MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 5044-5053
DOI: 10.12659/MSM.917382

Received: 2019.05.06
Accepted: 2019.06.03
Published: 2019.07.08

Overexpression of ClC-3 Chloride Channel in Chondrosarcoma: An *In Vivo* Immunohistochemical Study with Tissue Microarray

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1,2 Zhiqin Deng*
AG 2 Wencui Li*
E 2 Murad Alahdal
BC 2 Ningfeng Zhang
B 2 Junxiong Xie
B 2 Xiaotian Hu
B 2 Yang Chen
BF 2 Huankun Fang
A 2 Li Duan
A 1 Liqiang Gu
AG 2 Daping Wang

1 Department of Microsurgery and Orthopedic Trauma, The First Affiliated Hospital of Sun Yat-sen University, Sun Yat-sen University, Guangzhou, Guangdong, P.R. China
2 Guangdong Provincial Research Center for Artificial Intelligence and Digital Orthopedic Technology, Hand and Foot Surgery Department, Shenzhen Second People's Hospital (The First Hospital Affiliated to Shenzhen University), Shenzhen, Guangdong, P.R. China

* Zhiqin Deng and Wencui Li contributed equally to this work

Corresponding Author: Daping Wang, e-mail: dapingwang1963@qq.com

Source of support: This study was supported by the National Natural Science Foundation of China (81800785, 81272223, and 81273539), the China Postdoctoral Science Foundation (2018M633243), the Natural Science Foundation of Guangdong Province (2018A0303100027), the Sanming Project of Shenzhen Health and Family Planning Commission (SZSM201612086), Shenzhen Science and Technology Planning (JCYJ20180228163401333), and the Doctor Innovation Project of Shenzhen Health System (SZBC2018015)

Background: Recently, ClC-3 chloride channel expression has been noted to be high in some tumors. In chondrosarcoma, which is a malignant tumor with a high incidence in the bone, there has been no previous literature regarding ClC-3 chloride channel expression. Here we evaluated the expression of ClC-3 chloride channel in chondrosarcoma and explored its clinical significance.

Material/Methods: In this study, 75 chondrosarcoma and 5 normal cartilage tissues were collected. Thereafter, tissue microarray was performed. Immunohistochemistry was also used to observe the level of ClC-3 chloride channel expression between normal and chondrosarcoma tissues.

Results: Results showed that the expression of ClC-3 chloride channel in the normal chondrocyte was thinner, since it showed distinct differentiation among chondrosarcoma specimens. Interestingly, we noticed that the moderately-differentiated chondrosarcoma (MDC) and the poorly-differentiated chondrosarcoma (PDC) exhibited 94.44% of ClC-3 chloride channel. Besides, the subcellular localization of ClC-3 chloride channel was changed in association with malignant degree changes. The subcellular localization of ClC-3 chloride channel in the MDC and PDC tissue was localized in the cytoplasm and both nucleus and cytoplasm: 83.33% (5 out of 6 cases) and 91.66% (11 out of 12 cases) respectively. On the other hand, we noticed that patient age and gender could have a relation with ClC-3 chloride channel expression; 30- to 60-year-old males showed more expression.

Conclusions: These results demonstrated a high frequency of ClC-3 chloride channel overexpression and subcellular localization differences in MDC and PDC tissue, suggesting a specific role of ClC-3 chloride channel in the pathogenesis of chondrosarcoma.

MeSH Keywords: Chloride Channels • Chondrosarcoma • Immunohistochemistry • Tissue Array Analysis

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/917382

2465 4 3 40

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

The channel proteins of the ClC family are widely distributed in prokaryotes and eukaryotes. ClC family members are expressed in tissues and cells. It plays various and important roles in physiological functions, ranging from the control of cell volume to the regulation of information transfer between cells [1–3]. Loss of function of the ClC gene by gene mutation or other means can lead to a several congenital diseases [4]. Channel dysfunctions have been associated with Dent disease [5], osteosclerosis [4], Bartter syndrome [6], congenital epilepsy [7]. There is increasing evidence that ion channel dysfunction is also involved in cancers targeting ion channels, which is a promising strategy for the treatment of cancer [3,8,9]. ClC-3 is located on chromosome 4, 4q33. The ClC-3 chloride channel realizes anionic transport across the membrane in the form of dimers. The activation and inactivation of ClC-3 channels are regulated by various factors, such as anion concentration level, H^+ concentration level, calcium ion presence, osmotic pressure, energy, or anti-tumor drugs [8,10–12]. It is widely involved in the basic life processes [13–15].

Moreover, ClC-3 is a member of ClC voltage gated Cl^- channel gene super family [10]. However, the role of ClC-3 chloride channels as a component of volume-regulated anion channels (VRAC) are a controversial issue. A large amount of accumulated data indicates that ClC-3 is more highly expressed in cancer tissues such as glioma, nasopharyngeal carcinoma, breast cancer, and human cervical squamous cell carcinoma. It plays an important role in regulating cell migration and erosion, cell cycle and drug-induced apoptosis [2,8,14,16,17].

Chondrosarcoma is a chondrogenic bone tumor that can be classified into several types based on the presence of pathological lesions, preexisting lesions, and histological grading [18]. Most chondrosarcomas are primary, whereas secondary chondrosarcomas are caused by benign progenitor cell lesions such as osteochondromas [19]. Chondrosarcomas are usually a group of slow-growing, heterogeneous, primary bone malignancies characterized by the formation of hyaline cartilage tumor tissue. The prognosis of chondrosarcoma is related to the histologic grade and whether the tumor margin is removed completely. Most chondrosarcomas have a good prognosis, but once distant metastases, such as lung metastasis, occur, this is a red flag [19–21]. However, the expression of the ClC-3 chloride channel in chondrosarcoma has not been reported. To address this issue, we evaluated the immunoreactivity of the ClC-3 chloride channel in chondrosarcoma and explored its clinical significance.

