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ORIGINAL RESEARCH

Roles Of EAAT1, DHFR, And Fetuin-A In The Pathogenesis, Progression And Prognosis Of Chondrosarcoma

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Aims: Chondrosarcoma (CS) is a high-morbidity, relatively common bone malignancy without well-established biomarkers. The proteins EAAT1, DHFR, and fetuin-A have been investigated in many cancers, but their specific relationship to CS has not been reported. The present study examined EAAT1, DHFR, and fetuin-A expression in CS and the clinicopathological significance of these proteins in CS pathogenesis, progression, and prognosis.

Methods: EAAT1, DHFR, and fetuin-A protein levels in 80 CS and 25 chondroma specimens were measured by immunohistochemistry and related to histological and clinical factors with chi-squared tests. Following univariate survival analysis, ROC curves calculation, and multivariate analysis.

Results: EAAT1, DHFR, and fetuin-A expression levels were significantly higher in the CS group than in the chondroma group ($p < 0.05$). Their immunopositivity rates were significantly greater in tissues with moderate or poor tumor differentiation, AJCC stage III or IV, Enneking stage II or III, and metastasis ($p<0.05$ or $p<0.01$ or $p<0.001$). Kaplan–Meier survival analysis showed significantly shorter survival in patients with moderately or poorly differentiated tumors, AJCC stage III or IV CS, Enneking stage II or III CS, metastasis, invasion, or EAAT1, DHFR, and fetuin-A immunopositivity ($p < 0.05$ or $p < 0.001$). Cox regression analysis showed that moderate or poor tumor differentiation, AJCC stage III or IV, Enneking stage II or III, metastasis, invasion, and EAAT1, DHFR, or fetuin-A immunopositivity correlated negatively with postoperative survival and positively with mortality ($p < 0.05$). The AUCs for EAAT1, DHFR, and fetuin-A were 0.654 (95% CI: 0.532–0.776, $p = 0.025$), 0.638 (95% CI: 0.519–0.756, $p = 0.039$), and 0.670 (95% CI: 0.556–0.784, $p = 0.011$), respectively.

Conclusion: EAAT1, DHFR, and fetuin-A may be important biomarkers of the pathogenesis and progression of CS and predictors of its prognosis.

Keywords: chondrosarcoma, chondroma, EAAT1, DHFR, fetuin-A, immunohistochemistry

Introduction

Chondrosarcoma (CS) is the second most common primary solid malignant tumor in bone after osteosarcoma. There are approximately three new cases per million people per year.[1] Due to its resistance to chemotherapy and radiotherapy, surgical resection remains the standard treatment of CS, and the prognosis for patients is generally poor.[2] Reliable biomarkers related to its pathogenesis, progression, and prognosis are critical to improving treatment possibilities, but have yet to be established for clinical practice.[3]

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It has been widely reported that the glutamatergic (Glu) system acts as a primary regulator in bone tissue.[4] After activating its cognate receptors, released glutamate is taken up into astrocytes and neurons by cell membrane excitatory amino acid transporters (EAATs), resulting in termination of the glutamatergic signal. Recently, it was demonstrated that non-neuronal glutamatergic transmission occurs outside the central nervous system.[5] Cancer cells, including melanoma, colorectal carcinoma, hepatocellular carcinoma, and prostate carcinoma, have been shown to be modulated by a transmission system in which glutamate acted as an intercellular signaling factor.[6] Moreover, alterations in glutamate transport were found in malignant cells; repression of GLT-1/EAAT2[7] or mis-localization of excitatory amino acid transporter 1 (EAAT1) prevented cancer cells from taking up glutamate. The expression of EAATs was also found to be inversely correlated with tumor grade and restoring the function of EAATs decreased cancer cell proliferation and induced apoptosis in glioma cell lines.[8] In addition, EAAT1 expression was observed in osteosarcoma cell line MG-63 and shown to play an important role in bone pathophysiology.[9] In bone tissue, initial evidence for the presence of the Glu system was based on the expression of EAAT1 after mechanical loading on rat osteocytes.[10] Functional molecules of the Glu system have also been identified in T leukemia cells, thyroid carcinoma, melanoma and several other cancers.[11] However, the role of glutamatergic signaling in CS development and progression is still not well understood.

