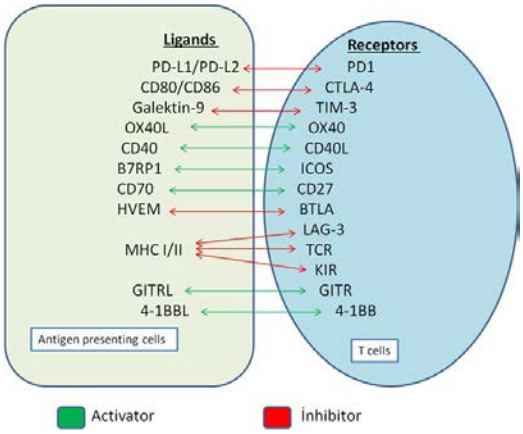

Figure 1. Immune checkpoint molecules.

Galectin-9 (Gal-9) was originally defined as a ligand for T cell immunoglobulin and mucin domain-containing molecule 3 (TIM -3)[9,10]. It regulates cell aggregation and adhesion. It also regulates apoptosis of tumour cells. Gal-9 plays an important role in the development of the adaptive immune system. Binding of Gal-9 to TIM -3 leads to apoptosis of T cells and negative regulation of the T cell response[11]. Gal-9 also binds to other immunomodulatory molecules[9,12]. Gal-9 also promotes the differentiation of regulatory T cells (Tregs) by binding to differentiation cluster 44 (CD44) and by binding to death receptor 3 to promote the expansion of Tregs[9,13,14].

Gal-9 specifically interacts with Tim-3 to induce apoptosis of CD8+ T cells[15]. Unlike other galectin members, it causes suppression of excessive immunity and inflammation[10,16]. It is a novel biomarker involved in both stimulation and inhibition of tumour growth depending on its interaction with receptors on T cells or tumour cells[17]. In addition, its concentration-dependent effects are also different. Gal-9 induces apoptosis of activated T cells at higher concentrations. On the other hand, at lower concentrations, it increases cytokine production by activated T cells[18].

Gal-9 stimulates apoptosis of malignant cells in many tumors, especially in hematological malignancies, gastrointestinal tumors and melanomas[19]. Gal-9 is highly expressed in some tumors such as oral,

pancreatic and colon cancer. On the other hand, it is expressed at low levels in breast, liver, lung, prostate, kidney cancer and melanoma[20,21]. Loss of Gal-9 in many solid tumors leads to tumor progression and an increased propensity for metastasis[22].

By investigating the Gal-9 expression status in meningiomas, we aim to contribute to the development of new personalized treatment methods. The aim of the study is to uncover the relationship between Gal-9 expression and the grade and prognosis of meningiomas and to determine the predictive value for a possible immunotherapeutic treatment.

MATERIALS AND METHODS

Study design and sample

This study is a retrospective study. The study was conducted in the Department of Pathology at Karadeniz Technical University. Our aim was to investigate the status of galectin-9 antibody in meningiomas.

Four hundred and two meningioma cases diagnosed in our laboratory between 2007 and 2020 were included in the study. All cases were re-evaluated by two neuropathologists and included in this study after the morphologic findings were confirmed. The clinical and radiologic data were obtained using the data processing unit of our hospital. The sample size was calculated using Openepi version 3.01. The total sample size was 370, with a confidence interval of 95, a power of 80%, a type 1 error margin of 0.05, a hazard ratio of 0.54 and a ratio of unexposed to exposed of 1.3.

Ethical approval was obtained from the Karadeniz Technical University Rectorate KTU Faculty of Medicine Scientific Research Ethics Committee Chairmanship with decision No. 2 dated 14.12.2020.

Preparation of the paraffin blocks

New blocks containing more than one tissue were prepared from the tumor-containing paraffin blocks of the cases using the tissue microarray method. For this purpose, 3 mm of tissue was taken from each block using a skin biopsy device, and new blocks containing tumor tissue from 9 cases were prepared. Sections were taken from these blocks for immunohistochemical staining.

Immunohistochemical antibody and staining

Immunohistochemical staining with Gal-9 antibody was performed using Ventana's BenchMark Ultra automatic staining device. The anti-Gal-9 100UL antibody (Abcam) was prepared at a dilution of 1/100. The slides stained with the antibody were analyzed using an Olympus BX51 light microscope.