Material and Methods

Patient specimens

Paraffin-embedded tissue microarrays (TMAs) used in the present study were purchased from Alenabio (Alenabio, Xian, China). The chondrosarcoma and normal cartilage tissue TMAs (cat. no. OS805, Table 1) contained 80 carcinoma samples, including 44 well-differentiated chondrosarcoma (WDC) cases, 6 moderately-differentiated chondrosarcoma (MDC) cases, 12 poorly-differentiated chondrosarcoma (PDC) cases, 4 dedifferentiation chondrosarcoma cases, 8 mesenchymal chondrosarcoma (MC) cases, 1 chondrosarcoma of degeneration (D-C) case and 5 normal cartilage tissue samples (C). One case of moderately-to-poorly differentiated osteosarcoma was classified as poorly-differentiated osteosarcoma for statistics conveniently. On the basis of morphology, the chondrosarcoma carcinoma samples were graded, according to the tumor-node-metastasis grading system [22] by the supplier, indicating well-, moderately- or poorly-differentiated tissue, respectively. In total, there were 80 tissue samples in the microarray, with 1 sample from each patient.

Immunohistochemistry

According to the previously described scheme, TMA was dewaxed with xylene, rehydrated with a graded alcohol series and antigen retrieval with 0.01 M sodium citrate buffer, in pH 6.0 at steady temperature. TMAs were then washed with phosphate-buffered saline (PBS, pH 7.4) 3 times and blocked with 3% H_2O_2 (v/v in PBS) for 15 minutes at room temperature. Glass slides were then incubated for 20 minutes with 2% normal goat serum (v/v in PBS) at room temperature to block the non-specific binding. Where after, the TMAs were incubated overnight at 4°C with a rabbit polyclonal antibody against to ClC-3 antibody 1: 250 dilution (Abcam, Cambridge, UK). Following several washes with PBS, the sections were incubated with secondary antibody (biotinylated goat anti-mouse/rabbit IgG, Ultra-Sensitive™ SP kit (CHEMBIO, China) for 10 minutes at room temperature. The sections were then washed with PBS and incubated with streptavidin-peroxidase for 10 minutes. Finally, DAB/ACE (DAB color development kit, CHEMBIO, China) developed color for 10 minutes and then we added distilled water to stop the color development. The equivalent procedure was conducted for the blank controls, with the primary antibody replaced by antibody diluent.

Image and data analysis graph

After immunohistochemical staining, images were captured with a DS-Ri2 digital camera (Nikon Corporation, Tokyo, Japan) mounted to a CX41 Nikon microscope (Nikon Corporation, Tokyo, Japan). The staining was scored according to the

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Clinicopathological information and expression of ClC-3 in the chondrosarcoma tissue microarray.

Pathological no.	Age (years)	Gender	Type	Tissue	Subcellular localization	AR
40054	24	M	WDC	Ribs	N/A	0
140010	54	F	WDC	Ribs	N/A	0
50114	46	F	WDC	Ribs	N/A	0
30121	47	M	WDC	Sacroiliac	N/A	0
30048	37	M	WDC	Ilium	N/A	0
40042	35	M	WDC	Ilium	N/A	0
70037	20	F	WDC	Ilium	N/A	0
30008	13	M	WDC	Ilium	Nucleus	1
140009	30	M	WDC	Ilium	Nucleus	1
40084	32	M	WDC	Ilium	N/A	0
30027	52	M	WDC	Ilium	Nucleus	1
30105	39	M	WDC	Ilium	N/A	0
140019	51	M	WDC	Ilium	Nucleus	1
40088	47	F	WDC	Ilium	Both	1
50021	28	F	WDC	Ilium	N/A	0
40014	50	M	WDC	Ilium	N/A	0
40071	42	F	WDC	Ilium	N/A	0
30067	47	F	WDC	Shoulder	N/A	0
70044	37	M	WDC	Shoulder	N/A	0
50100	16	M	WDC	Fibula	N/A	0
60046	37	M	WDC	Leg	N/A	0
150001	37	M	WDC	Leg	N/A	0
30091	22	M	WDC	Thigh	Both	1
50081	42	F	WDC	Thigh	N/A	0
40110	33	M	WDC	Thigh	N/A	0
40136	42	F	WDC	Pubic branch	N/A	0
40044	50	M	WDC	L3 centrum	Nucleus	1
20030	46	M	WDC	sternum	N/A	0
140021	46	M	WDC	Chest wall	N/A	0
90024	22	M	WDC	Chest wall	N/A	0
30132	40	M	WDC	Neck	Both	2
60043	29	M	WDC	Basalis	N/A	0
60085	53	F	WDC	Basalis	Cytoplasm	1
90002	39	M	WDC	Knee	Nucleus	2
40049	33	M	WDC	Lumbar part	N/A	0
30065	52	M	WDC	Sacroiliac	Cytoplasm	2
140027	42	M	WDC	Knee joint	N/A	0
40079	54	M	WDC	Hipbone	N/A	0
40111	34	M	WDC	Upper arm	N/A	0
40119	41	F	WDC	Upper arm	N/A	0
40093	70	M	WDC	Ankle	N/A	0

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1 continued. Clinicopathological information and expression of ClC-3 in the chondrosarcoma tissue microarray.