Dihydrofolate reductase (DHFR) is a folate metabolism enzyme critical to the processes of DNA synthesis, repair, and methylation.[12] Altered cellular folate levels are associated with aberrant DNA repair and methylation, including elevated hypo- and hypermethylation of tumor suppressor gene promotors.[13] DHFR is a known target of chemotherapeutic agents such as methotrexate (MTX), which reduces DNA synthesis and cell proliferation rates in cancer cells. Increasing cellular expression of DHFR results in tumor resistance to MTX. Possible mechanisms underlying MTX resistance include DHFR mutations that decrease the affinity of DHFR protein to MTX, and reduced uptake of MTX due to impaired transport.[14] These events have been reported to play a role in carcinogenesis in the colon.[13] However, reported associations between DHFR and cancers are often conflicting. Several studies have revealed, in various diseases, that deletion of DHFR is associated with higher morbidity.[15,16] DHFR expression was found to affect breast cancer cell proliferation and MTX sensitivity.[17] On the contrary, Xu et al[16] reported that there was no correlation between breast cancer susceptibility and DHFR genotype. The discrepancy between these studies may be the result of differences in the studied populations and their exposure to diverse carcinogens. Regardless, DHFR has not been well studied in CS.

Fetuin-A is a 63-kDa glycoprotein that is synthesized mainly by the liver and secreted into the serum. Of its multiple functions,[18] the most widely accepted one is bone remodeling and inhibiting excess systemic ectopic calcification. The role of fetuin-A in tumor progression is that of mediator of tumor cell adhesion, which is an important step in tumor growth and development of metastases.[19] Moreover, activation of resident osteoclasts to break down bone enables tumor cell colonization. The mineral component of bone, hydroxyapatite, has a strong affinity for fetuin-A. Breast cancer[20] and multiple myeloma[21] are regarded as osteoclastic tumors because they are associated with greater osteoclast activity relative to osteoblast activity. Being a chemoattractant,[22] fetuin-A could play a role in attracting tumor cells to the bone metastatic niche. Indeed, prostate cancer cells that colonize the bone have been shown to synthesize and secrete ectopic fetuin-A,[23] and fetuin-A has been shown to promote breast cancer and lung carcinoma progression.[24,25] Additionally, Thompson et al[26] suggested that fetuin-A plays a role in tumorigenesis of head and neck cancer. To our knowledge, there are no reports about the role of fetuin-A in CS.

In the present study, we used immunohistochemistry that investigated the expression of EAAT1, DHFR, and fetuin-A in CS and chondroma tissues, a common benign bone tumor. The clinical and pathological significance of these proteins in CS and their roles in the pathogenesis, progression, and prognosis of this malignancy were analyzed.

Materials And Methods

Specimens And Clinical Data

This study was conducted with resected tumor specimens from 80 patients with CS and 25 patients with chondroma, collected between January 2011 and June 2015 at the Second and Third Xiangya Hospitals, Central South University in Changsha, China. Diagnoses were confirmed by histopathology. Of the 80 CS tissues, 73 tissues were conventional CS (91.2%), 1 tissue was clear cell CS (1.3%), 2 tissues were mesenchymal CS (2.5%), and 4 tissues were

dedifferentiated CS (5.0%). Tissues were formalin-fixed and paraffin-embedded using standard procedures.

Clinicopathological data collected included patient age and sex, extent of tumor differentiation, tumor size, American Joint Committee on Cancer (AJCC) and Enneking stages, and the presence of metastasis and/or invasion. Survival information for patients with CS was obtained by phone or email.

Ethics Statement

This study was approved by the Medical Ethics Committee of the Second Xiangya Hospital, Central South University. Written, informed consent was obtained from each participant or their legal custodian, in accordance with the Declaration of Helsinki.