Immunohistochemical evaluation

Gal-9 was scored using the H-score method. Cytoplasmic and nuclear membrane staining was evaluated. For the H-score method, each slide was examined by dividing it into four quadrants and the staining intensity and percentage of all slides were evaluated. The staining intensity was scored semi-quantitatively as 0 (no immunoreactivity), 1+ (weak immunoreactivity), 2++ (moderate immunoreactivity), 3+++ (strong immunoreactivity). The percentage of staining is the ratio between the number of stained cells and the total number of cells in the same area. By applying the H-score formula (3 x 3 percentage of positive cells + 2 x 2 percentage of positive cells + 1 x 1 percentage of positive cells + 0 x percentage of unstained cells), 4 separate H-scores (between 0 and 300) were determined for each case and the average was taken[23,24,25].

Statistical analysis

The statistical programme IBM SPSS Statistics for Windows Version 23.0 was used. Descriptive statistics of the evaluation results; numbers and percentages for categorical variables, mean, standard deviation, minimum and maximum for numerical variables. Kolmogorov-Smirnov or Shapiro-Wilk tests were used to test the agreement of the measured data with the normal distribution.

The T-test in independent groups was used for the measured variables that conformed to the normal distribution, and the Mann Whitney U-test or Kruskall Wallis analysis of variance was used for the measured variables that did not conform to the normal distribution.

The Dunn test and Bonferroni correction were performed on the Kruskal-Wallis test and post-hoc analysis. The Spearman correlation test was used for the data that did not correspond to the normal distribution in the correlation analysis of the measurement data. The chi-square test was used to

analyse the categorical data. All statistical analyses were performed in two ways. A significance value of $p<0.05$ was accepted for all statistical analyses.

RESULTS

Of the 402 cases in the study, 289 were female and 113 were male. 271 (67.4%) cases were WHO grade 1, 121 (30.1%) were grade 2 and 10 (2.5%) were grade 3. Brain invasion was observed in 28 (7.0%) cases. 336 (83.6%) were located in the supratentorium, 43 (10.7%) in the spinal cord and 23 (5.7%) in the posterior fossa. The distribution of cases by tumour type and grade is shown in Table 1. Hypercellularity was present in 40 (10%) cases, small nuclei in 57 (14.2%), macronucleoli in 11 (2.7%), pattern loss in 31 (7.7%), and spontaneous necrosis in 37 (9.2%) cases. Brain invasion was observed in a total of 28 (7.0%) cases. Lymphocytes were easily detected at 10x magnification in 300 of the cases, and in the remaining 102 cases they were found at 40x magnification.

Table 1. Distribution of cases according to histological type and tumor grade

Histological grade	Histological type	Number of cases	%
Grade 1	Transitional	85	21.1
	Fibrous	74	18.4
	Meningothelial	68	16.9
	Angiomatous	18	4.5
	Psammomatous	17	4.2
	Secretory	7	1.7
	Microcystic	4	1.0
	Metaplastic	3	0.7
Grade 2	Atypical	116	28.9
Grade 3	Anaplastic	10	2.5
Total		402	100.0

The comparison of Gal-9 expression with tumor grade and morphological parameters was performed by H-score. The Gal-9 expressions in different grades of meningiomas are shown in Figure 2. The cases were first compared according to the mean H-score (Table 2). A statistically significant difference was found between Gal-9 H-score and tumor grade (p-value 0.042).

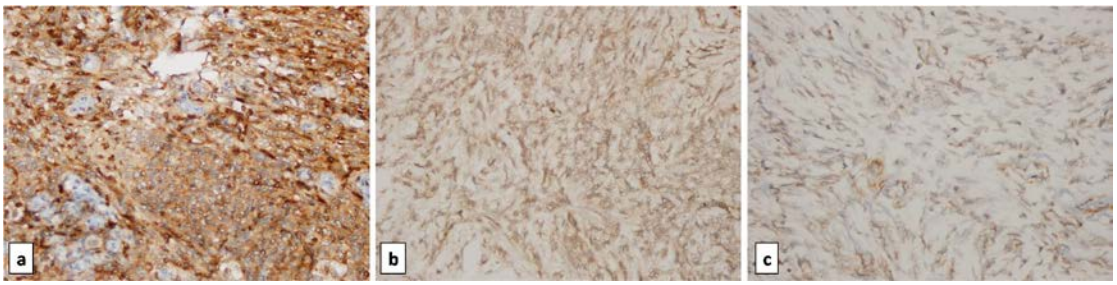

Figure 2. Galectin-9 x 400 (a:Grade 1, b:Grade 2, c: Grade 3).