Pathological no.	Age (years)	Gender	Type	Tissue	Subcellular localization	AR
40089	49	F	WDC	Upper limb	N/A	0
30113	50	M	WDC	Thigh	Both	1
90005	39	M	WDC	Shoulder	Cytoplasm	1
30012	16	M	MDC	Tibia	Both	1
140031	60	M	MDC	Buttock	N/A	0
50120	52	M	MDC	Nasal vestibule	Cytoplasm	2
140022	38	M	MDC	Sacroiliac joint	Both	2
90026	52	M	MDC	Sacroiliac	Both	3
90023	47	F	MDC	Femur	Cytoplasm	1
120003	15	M	PDC	Knee	Both	3
60020	27	F	PDC	Femur	Cytoplasm	1
20032	48	F	PDC	Femur	Cytoplasm	1
70009	56	M	PDC	Thoracic vertebrae	Cytoplasm	3
140028	42	M	PDC	Thigh	Cytoplasm	3
40008	35	M	PDC	Femur	Cytoplasm	1
30093	13	F	PDC	Tibia	Both	3
30114	41	M	PDC	Femur	Nucleus	2
60053	20	M	PDC	Tibia	Both	1
30148	12	F	PDC	Femur	Both	1
50001	40	M	PDC	Femur	Both	1
40127	35	F	M-PDC	Pars sacralis	Both	3
40140	58	F	D-C	Shoulder	Nucleus	1
50094	38	M	DDC	Face	Both	1
40009	40	M	MC	First metatarsal	Both	1
60063	33	M	MC	Fifth ribs	Both	2
30140	28	M	DDC	Sole of foot	Cytoplasm	2
30038	38	M	DDC	Hip	Cytoplasm	1
10023	47	M	DDC	Pars sacralis	Cytoplasm	1
30088	30	M	MC	Leg	Cytoplasm	2
60072	46	F	MC	Sacrum	Nucleus	1
70001	84	F	MC	Sacrum	Cytoplasm	1
60099	65	M	MC	Sacrum	Cytoplasm	1
60093	46	F	MC	Sacrum	Cytoplasm	1
70015	18	M	MC	Sacrum	Cytoplasm	1
06N023	21	F	C	Cartilage	N/A	0
06N002	15	F	C	Cartilage	N/A	0
07N001	41	F	C	Cartilage	N/A	0
07N026	33	M	C	Cartilage	N/A	0
07N025	27	F	C	Cartilage	N/A	0

M – male; F – female; WDC – well-differentiated chondrosarcoma; MDC – moderately-differentiated chondrosarcoma; PDC – poorly-differentiated chondrosarcoma; MC – mesenchymal chondrosarcoma; DDC – dedifferentiation chondrosarcoma; C – normal cartilage tissue.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

previously described 4-point system (score 0–3) [23,24] by a pathologist (double-blinded) as follows: score 3, dark staining that is easily visible and present in >50% of cells; score 2, focal areas of dark staining (<50% of cells) or moderate staining of >50% of cells; score 1, focal moderate staining in <50% of cells or pale staining in any proportion of cells not easily observable at low power; and score 0, none of the aforementioned. A high level of expression was defined as a score of 2–3 and low level of expression was defined as a score of 0–1, as described previously. Considering the comparatively small sample size, an early tumor stage was defined as stages I and II, and an advanced stage was defined as stages III and IIIb. Well-differentiated carcinoma (WDC) was defined as grade 1, moderately-differentiated carcinoma (MDC) as grade 2 and poorly-differentiated carcinoma (PDC) was defined as grade 3 [24].

Statistical analysis

All data are expressed as n (%) and were compared using a χ^2 test. Fisher's exact test was used for correction. Statistical analysis was performed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Overexpression of ClC-3 chloride channel in moderately- and poorly- differentiated chondrosarcoma

Immunohistochemical analysis demonstrated that ClC-3 chloride channel expression in normal cartilage tissue was significantly thinner in normal chondrocyte and well-differentiated chondrosarcoma (WDC) (Figure 1A, 1B) in comparison to moderately- and poorly- differentiated chondrosarcoma that presented high expression of ClC-3 chloride channel. As shown in Table 2, among the 44 cases of WDC, only 29.55% exhibited low expression levels of ClC-3 chloride channel (Figure 1A, 1B). However, among the 18 cases of MDC and PDC, 94.44% exhibited high levels of ClC-3 chloride channel expression. Besides, the rate of grade >3 (high expression of ClC-3) was 0%, 16.66%, and 41.66% in well-, moderately- and poorly-differentiated chondrosarcoma, respectively (Figure 1C–1F). The statistical analysis demonstrated significant difference among the 3 groups (Table 2, $\chi^2=33.53$, $P<0.05$).

Subcellular localization of ClC-3 chloride channel immunohistochemical staining

The investigation of ClC-3 chloride channel subcellular localization in the chondrosarcoma tissues showed that among the 44 cases of WDC, which exhibited low expression levels of ClC-3 chloride channel expression, ClC-3 was noticed in the nucleus, (Figure 2A, 2B). In the 6 cases of MDC and 12 cases of PDC, ClC-3 chloride channel subcellular localization presented overexpression 83.33% (5 out of 6 cases) and 91.66% (11 out of 12 cases). It mainly localized in the cytoplasm and in both nucleus and cytoplasm, respectively (Figure 2C, 2D). Additionally, the immunohistochemical staining of mesenchymal chondrosarcoma (MC) tissues and dedifferentiation chondrosarcoma (DDC) tissues showed that subcellular localization of ClC-3 chloride channel also in the cytoplasm and in both nucleus and cytoplasm among the 6 and 4 cases, respectively (Figure 2E, 2F). We also noticed that ClC-3 chloride channel localization changes with the change of malignant degree, and showed significant difference among the groups (Table 3, $\chi^2=48.51$, $P<0.05$).