Immunohistochemistry

Four-micrometer-thick sections were cut from paraffin-embedded tissues with a cryostat. EAAT1, DHFR, and fetuin-A were labeled with the EnVision™ detection kit (Dako Laboratories, Carpinteria, CA) according to the manufacturer's protocol. Briefly, sections were deparaffinized and endogenous peroxidase was blocked by incubation in 3% H_2O_2 in the dark for 15 mins. Following a 20-min sitting in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) at room temperature for 20 mins, the sections were incubated with rabbit anti-human EAAT1 [1:50], DHFR [1:50], or fetuin-A [1:50] antibodies (Boster Biological Technology Co., Ltd., California, USA) for 2 hrs and then rinsed with 1× phosphate buffered saline three times for 5 mins each. Sections were then incubated at room

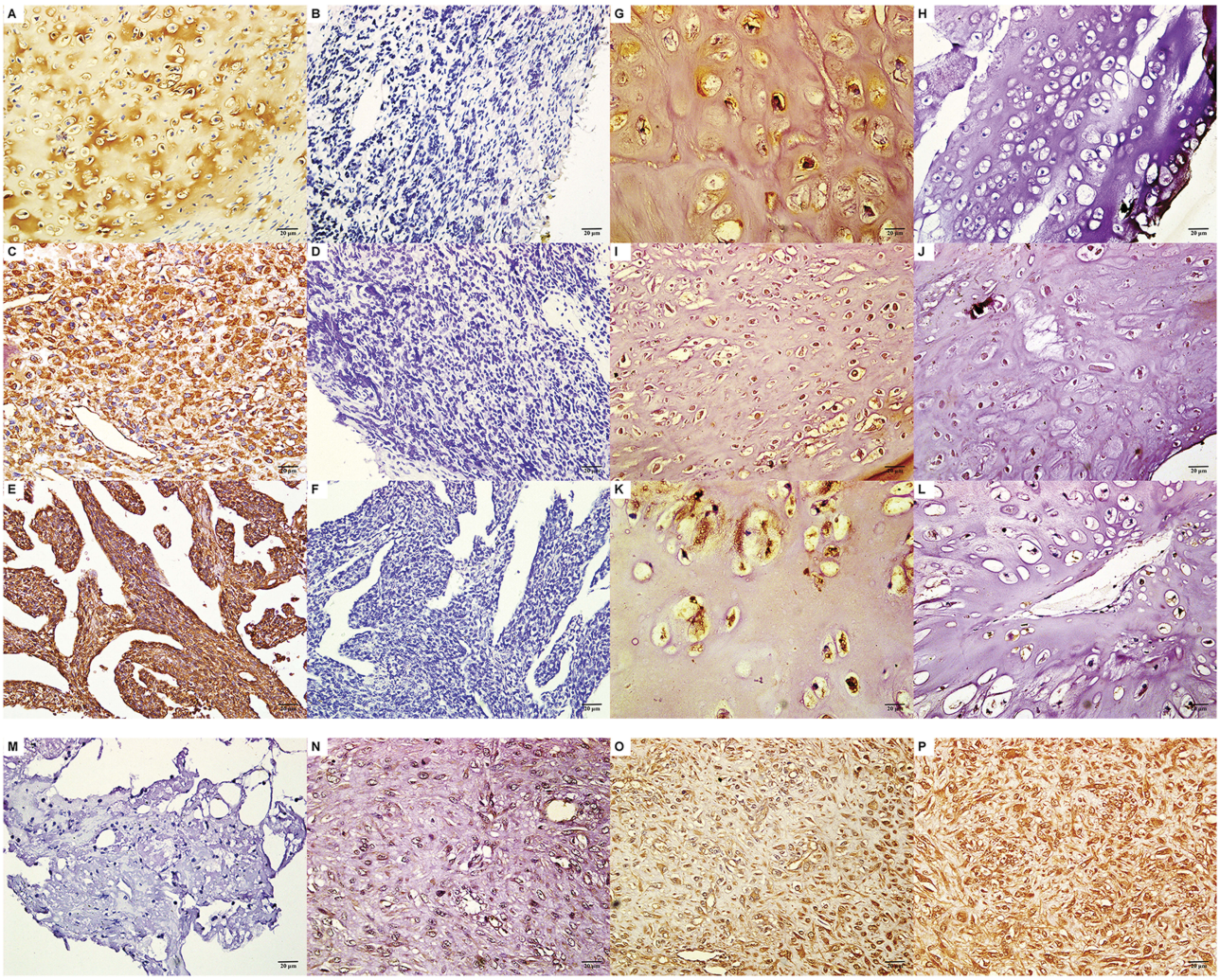


Figure 1 EAAT1, DHFR, and fetuin-A expression in chondrosarcoma (CS) and chondroma tissues. Representative patterns of EAAT1 expression in CS (positive [**A**] and negative [**B**]) and chondroma (positive [**G**] and negative [**H**]), DHFR expression in CS (positive [**C**] and negative [**D**]) and chondroma (positive [**I**] and negative [**J**]), and fetuin-A expression in CS (positive [**E**] and negative [**F**]) and chondroma (positive [**K**] and negative [**L**]) are shown (400×). Representative patterns of different staining intensities are shown: intensity 0 (**M**), intensity 1 (**N**), intensity 2 (**O**), intensity (**P**).