There was no significant correlation between brain invasion and Gal-9 expression (p-value 0.395). No significant correlation was found between the minor criteria used in tumor grading (small nucleus, hypercellularity, macronucleolus, spontaneous necrosis, pattern loss) and Gal-9 expression (Table 2). There was no statistically significant correlation between Gal-9 and tumor diameter, age and gender of patients (p-values 0.602 - 0.099 - 0.548, respectively).

Table 2. Comparison of the mean H score of Galectin-9 with tumor grade and morphological parameters

Parameter		Number of cases	Galectin-9 H score					
			Mean	Standard deviation	Median	Percentile 25	Percentile 75	p value
Hypercellularity*	No	362	92.36	42.64	95	70	117	0.548
	Yes	40	95.75	38.18	99	81.5	110	
Small nucleus*	No	345	92.99	44.20	95	70	120	0.640
	Yes	57	90.89	27.10	94	72	105	
Macronukleolus*	No	391	92.13	41.43	95	70	115	0.154
	Yes	11	113.00	62.91	120	60	177	
Pattern loss*	No	371	92.28	42.43	98	81	124	0.387
	Yes	31	97.65	39.37	95	70	115	
Spontaneous Necrosis*	No	365	93.03	42.37	95	70	114	0.847
	Yes	37	89.38	40.69	100	60	120	
Brain invasion*	No	374	93.37	42.56	95	70	118	0.395
	Yes	28	83.71	36.22	99	57.5	108	
Grade**	Grade1[a]	271	93.38	43.68	95	70	120	0.042
	Grade2[a]	121	93.91	38.23	96	70	115	
	Grade3[b]	10	59.40	35.72	62.50	27	90	

[a,b]There is a significant difference between the different letters for the tumor grade.; *Mann-Whitney U Test.; ** Dunn Test and Bonferroni correction were performed in Kruskal-Wallis Test and Post hoc analysis.

DISCUSSION

High-grade or subtotally resected meningiomas have a relatively high recurrence rate. However, there are few biomarkers for follow-up, treatment decision and progression of meningiomas. There are studies in the literature that show that monitoring changes in Gal-9 levels and using them as biomarkers in clinical practice will be an important tool for monitoring disease activity and facilitating personalized treatment decisions[3].

In the study by Jafari et al, Gal-9 was shown to induce apoptosis in ovarian cancer cells[26]. In the study by Nobumoto et al., Gal-9 was shown to prevent metastasis of melanoma and colon cancer in mouse models by suppressing extravasation and adhesion of circulating tumor cells[27]. There are studies showing that Gal-9 increases antitumor immunity and prolongs survival in mouse models[28,29].

There is no study in the literature on Gal-9 in meningiomas. However, there are studies on other solid tumors and gliomas. In the study by Liu et al. the expression of Gal-9 was significantly low in normal brain, while increased expression was found in gliomas. In this study, Gal-9 expression correlated with glioma grade and was significantly increased in high-grade gliomas (grade 4) compared to low-grade gliomas (grade 2-3). In addition to Gal-9, the expression of TIM-3 is also increased[11].

It has been reported in the literature that as Gal-9 expression decreases in many solid tumors, tumor progression and its propensity to metastasize increase[22]. In our study, a high H-score was observed in grade 1 and 2 tumors and a low H-score in grade 3 tumors. In meningiomas, high Gal-9 expression was observed in grade 1 and 2 tumors and low expression in grade 3 tumors. In contrast to gliomas, Gal-9 expression decreased with increasing tumor grade[11]. The result of our study, the decrease in Gal-9 expression (low H-score) in high-grade meningiomas, is consistent with the literature.

On the other hand, the study by Zhou et al. reported that high Gal-9 expression was associated with longer survival and good prognosis in hepatocellular, colorectal, gastric and non-small cell lung cancers. High expression of Gal-9 has also been associated with lower tumor invasion depth and earlier tumor staging[30]. In our study, high Gal-9 expression was found in low-grade meningiomas. Loss of Gal-9 expression in many solid tumors such as breast, liver, lung, prostate, kidney cancer and melanoma indicates tumor progression and increased propensity to metastasis[20,21,30]. Our study suggests that low Gal-9

expression in grade 3 meningiomas is associated with poor prognosis.