Age and gender associate differences in varying degrees of differentiated chondrosarcoma

The present study also investigated the effect of age on ClC-3 immunoreactivity in varying degrees of differentiated chondrosarcoma. The occurrence of chondrosarcoma diseases exhibits a certain degree of age bias, with a higher percentage detected in young patients. As shown in Figure 3, the mean age of the 75 patients was 38.8 years old. The ratio of age in the 75 chondrosarcoma tissues significantly indicated that 21.33%, (16 out of 75 samples) were from patients aged <30 years, 73.33% (55 out of 75 samples) were from patients 30≤ age <60 years, and 5.33% (4 out of 75 samples) were from patients age ≥60 years. The percentage of samples with ClC-3 chloride channel high expression level (>2) was 18.75% (3 out of 16 samples) from patients aged <30 years, 21.81% (12 out of 55 samples) in those 30≤ age <60 years, and 25.00% (1 out of 4 samples) in those age ≥60 years. However, chi-square statistical analysis for the 3 age groups demonstrated no significant difference among the 3 age groups (Table 4, $\chi^2=0.271$, $P>0.05$).

There were sex-associated differences in ClC-3 chloride channel in chondrosarcoma tissue. The occurrence of chondrosarcoma also exhibits a certain degree of sex bias, with a higher percentage detected in males. Thus, the present study compared the expression of ClC-3 chloride channel in various degrees of chondrosarcoma tissues from male and female. Among the 75 cases of chondrosarcoma tissue, the percentage of samples with high ClC-3 expression level (>2) was 9.09% (2 out of 22 cases) in females and 24.52% (13 out of 53 cases) in males. However, the chi-square statistical analysis of gender groups demonstrated no significant differences (Table 4, $\chi^2=2.32$, $P>0.05$).

Discussion

Primary chondrosarcoma is a malignant chondrogenic tumor characterized by the formation of cartilage matrix by tumor cells and the direct formation of nidus. Primary chondrosarcomas are

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

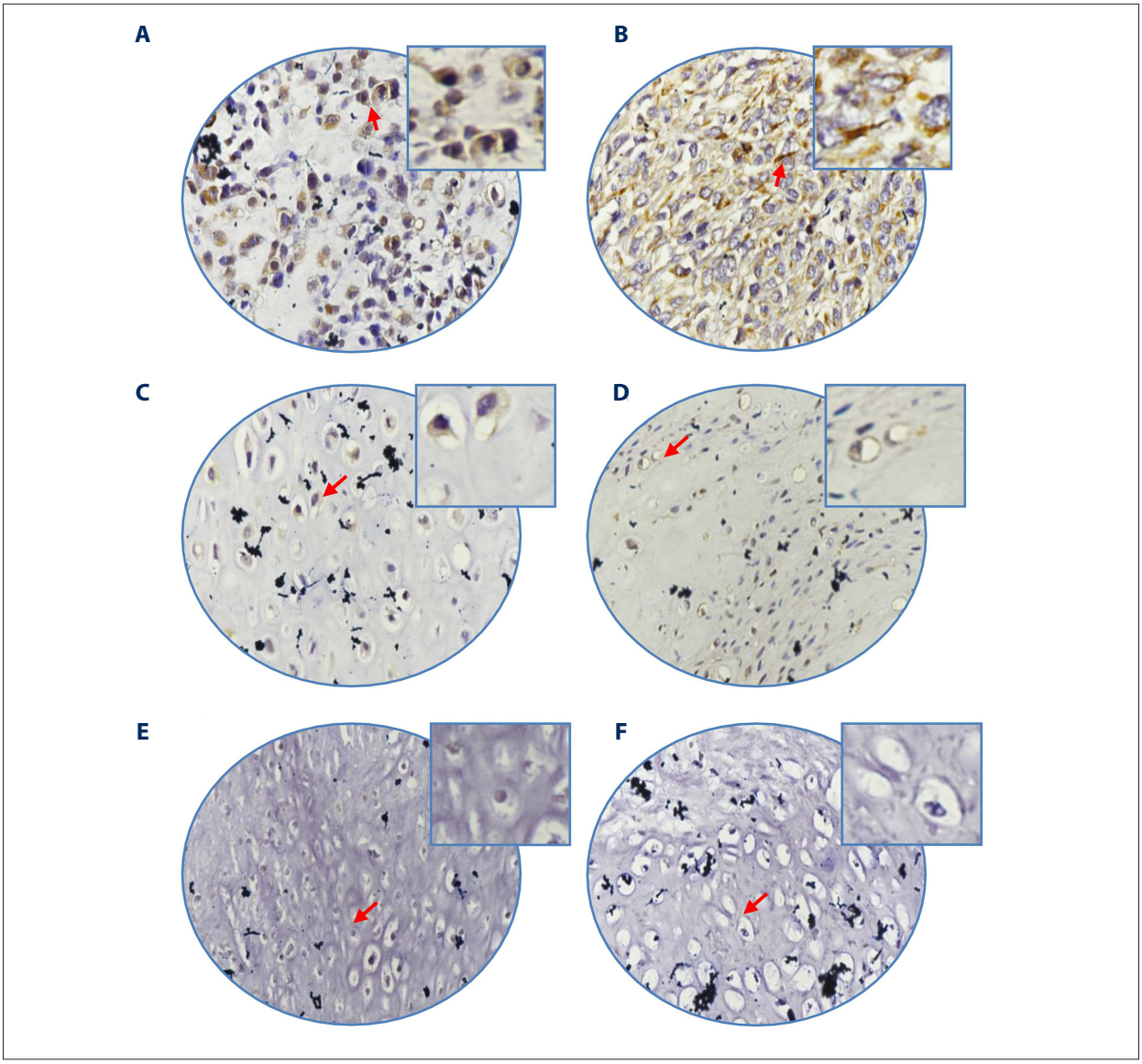

Figure 1. ClC-3 expression in different types of chondrosarcoma. Poorly-differentiated chondrosarcoma tissue from (**A**) a 15-year-old male and (**B**) a 56-year-old male in the left knee and the fourth thoracic vertebrae, respectively. Moderately-differentiated chondrosarcoma tissue from (**C**) a 38-year-old male and (**D**) a 52-year-old male in the left sacroiliac joint and sacroiliac, respectively. Well-differentiated chondrosarcoma tissue from (**E**) a 13-year-old male and (**F**) a 20-year-old male in the left ilium and right ilium, respectively.