temperature for 30 mins in Solution A, which contained horseradish peroxidase-conjugated anti-rabbit secondary antibody (400 μg/mL) (Santa Cruz Biotechnology). After rinsing with 1× PBS three times, for 5 mins each, sections were stained with 3,3′-diaminobenzidine, counterstained with hematoxylin, dehydrated with alcohol, soaked in xylene, and mounted with neutral balsam. Positivity for EAAT1, DHFR, or fetuin-A was defined as ≥20% of cells with a staining intensity ≥2 or 10–20% of cells with a staining intensity of 3. The staining intensity was graded as follows: 0, no staining; 1, yellow; 2, brownish yellow; and 3, brown.[27](Figure 1).

Statistical Analysis

Data were analyzed in the Statistical Package for the Social Sciences version 18.0 (SPSS Inc., Chicago, IL). Protein expression levels in CS and chondroma specimens and their relationships with histological and clinical factors were analyzed with chi-squared tests. Kaplan–Meier and log rank tests were used for univariate survival analysis, with calculation of receiver operating characteristic (ROC) curves and areas under the curves (AUCs). A Cox proportional-hazard model was used for multivariate analysis and determination of 95% confidence intervals (CIs). A *p*-value less than 0.05 was considered significant.

Results

EAAT1, DHFR, And Fetuin-A Protein Expression In CS And Chondroma Tissues

EAAT1, DHFR, and fetuin-A immunopositivity was located mainly in the cytoplasm and/or plasma membrane and/or nuclear of tumor cells (Figure 1). EAAT1, DHFR, and fetuin-A immunopositivity was observed in 42/80 (52.5%), 38/80 (47.5%), and 40/80 (50.0%) CS specimens, respectively. EAAT1, DHFR, and fetuin-A immunopositivity was observed in 5/25 (20.0%), 5/25 (20.0%), and 4/25 (16.0%) chondroma specimens. For all three proteins, levels were significantly higher in CS than in chondroma specimens (EAAT1 $p = 0.004$, DHFR $p = 0.015$, fetuin-A $p = 0.003$; Table 1).

Table 1 EAAT1, DHFR, And Fetuin-A Expression In CS And Chondroma

Protein	CS		Chondroma		Chi-square	
	N	%	N	%	χ^2	*p*
EAAT1						
–	38	47.5	20	80.0	8.137	0.004
+	42	52.5	5	20.0		
DHFR						
–	42	52.5	20	80.0	5.957	0.015
+	38	47.5	5	20.0		
Fetuin-A						
–	40	50.0	21	84.0	9.045	0.003
+	40	50.0	4	16.0		

Abbreviations: CS, chondrosarcoma; EAAT1, excitatory amino acid transporter 1; DHFR, dihydrofolate reductase; N, number.

Association Of EAAT1, DHFR, And Fetuin-A Expression With CS Clinicopathological Features

EAAT1, DHFR, and fetuin-A expression in CS samples was not significantly associated with patient age, sex, or tumor size (Table 2). Immunopositivity percentages were higher in tissues with moderate or poor tumor differentiation, AJCC stage III or IV, Enneking stage II or III, and metastasis than in those with good differentiation, AJCC stage I or II, Enneking stage I, and no metastasis ($p<0.05$ or $p<0.01$ or $p<0.001$). In addition, EAAT1 immunopositivity was higher in CS tissues with invasion than in those without ($p = 0.020$).

Correlations Of EAAT1, DHFR, And Fetuin-A Expression In CS

Of the 42 CS samples positive for EAAT1, 30 were also DHFR-positive and 27 were fetuin-A-positive. Of the 38 tissues negative for EAAT1 expression, 30 were DHFR-negative and 25 were fetuin-A-negative. Of the 38 samples positive for DHFR expression, 28 were fetuin-A-positive. Of the 42 samples negative for DHFR expression, 30 were fetuin-A-negative. There was a significant positive correlation between the expression of EAAT1 and DHFR ($\chi^2 = 20.302$, $p < 0.001$), EAAT1 and fetuin-A ($\chi^2 = 7.218$, $p = 0.007$), and DHFR and fetuin-A ($\chi^2 = 16.241$, $p < 0.001$).