The expression and function of Gal-9 varies considerably in different types of cancer. Detection of Gal-9 expression in a tumor provides clues for treatment and prognosis. It can be predicted that tumors in which high Gal-9 expression is associated with poor prognosis and high severity may benefit from targeted Gal-9 therapy[18]. For tumors in which low Gal-9 expression is associated with poor prognosis and high severity, induction of Gal-9 expression may support tumor treatment[18].

Induction of Gal-9 expression in meningiomas with low Gal-9 expression, as in our study, may be beneficial for treatment. In addition, low Gal-9 expression can be used as a non-invasive biomarker that can be easily measured by immunohistochemistry along with other parameters as an indicator of high-grade and aggressive course in meningiomas.

The study has several limitations. It was a retrospective study involving a single center. The second limitation of the study is the small diameter of the tumor tissue in which the immunohistochemical examination was performed (3 mm in diameter). Despite these limitations, the results of our study shed new light on the treatment of meningiomas.

In our study, Gal-9 showed a lower H-score and expression level in high-grade meningiomas. These results suggest that Gal-9, an immune checkpoint molecule with different expression levels, can be used as a prognostic and predictive biomarker and may also be an important tool for treatment.

In conclusion, understanding the status of immune checkpoint molecules, accurate grading, careful selection of patients for appropriate treatment, and increasing clinical experience with immune checkpoint blockers may lead to the discovery of effective treatments for patients with aggressive meningiomas. In addition, clinical trials can be conducted in selected suitable patients with an immunotherapeutic agent that may be developed in the future.

Author Contributions: Concept/Design : İS, EC; Data acquisition: İS, SNK, İE; Data analysis and interpretation: İS, ARG, SNK; Drafting manuscript: İS, EC; Critical revision of manuscript: İS, EC, İE; Final approval and accountability: İS, EC, SNK, ARG, İE, MMU; Technical or material support: İS, EC, SNK; Supervision: MMU, ARG; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained from the Karadeniz Technical University Rectorate KTU Faculty of Medicine Scientific Research Ethics Committee Chairmanship with decision No. 2 dated 14.12.2020.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: The authors have declared that they have received financial support
Thanks: They thank the Scientific and Technological Research Council of Turkey (TUBITAK) for their financial support to the Scientific Research Project Financing (TUBITAK, Project no: 220S403).

REFERENCES

1. Ostrom QT, Gittleman H, Fulop J, Liu M, Blanda R, Kromer C et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2008-2012. Neuro Oncol. 2015;17:iv1-62.
2. Al-Rashed M, Foshay K, Abedalthagafi M. Recent advances in meningioma immunogenetics. Front Oncol. 2020;9:1472.
3. Moar P, Tandon R. Gal-9 as a biomarker of disease severity. Cell Immunol. 2021;361:104287.
4. Torp SH, Solheim O, Skjulsvik AJ. The WHO 2021 Classification of central nervous system tumours: a practical update on what neurosurgeons need to know-a minireview. Acta Neurochir (Wien). 2022;164:2453-64.
5. Gupta S, Bi WL, Dunn IF. Medical management of meningioma in the era of precision medicine. Neurosurg Focus. 2018;44:E3.
6. Shahbandi A, Shah DS, Hadley CC, Patel AJ. The role of pharmacotherapy in treatment of meningioma: a systematic review. Cancers. 2023;12;15:483.
7. Foy SP. Mandl SJ. Dela Cruz T et al. Poxvirus-based active immunotherapy synergizes with CTLA-4 blockade to increase survival in a murine tumor model by improving the magnitude and quality of cytotoxic T cells. Cancer Immunol Immunother. 2016;65:537-49.
8. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T et al. Check point blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature. 2014;515:577-81.
9. Yang R, Sun L, Li CF, Wang YH, Xia W, Liu B et al. Development and characterization of anti-gal-9 antibodies that protect T cells from galectin-9-induced cell death. J Biol Chem. 2022;298:101821.
10. Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ et al. The Tim-3 ligand gal-9 negatively regulates T helper type 1 immunity. Nat Immunol. 2005;6:1245-52.
11. Liu Z, Han H, He X, Li S, Wu C, Yu C et al. Expression of the gal-9 Tim-3 pathway in glioma tissues is associated with the clinical manifestations of glioma. Oncol Lett. 2016;11:1829-34.
12. Daley D, Mani VR, Mohan N, Akkad N, Ochi A, Heindel DW et al. Dectin 1 activation on macrophages by gal-9 promotes pancreatic carcinoma