Table 2. Statistics of ClC-3 expression in different types of chondrosarcoma.

Type	Expression level				Total	Rate of grade >3 (high expression of ClC-3) (%)	χ^2	*P*-value
	0	1	2	3				
WDC	31	10	3	0	44	0	33.53	0.00
MDC	1	2	2	1	6	16.66		
PDC	0	6	1	5	12	41.66		

WDC – well-differentiated chondrosarcoma; MDC – moderately-differentiated chondrosarcoma; PDC – poorly-differentiated chondrosarcoma.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

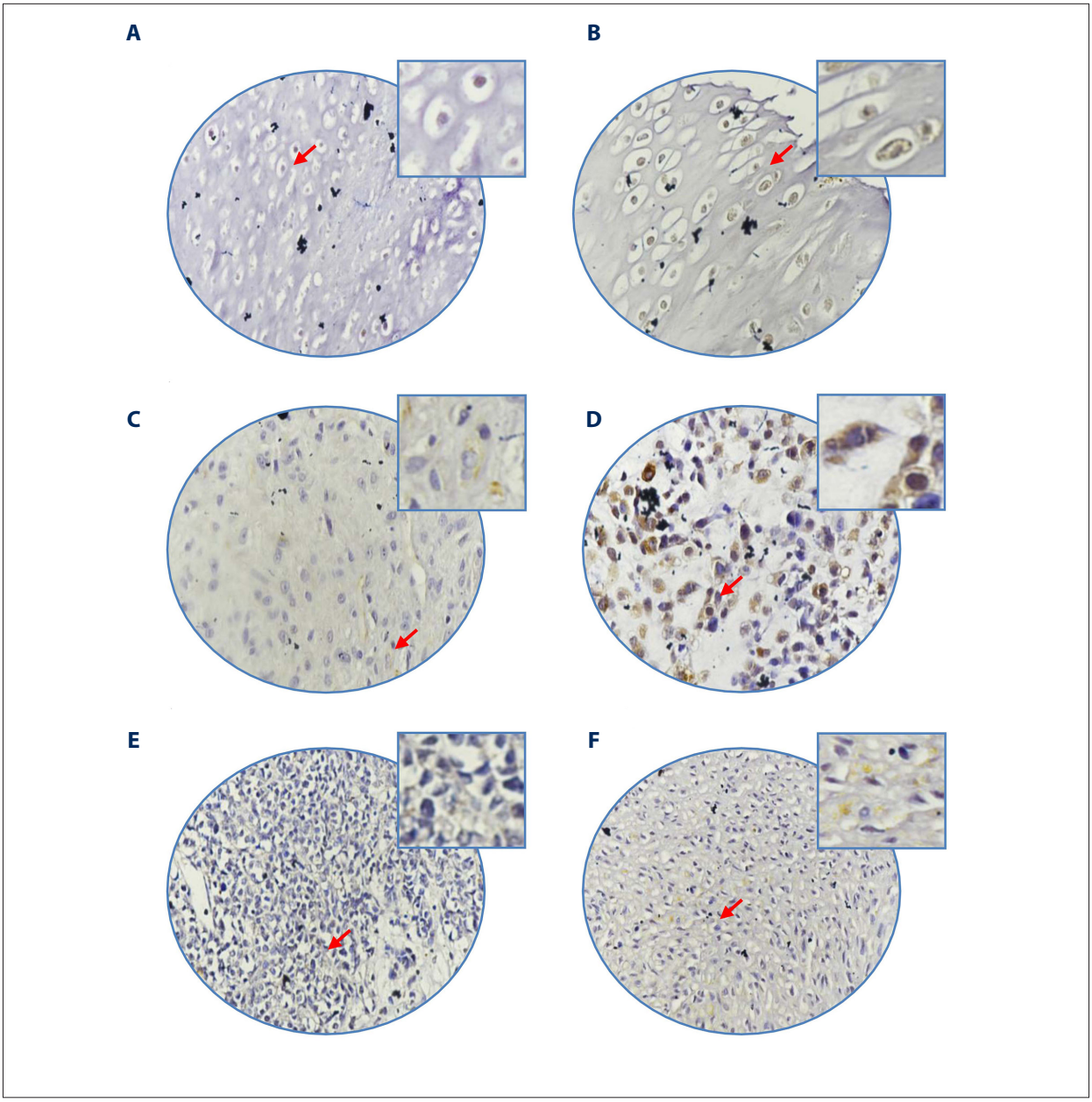

Figure 2. Subcellular localization of ClC-3 in different types of chondrosarcoma. The location of ClC-3 chloride channel is N/A of well-differentiated chondrosarcoma (**A**) and in the nucleus of well-differentiated chondrosarcoma (**B**). The location of ClC-3 chloride channel is in the cytoplasm and in both of the nucleus and cytoplasm in moderately-differentiated chondrosarcoma (**C**) and poorly-differentiated chondrosarcoma (**D**). The mesenchymal chondrosarcoma tissues (**E**) and dedifferentiation chondrosarcoma (**F**) tissues showed the subcellular localization of ClC-3 chloride channel was in the cytoplasm.

classified into 7 histological subtypes, including conventional intramedullary chondrosarcoma, clear cell chondrosarcoma, juxtacortical chondrosarcoma, myxoid chondrosarcoma, and dedifferentiated chondrosarcoma [25,26]. Chondrosarcoma is a common malignant bone tumor in the skeletal system, and its treatment is challenging for orthopedists. Since it is not sensitive to radiotherapy and chemotherapy, the only effective treatment at present is surgical excision. However, incomplete surgical resection or metastasis is associated with poor survival. Therefore, it is of great significance to study the risk factors of chondrosarcoma and more effective treatment methods for improving the survival rate of patients [26,27].