Correlations Of Clinicopathological Parameters, EAAT1, DHFR, And Fetuin-A Expression With The Mean Survival Of Patients With CS

In an 84-month follow-up period, 53/80 (66.3%) patients with CS died. Patients who had died of other causes or who had disenrollment or who were alive at the time of the last follow-up were censored. Kaplan–Meier survival analysis revealed significantly shorter survival in patients with moderately or poorly differentiated tumors, AJCC stage III or IV CS,

Table 2 Association Of EAAT1, DHFR, And Fetuin-A Expression With Clinicopathological Characteristics Of CS

Characteristic	N	EAAT1 Positive N (%)	χ^2	p	DHFR Positive N (%)	χ^2	p	Fetuin-A Positive N (%)	χ^2	p
Age, years										
<45	34	20 (58.8)	0.948	0.330	20 (58.8)	3.040	0.081	20 (58.8)	1.841	0.175
≥45	46	22 (47.8)			18 (39.1)			20 (43.5)		
Sex										
Male	43	22 (51.2)	0.067	0.796	21 (48.8)	0.067	0.796	23 (53.5)	0.453	0.501
Female	37	20 (54.1)			17 (45.9)			17 (45.9)		
Differentiation										
Well	58	24 (41.4)	10.484	0.005	21 (36.2)	10.841	0.004	21 (36.2)	16.347	<0.001
Moderate	12	10 (83.3)			9 (75.0)			11 (91.7)		
Poor	10	8 (80.0)			8 (80.0)			8 (80.0)		
Tumor size										
<5 cm	24	10 (41.7)	1.614	0.204	10 (41.7)	0.468	0.494	11 (45.8)	0.238	0.626
≥5 cm	56	32 (57.1)			28 (50.0)			29 (51.8)		
AJCC stage										
I	29	10 (34.5)	11.987	0.007	7 (24.1)	24.108	<0.001	5 (17.2)	24.812	<0.001
II	32	16 (50.0)			13 (40.6)			18 (56.3)		
III	8	6 (75.0)			8 (100)			7 (87.5)		
IV	11	10 (90.9)			10 (90.9)			10 (90.9)		
Enneking stage										
I	55	22 (40.0)	11.696	0.003	17 (30.9)	19.667	<0.001	17 (30.9)	25.685	<0.001
II	15	11 (73.3)			12 (80.0)			14 (93.3)		
III	10	9 (90.0)			9 (90.0)			9 (90.0)		
Metastasis										
No	69	33 (47.8)	4.396	0.036	28 (40.6)	9.637	0.002	30 (43.5)	8.538	0.003
Yes	11	9 (81.8)			10 (90.9)			10 (90.9)		
Invasion										
No	13	3 (23.1)	5.389	0.020	5 (38.5)	0.509	0.476	4 (30.8)	2.296	0.130
Yes	67	39 (58.2)			33 (49.3)			36 (53.7)		

Abbreviations: CS, chondrosarcoma; EAAT1, excitatory amino acid transporter 1; DHFR, Dihydrofolate reductase; N, number; AJCC, American Joint Committee on Cancer.

Enneking stage II or III CS, metastasis, invasion, or EAAT1, DHFR, and fetuin-A immunopositivity ($p < 0.05$ or $p < 0.001$). Mean survival was not associated with patient age, sex, or tumor size (Figure 2, Table 3).