and peritumoral immune tolerance. Nat Med. 2017;23:556-67.
13. Wu C, Thalhamer T, Franca RF, Xiao S, Wang C, Hotta C et al. Gal-9 - CD44 interaction enhances stability and function of adaptive regulatory T cells. Immunity. 2014;41:270-82.
14. Madireddi S, Eun SY, Mehta AK, Birta A, Zajonc DM, Niki T et al. Regulatory t cell-mediated suppression of inflammation induced by DR3 signaling is dependent on Gal-9. J Immunol. 2017;199:2721-28.
15. Wang Y, Song L, Sun J, Sui Y, Li D, Li G, et al. Expression of Galectin-9 and correlation with disease activity and vascular endothelial growth factor in rheumatoid arthritis. Clin Exp Rheumatol. 2020;38:654-61.
16. Seki M, Oomizu S, Sakata KM, Sakata A, Arikawa T, Watanabe K et al. Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis. Clin Immunol. 2008;127:78e88.
17. Chou FC, Chen HY, Kuo CC, Sytwu HK. Role of galectins in tumors and in clinical immunotherapy. Int J Mol Sci. 2018;19:430.
18. Lv Y, Ma X, Ma Y, Du Y, Feng J. A new emerging target in cancer immunotherapy: Galectin-9 (LGALS9). Genes Dis. 2022 Jun 4;10:2366-82.
19. Fujita K, Iwama H, Oura K, Tadokoro T, Samukawa E, Sakamoto T et al. Cancer therapy due to apoptosis: Gal-9. Int J Mol Sci. 2017;18:74.
20. Liu D, Zhu H, Li C. Galectins and galectin-mediated autophagy regulation: new insights into targeted cancer therapy. Biomark Res. 2023;11:22.
21. Wdowiak K, Francuz T, Gallego-Colon E, Ruiz-Agamez N, Kubeczko M, Grochoła I et al. Galectin targeted therapy in oncology: current knowledge and perspectives. Int J Mol Sci. 2018;19:210.
22. Fujihara S, Mori H, Kobara H, Rafiq K, Niki T, Hirashima M et al. Gal-9 in cancer therapy. Recent Pat Endocr Metab Immune Drug Discov. 2013;7:130-7.
23. McCarty KS Jr, Miller LS, Cox EB, Konrath J, McCarty KS Sr. Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch Pathol Lab Med. 1985;109:716-21.
24. Rakha EA, Soria D, Green AR, Lemetre C, Powe DG, Nolan CC, et al. Nottingham Prognostic Index Plus (NPI+): a modern clinical decision making tool in breast cancer. Br J Cancer. 2014;110:1688-97.
25. McCarty KS Jr, Szabo E, Flowers JL, Cox EB, Leight GS, Miller L, et al. Use of a monoclonal anti-estrogen receptor antibody in the immunohistochemical evaluation of human tumors. Cancer Res. 1986;46:4244-48.
26. Jafari SM, Nazri A, Shabani M, Balajam NZ, Aghaei M. Gal-9 induces apoptosis in OVCAR-3 ovarian cancer cell through mitochondrial pathway. Res Pharm Sci. 2018;13:557-65
27. Nobumoto A, Nagahara K, Oomizu S, Katoh S, Nishi N, Takeshita K et al. Gal-9 suppresses tumor metastasis by blocking adhesion to endothelium and extracellular matrices. Glycobiology. 2008;18:735-44.
28. Nagahara K, Arikawa T, Oomizu S, Kontani K, Nobumoto A, Tateno H et a. Gal-9 increases Tim-3+ dendritic cells and CD8+ T cells and enhances antitumor immunity via galectin-9-Tim-3 interactions. J Immunol. 2008;181:7660-69.
29. Nobumoto A, Oomizu S, Arikawa T, Katoh S, Nagahara K, Miyake M et al. Gal-9 expands unique macrophages exhibiting plasmacytoid dendritic cell-like phenotypes that activate NK cells in tumor-bearing mice. Clin Immunol. 2009;130:322-30.
30. Zhou X, Sun L, Jing D, Xu G, Zhang J, Lin L et al. Gal-9 expression predicts favorable clinical outcome in solid tumors: a systematic review and meta-analysis. Front Physiol. 2018;9:452.