TMA studies of histopathological materials are often used to study malignant diseases. In this study, we reported for the first time the immunoreactivity of ClC-3 chloride

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. Statistics on subcellular localization of ClC-3 in different types of chondrosarcoma.

Type	Subcellular localization				Total	χ2	*P*-value
	N/A	Nucleus	Cytoplasm	Both			
WDC	31	6	3	4	44	48.51	0.00
MDC	1	0	2	3	6		
PDC	0	1	5	6	12		
MC	0	1	5	0	6		
DDC	0	0	3	1	4		

WDC – well-differentiated chondrosarcoma; MDC – moderately-differentiated chondrosarcoma; PDC – poorly-differentiated chondrosarcoma; MC – mesenchymal chondrosarcoma; DDC – dedifferentiation chondrosarcoma.

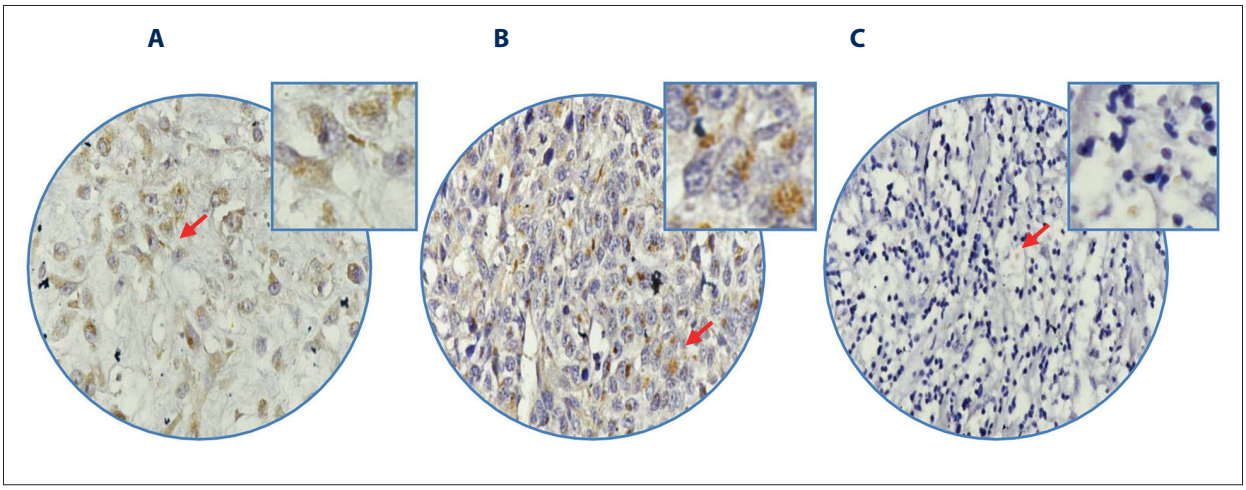

Figure 3. Age- and gender-associated differences in varying degrees of differentiated chondrosarcoma. (**A**) A 13-year-old female with left tibia of poorly-differentiated chondrosarcoma tissue. (**B**) A 42-year-old male with left thigh of poorly-differentiated chondrosarcoma tissue. (**C**) A 84-year-old female with sacrum of mesenchymal chondrosarcoma tissue.

Table 4. Statistics of ClC-3 expression in different types of chondrosarcoma.

	Type	Expression level		Total	χ^2	*P*-value
		Low	High			
Age (years)	Age <30	13	3	16	0.271	1.00
	30≤ age <60	43	12	55		
	Age ≥60	7	1	4		
Gender	Female	20	2	22	2.32	0.128
	Male	40	13	53		

channel in TMA-based chondrosarcoma. Among the 18 cases of moderately- and poorly-differentiated chondrosarcoma (MDC and PDC), 94.44% exhibited high levels of ClC-3 chloride channel expression. With the increase of tumor malignancy (from high differentiation to low differentiation), the expression level of ClC-3 chloride channel was significantly increased. The size of samples for MDC and PDC was small in this study. However, we have used these differences just as indication for further studies. The survival rate of chondrosarcoma patients was closely related to the grade of metastasis and malignancy. Nie et al. analysis of 3737 patients and found that the 5-year overall survival rate of chondrosarcoma was 73.9%. However, if there was remote metastasis, non-differentiation or single radiation, the 5-year survival rate was greatly reduced, below 30%. The characteristic of "distant" stage, "undifferentiated" grade, and single radiation had very

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

low 5-year survival rates [28]. Wang et al. considered that high histological grade was an independent risk factor for chondrosarcoma [29]. Fiorenza et al. also considered that high histological grade was an independent risk factor for survival and prognosis evaluation, but the location and surgical type was not, after they analyzed 153 patients with non-metastatic chondrosarcoma [30].