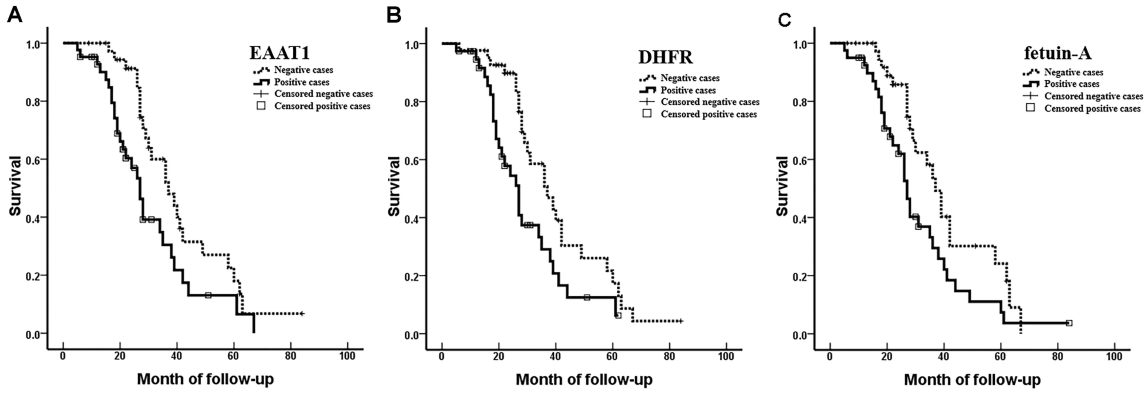


Figure 2 Associations of EAAT1, DHFR, and fetuin-A expression with survival, represented by Kaplan–Meier curves, in patients with CS. (**A**) EAAT1 expression (mean survival, positive 30.48 vs. negative 41.64 months; $p = 0.017$). (**B**) DHFR expression (mean survival, positive 29.77 vs. negative 40.56 months; $p = 0.015$). (**C**) Fetuin-A expression (mean survival, positive 31.07 vs. negative 40.66 months; $p = 0.022$).

Table 3 Relationship Of Clinicopathological Characteristics With Survival In Patients With CS

Characteristic	N	Mean Survival (Range), Months	χ^2	*p*
Age			0.278	0.598
<45	34	36.75 (5–67)		
≥45	46	34.74 (13–84)		
Sex			0.865	0.352
Male	43	38.65 (6–84)		
Female	37	33.77 (5–67)		
Differentiation			22.017	<0.001
Well	58	40.51 (6–84)		
Moderate	12	28.39 (12–42)		
Poor	10	20.50 (5–38)		
Tumor size			0.999	0.318
<5 cm	24	39.58 (12–62)		
≥5 cm	56	33.93 (5–84)		
AJCC stage			35.621	<0.001
I	29	49.47 (20–84)		
II	32	32.53 (6–67)		
III	8	17.20 (13–22)		
IV	11	25.40 (5–61)		
Enneking stage			17.776	<0.001
I	55	40.85 (6–84)		
II	15	23.83 (13–35)		
III	10	25.83 (5–61)		
Metastasis			5.459	0.019
No	69	37.69 (6–84)		
Yes	11	25.74 (5–61)		
Invasion			6.134	0.013
No	13	76.08(18–84)		
Yes	67	57.82(5–67)		
EAAT1			5.682	0.017
–	38	41.64 (16–84)		
+	42	30.48 (5–67)		
DHFR			5.942	0.015
–	42	40.56 (6–84)		
+	38	29.77 (5–62)		
Fetuin-A			5.212	0.022
–	40	40.66 (16–67)		
+	40	31.07 (5–84)		

Abbreviations: CS, chondrosarcoma; EAAT1, excitatory amino acid transporter 1; DHFR, dihydrofolate reductase; N, number; AJCC, American Joint Committee on Cancer.

Multivariate Analysis

Multivariate Cox regression analysis illustrated that moderate or poor tumor differentiation, AJCC stage III or IV, Enneking stage II or III, metastasis, invasion, or positive staining for EAAT1, DHFR, and fetuin-A correlated negatively with postoperative survival rate and positively with mortality. Expressions of EAAT1, DHFR, and fetuin-A were found to be independent predictors of CS ($p < 0.05$; Table 4). AUCs for EAAT1, DHFR, and fetuin-A were 0.654 (95% CI: 0.532–0.776, $p = 0.025$), 0.638 (95% CI: 0.519–0.756, $p = 0.039$), and 0.670 (95% CI: 0.556–0.784, $p = 0.011$), respectively (Figure 3).