Our results also demonstrated that localization of ClC-3 chloride channel changed with the change of malignant degree. Among the 44 cases of WDC, ClC-3 level was low expression and present in the nucleus. In the 6 cases of MDC and 12 cases of PDC, the subcellular localization of ClC-3 chloride channel was overexpression and mainly localized in the cytoplasm and in both of nucleus and cytoplasm. The significance of the different localization in different malignant degrees remains unclear. It has been suggested that ClC-3 chloride channels may perform different functions with difference distributions. Some of the data have showed that ClC-3 chloride channels were distributed on the cell membrane and were related to cell cycle and apoptosis [31,32]. ClC-3 chloride channels have also been located in the intracellular compartment and associated with changes in the intracellular acidic environment, thereby increasing the isolation of anti-tumor drugs and leading to resistance to chemotherapy drugs [33,34]. It has been shown that ClC-3 induces tumor mediating multidrug resistance by activating the nuclear factor kappa B signaling pathway [17].

Chondrosarcoma, as a common malignant bone tumor, mostly occurs in patients younger than 30 years of age, and the incidence of chondrosarcoma increases gradually over the age of 35 years. Our study found that the grading of chondrosarcoma increased with age. But the ratio of age in chondrosarcoma significantly showed that the incidence of chondrosarcoma increased gradually in the age range of 30≤ age <60 years. Interestingly, marital status was identified as an independent prognostic factor for chondrosarcoma. Widowed patients have been reported to show the worst chondrosarcoma cancer-specific survival performance compared with married, divorced, and single controls [35]. Several studies have confirmed that marital status is an independent prognostic factor for cancer patients [36–38].

We also looked at gender differences in the incidence of chondrosarcoma in this study. The occurrence of chondrosarcoma also exhibits a certain degree of sex bias, with a high percentage detected in males. We found a 2.4: 1 prevalence predilection in males versus females. Among the 75 cases of chondrosarcoma tissue, the percentage of samples with high ClC-3 expression level (>2) was also significantly higher in males. Gender differences in chondrosarcoma disease have been well established. Arshi et al. analyzed 973 cases of chondrosarcoma and found a 3: 2 prevalence predilection in males versus females [39]. Shao et al. analyzed 40 Chinese cases of extra skeletal myxoid chondrosarcoma and found a gender ratio of 1.7: 1 [40].

Moreover, the etiology of osteosarcoma has not been fully illuminated. Tumor characteristics understanding may play an important role in guiding development of targeted therapy for these diseases. In this study, we found that the ClC-3 chloride channels were significantly increased in chondrosarcoma in highly malignant tissue. The high expression of ClC-3 chloride channels may be related to the malignant proliferation and erosion in chondrosarcoma diseases. We and others have found that high expression of ClC-3 chloride channels are associated with endometriosis, nasopharyngeal cancer, breast cancer, and peritoneal adhesion. However, the mechanism of ClC-3 chloride channels in regulating the pathogenesis of chondrosarcoma needs further study.

Conclusions

We examined the expression of ClC-3 chloride channel in different types of chondrosarcoma and normal cartilage tissues. Our data provided the first evidence that the ClC-3 chloride channels were significantly increased in chondrosarcoma in highly malignant tissue. Besides, the subcellular localization of ClC-3 chloride channel was changed in association with malignant degree changes. Our results revealed the potential of ClC-3 chloride channel in the treatment of chondrosarcoma.

Conflicts of interest

None.

References:

1. Rossow CF, Duan D, Hatton WJ et al: Functional role of amino terminus in ClC-3 chloride channel regulation by phosphorylation and cell volume. Acta Physiologica, 2006; 187(1–2): 5–19
2. Zhang H, Deng Z, Yang L et al: The AQP-3 water channel is a pivotal modulator of glycerol-induced chloride channel activation in nasopharyngeal carcinoma cells. Int J Biochem Cell Biol, 2016; 72: 89–99
3. Yang H, Ma L, Wang Y et al: Activation of ClC-3 chloride channel by 17beta-estradiol relies on the estrogen receptor alpha expression in breast cancer. J Cell Physiol, 2018; 233(2): 1071–81
4. Kornak U, Kasper D, Bosl MR et al: Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Cell, 2001; 104(2): 205–15
5. Christensen EI, Devuyst O, Dom G et al: Loss of chloride channel ClC-5 impairs endocytosis by defective trafficking of megalin and cubilin in kidney proximal tubules. Proc Natl Acad Sci USA, 2003; 100(14): 8472–77
6. Konrad M, Vollmer M, Lemmink HH et al: Mutations in the chloride channel gene CLCNKB as a cause of classic Bartter syndrome. J Am Soc Nephrol, 2000; 11(8): 1449–59
7. Berkovic SF, Kapur J: Are myotonia and epilepsy linked by a chloride channel? Neurology, 2013; 80(12): 1074–75

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

8. Zhou C, Tang X, Xu J et al: Opening of the CLC-3 chloride channel induced by dihydroartemisinin contributed to early apoptotic events in human poorly differentiated nasopharyngeal carcinoma cells. J Cell Biochem, 2018; 119(11): 9560–72
9. Zhu L, Yang H, Zuo W et al: Differential expression and roles of volume-activated chloride channels in control of growth of normal and cancerous nasopharyngeal epithelial cells. Biochem Pharmacol, 2012; 83(3): 324–34
10. Wang L, Chen L, Jacob TJ: The role of ClC-3 in volume-activated chloride currents and volume regulation in bovine epithelial cells demonstrated by antisense inhibition. J Physiol, 2000; 524(1): 63–75
11. Deng Z, Peng S, Zheng Y et al: Estradiol activates chloride channels via estrogen receptor-alpha in the cell membranes of osteoblasts. Am J Physiol Cell Physiol, 2017; 313(2): C162–72
12. Yang L, Zhu L, Xu Y et al: Uncoupling of K+ and Cl- transport across the cell membrane in the process of regulatory volume decrease. Biochem Pharmacol, 2012; 84(3): 292–302
13. Zheng Y, Chen Z, Gu Z et al: Starvation-induced autophagy is upregulated via ROS-mediated ClC-3 chloride channel activation in the nasopharyngeal carcinoma cell line CNE-2Z. Biochemical Journal, 2019 [Accepted manuscript]
14. Guan Y, Luan Y, Xie Y et al: Chloride channel-3 is required for efficient tumour cell migration and invasion in human cervical squamous cell carcinoma. Gynecol Oncol, 2019; 153(3): 661–69
15. Deng Z, Li W, Xu J et al: ClC-3 chloride channels are involved in estradiol regulation of bone formation by MC3T3-E1 osteoblasts. J Cell Biochem, 2018 [Epub ahead of print]
16. Wang LW, Chen LX, Jacob T: ClC-3 expression in the cell cycle of nasopharyngeal carcinoma cells. Sheng Li Xue Bao, 2004; 56(2): 230–36
17. Chen Q, Liu X, Luo Z et al: Chloride channel-3 mediates multidrug resistance of cancer by upregulating P-glycoprotein expression. J Cell Physiol, 2019; 234(5): 6611–23
18. Jeong W, Kim HJ: Biomarkers of chondrosarcoma. J Clin Pathol, 2018; 71(7): 579–83
19. Chow WA: Chondrosarcoma: Biology, genetics, and epigenetics. F1000Res, 2018; 7: pii: F1000 Faculty Rev-1826
20. Heck RK Jr., Peabody TD, Simon MA: Staging of primary malignancies of bone. Cancer J Clin, 2006; 56(6): 366–75
21. van Oosterwijk JG, Anninga JK, Gelderblom H et al: Update on targets and novel treatment options for high-grade osteosarcoma and chondrosarcoma. Hematol Oncol Clin North Am, 2013; 27(5): 1021–48
22. Edge SB, Compton CC: The American Joint Committee on Cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol, 2010; 17(6): 1471–74
23. Yu Y, Li S, Zhang H et al: NRSF/REST levels are decreased in cholangiocellular carcinoma but not hepatocellular carcinoma compared with normal liver tissues: A tissue microarray study. Oncol Lett, 2018; 15(5): 6592–98
24. Liu C, Zhang Y, Zhang K et al: Expression of estrogen receptors, androgen receptor and steroid receptor coactivator-3 is negatively correlated to the differentiation of astrocytic tumors. Cancer Epidemiol, 2014; 38(3): 291–97
25. Aigner T: Towards a new understanding and classification of chondrogenic neoplasias of the skeleton – biochemistry and cell biology of chondrosarcoma and its variants. Virchows Archiv, 2002; 441(3): 219–30
26. Gelderblom H, Hogendoorn PC, Dijkstra SD et al: The clinical approach towards chondrosarcoma. Oncologist, 2008; 13(3): 320–29
27. Nazeri E, Gouran Savadkoohi M, Majidzadeh AK et al: Chondrosarcoma: An overview of clinical behavior, molecular mechanisms mediated drug resistance and potential therapeutic targets. Crit Rev Oncol Hematol, 2018; 131: 102–9
28. Nie Z, Lu Q, Peng H: Prognostic factors for patients with chondrosarcoma: A survival analysis based on the Surveillance, Epidemiology, and End Results (SEER) database (1973–2012). J Bone Oncol, 2018; 13: 55–61
29. Wang JW, Ger LP, Shih CH et al: Chondrosarcoma of bone: A statistical analysis of prognostic factors. J Formos Med Assoc, 1991; 90(10): 998–1003
30. Fiorenza F, Abudu A, Grimer RJ et al: Risk factors for survival and local control in chondrosarcoma of bone. J Bone Joint Surg Br, 2002; 84(1): 93–99
31. Zhang H, Li H, Yang L et al: The ClC-3 chloride channel associated with microtubules is a target of paclitaxel in its induced-apoptosis. Sci Rep, 2013; 3: 2615
32. Zhang H, Zhu L, Zuo W et al: The ClC-3 chloride channel protein is a downstream target of cyclin D1 in nasopharyngeal carcinoma cells. Int J Biochem Cell Biol, 2013; 45(3): 672–83
33. Hong S, Bi M, Wang L et al: CLC-3 channels in cancer (review). Oncol Rep, 2015; 33(2): 507–14
34. Wang L, Ma W, Zhu L et al: ClC-3 is a candidate of the channel proteins mediating acid-activated chloride currents in nasopharyngeal carcinoma cells. Am J Physiol Cell Physiol, 2012; 303(1): C14–23
35. Gao Z, Ren F, Song H et al: Marital status and survival of patients with chondrosarcoma: A population-based analysis. Med Sci Monit, 2018; 24: 6638–48
36. Shi RL, Qu N, Lu ZW et al: The impact of marital status at diagnosis on cancer survival in patients with differentiated thyroid cancer. Cancer Med, 2016; 5(8): 2145–54
37. McLaughlin JM, Fisher JL, Paskett ED: Marital status and stage at diagnosis of cutaneous melanoma: Results from the Surveillance Epidemiology and End Results (SEER) program, 1973–2006. Cancer, 2011; 117(9): 1984–93
38. Alamanda VK, Song Y, Holt GE: Effect of marital status on treatment and survival of extremity soft tissue sarcoma. Ann Oncol, 2014; 25(3): 725–29
39. Arshi A, Sharim J, Park DY et al: Chondrosarcoma of the osseous spine: An analysis of epidemiology, patient outcomes, and prognostic factors using the SEER Registry From 1973 to 2012. Spine, 2017; 42(9): 644–52
40. Shao R, Lao IW, Wang L et al: Clinicopathologic and radiologic features of extraskeletal myxoid chondrosarcoma: A retrospective study of 40 Chinese cases with literature review. Ann Diagn Pathol, 2016; 23: 14–20

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]