Discussion And Conclusion

In the present study, EAAT1, DHFR, and fetuin-A expression levels revealed by immunohistochemistry were found to be higher in CS tumors than in chondromas, indicating that these proteins might play an important role in the pathogenesis of CS. These findings are consistent with other studies showing that EAAT1,[9] DHFR,[28] and fetuin-A[19] are over-expressed in malignant tissues. Moreover, expression of these proteins was associated with CS severity, progression, and poor prognosis. These findings suggest that EAAT1, DHFR, and fetuin-A may be useful biomarkers for this malignancy. Notably, our finding showing that EAAT1 expression was significantly higher in CS tissues with invasion than in those without suggests that EAAT1 may serve as a specific marker of invasive CS. We also detected significant positive correlations between the expression of EAAT1 and DHFR, EAAT1 and fetuin-A, and DHFR and fetuin-A, suggesting that these proteins may mutually regulate each other or that their expression is regulated through the same pathway. Finally, our AUC analyses indicated that EAAT1, DHFR, or fetuin-A immunopositivity are associated with a higher risk of CS.

The present EAAT1 data fit with prior studies,[8,9] implicating this protein in the pathogenesis, progression, and prognosis of CS. There are multiple mechanisms by which EAAT1 may come to be over-expressed in malignant tumors. First, the expression and activity of EAATs are modulated by caspase-3, which have been shown to be responsible for impairment of glutamate uptake in amyotrophic lateral sclerosis.[29] Second, the promoter that regulates EAAT expression has four binding sits for the transcription factor NF-κB.[30] NF-κB also regulates the expression of growth factors that are overexpressed in malignant tumors. Karki et al[31] demonstrated that upregulating EAAT1 may in turn activate NF-κB via ERK and PI3K/Akt signaling pathways. Finally, overexpression of the transcription factor Ying Yang 1, which can up- or down-regulate transcription depending on the cellular context, reduces EAAT1 mRNA levels and glutamate uptake

Table 4 Multivariate Cox Regression Analysis Of Clinicopathologic Characteristics With Overall Survival In Patients With CS

Factor	Compared Subgroups	*p*	Relative Risk	95% CI	
				Lower	Upper
Age, years	<45/≥45	0.451	1.269	0.683	2.357
Sex	Male/Female	0.919	1.033	0.558	1.911
Differentiation	I/II/III	0.001	2.581	1.491	4.467
Tumor size	<5 cm/≥5 cm	0.147	0.519	0.214	1.258
Metastasis	No/Yes	0.029	6.640	1.216	36.247
Invasion	No/Yes	0.026	3.274	1.150	9.324
AJCC stage	I/II/III/Ⅳ	0.008	3.414	1.372	8.494
Enneking stage	I/II/III	0.009	3.418	1.358	8.603
EAAT1	–/+	0.005	2.912	1.369	6.194
DHFR	–/+	0.020	2.732	1.174	6.358
Fetuin-A	–/+	0.029	2.241	1.087	4.619

Abbreviations: CS, chondrosarcoma; EAAT1, excitatory amino acid transporter 1; DHFR, dihydrofolate reductase; N, number; AJCC, American Joint Committee on Cancer.

activity.[32] Further studies are required to find out the individual contributions and significance of these pathways.

DHFR is important for DNA synthesis and repair because the product of its enzymatic action, tetrahydrofolate, is essential for de novo purine and thymidylate synthesis. Therefore, DHFR has become a target of interest for cancer drug development.[33] For example, MTX was approved by the US Food and Drug Administration in 1985, raltitrexed in 1998, pemetrexed in 2001, and pralatrexate in 2009. In agreement with our results, other studies have also described a role for DHFR as a potential biomarker in the development and progression of cancer.[34] Zaiwiah et al[35] found that the presence of DHFR predicted drug response and early relapse in colorectal cancer, and likely operated via the 5-fluorouracil pathway. Ovarian cancer cell resistance to cisplatin was found to be increased with higher expression of DHFR.[36] However, DHFR expression was found to be not related to breast cancer.[37] The above studies showed that the role of DHFR in cancers is complex, and the seemingly contradictory conclusions may be a result of cancer heterogeneity.

DHFR expression is regulated by multiple mechanisms, including gene amplification,[38] transcriptional upregulation,[39] and microRNA-mediated transcriptional repression.[40] The chemotherapeutic agent fluorouracil reduces the ability of nascent DHFR mRNA to relocate to the cytoplasm, probably due to inhibition of mRNA processing or transport.[41] A 19-bp deletion polymorphism (D-allele) in intron-1 of the DHFR gene leads to lowered levels of DHFR and reduced folate in the cell.[42] Given the complexity of DHFR regulation, further studies are required to understand its role in cancer progression.

Fetuin-A affects insulin resistance, which is associated with increased risk of cancer. The progression of several cancers, such as pancreatic cancer,[43] prostate cancer,[23] and glioblastoma multiforme,[44] is driven by synthesis of ectopic fetuin-A. Research has shown that a higher level of fetuin-A is associated with a modestly higher risk of colorectal cancer.[45]

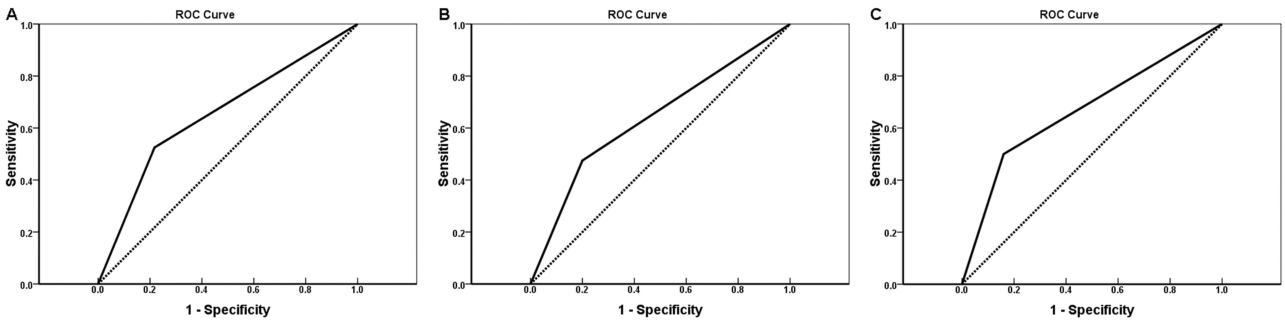


Figure 3 EAAT1, DHFR, and fetuin-A ROC curves for overall survival in CS patients (N = 80) from January 2011 to June 2015. AUCs for EAAT1 (**A**), DHFR (**B**), and fetuin-A (**C**) were 0.654, 0.638, and 0.670, respectively, indicating good predictive performance.

Fetuin-A has also been shown to be a major driver of cell proliferation both in vitro and in vivo.[46] Guillory et al[24] reported that a lack of murine fetuin-A delays the growth of breast cancer. Additionally, fetuin-A plays a role in cell attachment,[47] a key process in cancer growth and metastasis. All these findings are in consisteny with ours, indicating fetuin-A has an important role in pathogenesis, progression and prognosis of CS. However, contradictory results have also been reported. For instance, because fetuin-A is a competitive inhibitor of TGF-β, its increased expression in cancer is likely to neutralize TGF-β signaling, which has been shown to be stimulatory in advanced and metastatic tumors.[48] A study demonstrated that fetuin-A knockout mice developed more intestinal tumors, due to a lack of fetuin-A allowing TGF-β to drive tumorigenicity.[24] Therefore, the complex mechanisms by which fetuin-A promotes adhesion, motility, and cell proliferation required further study.[19,24,46]

It is noteworthy that the results of this study should be interpreted with caution since there are some limitations. They include its small sample size, its performance at only two academic medical centers and the lack of validation of the presented results in an independent patient cohort. However, we believe that the results of this study provide a useful reference for further research.

In conclusion, in the present study, EAAT1, DHFR, and fetuin-A were highly expressed in CS compared to chondroma. Over-expression of these proteins was observed in patients with moderate or poor differentiation, AJCC stage III or IV, Enneking stage II or III, and metastasis. Moreover, expression levels of EAAT1, DHFR, and fetuin-A were found to be associated with shorter patient survival. Demonstrated by AUC analyses, immunopositivity of these proteins is related to a higher risk of CS. Together these results indicate that EAAT1, DHFR, and fetuin-A proteins may serve as biomarkers of pathogenesis and progression, and predictors of prognosis, in CS.

Abbreviations

CS, chondrosarcoma; EAAT1, excitatory amino acid transporter 1; DHFR, Dihydrofolate reductase. AJCC, American Joint Committee on Cancer; ROC, receiver operating characteristic; AUC, areas under the curves.

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Disclosure

The authors report no conflicts of interest in this work.

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