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Correlation of MRI apparent diffusion coefficient of invasive breast cancer with tumor tissue growth and angiogenesis

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ABSTRACT

Objective: To study the correlation of MRI apparent diffusion coefficient (ADC value) of invasive breast cancer with tumor tissue growth and angiogenesis. **Methods:** Patients with breast mass who were treated in Wuhan No. 6 Hospital between March 2014 and May 2017 were selected as the research subjects and divided into group A with invasive ductal carcinoma, group B with intraductal carcinoma and group C with benign lesion according to the biopsy results, magnetic resonance diffusion-weighted imaging was conducted to determine ADC values, and biopsy tissue was taken to determine the expression of proliferation genes and angiogenesis genes. **Results:** USP39, CyclinD1, VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression levels in lesions of group A and group B were significantly higher than those of group C while ADC value as well as ALEX1 and Bax protein expression levels were significantly lower than those of group C; USP39, CyclinD1, VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression levels in lesions of group A were significantly higher than those of group B while ADC value as well as ALEX1 and Bax protein expression levels was significantly lower than those of group B; USP39, CyclinD1, VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression levels in invasive breast cancer tissue with high ADC value were significantly lower than those in invasive breast cancer tissue with low ADC value while ALEX1 and Bax protein expression levels were significantly higher than those in invasive breast cancer tissue with low ADC value. **Conclusion:** The decrease of ADC value of invasive breast cancer is closely related to cancer cell proliferation and angiogenesis.

1. Introduction

Breast cancer is one of the common malignant tumors in women, and the incidence is increasing year by year. At present, pathological biopsy is still the gold standard for diagnosing breast cancer, and it can accurately assess the pathological type, differentiation of breast cancer and so on. Imageological examination is a common clinical method to determine the malignancy and prognosis of breast cancer, and it can also be used to evaluate the effect of radiotherapy and chemotherapy. Magnetic resonance diffusion-weighted imaging (DW-MRI) is the means of magnetic resonance imaging developed in recent years, which determines apparent diffusion coefficient (ADC) to reflect the activity of water exchange between tissue compositions[1,2]. The ADC value is closely related to the cell density in the tissue, the abnormal proliferation of cancer cells in the breast cancer lesion can result in increased cell density and restricted free movement of water molecules, and thus the ADC value changes[3]. In the following studies, we analyzed the correlation of ADC value of invasive breast cancer with tumor tissue growth and angiogenesis.

2. Case information and research methods

2.1. General case information

Patients with breast mass who were treated in Wuhan No. 6 Hospital between March 2014 and May 2017 were selected as the research subjects, all patients were admitted to the hospital for

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breast mass, breast ultrasound and MRI demonstrated that there was breast mass, and they were divided into group A with invasive ductal carcinoma, group B with intraductal carcinoma and group C with benign lesion according to the biopsy results. Group A included 38 cases that were 42-68 years old; group B included 23 cases that were 39-66 years old; group C included 35 cases that were 43-64 years old. There was no significant difference in the general data of the three groups (P>0.05).

2.2 ADC value detection

Siemens 1.5 T magnetic resonance scanner was used for conventional MRI scan of three groups of patients, then DW-MRI scan was conducted, sensitive coefficient was 1 000 s/mm^2, the matrix was 256 256, number of excitation was 4, diffusion directions were 3, ROI was manually placed after image was obtained, it included tumor parenchyma area, but did not include cystic or necrotic area, and ADC value was automatically calculated after ROI was selected.

2.3 Gene expression detection

Pathological biopsy tissue of three group of patients was taken and added in RIPA lysate to extract the total protein in tissue, it was centrifuged to separate upper clear protein liquid, and enzyme-linked immunosorbent assay was used to determine USP39, CyclinD1, ALEX1, Bax, VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 levels.

2.4 Statistical methods

SPSS 19.0 software was used to input data, the median of ADC of invasive breast cancer was calculated, ADC value > the median was judged as high ADC value and ADC value < the median was judged as low ADC value; data among three groups were by variance analysis, data between two groups were by t test and P< 0.05 indicated statistically significance in differences in test results.

3. Results

3.1 Lesion ADC of three groups of patients

ADC value of group A was (1.15±0.15) 10^{-3} mm^2/s, ADC value of group B was (1.42±0.18) 10^{-3} mm^2/s and ADC value of group C was (1.77±0.22) 10^{-3} mm^2/s. Variance analysis showed that ADC value of group A and group B were significantly lower than that of group C, and ADC value of group A was significantly lower than that of group B. Differences in pair-wise comparison of ADC value were statistically significant among three groups of patients (P<0.05).

3.2 Proliferation gene expression

Analysis of proliferation genes USP39 (pg/mL), CyclinD1 (ng/mL), ALEX1 (pg/mL) and Bax (ng/mL) expression in lesions among three groups of patients was as follows: USP39 and CyclinD1 protein expression levels in lesions of group A and group B were significantly higher than those of group C while ALEX1 and Bax protein expression levels were significantly lower than those of group C; USP39 and CyclinD1 protein expression levels in lesions of group A were significantly higher than those of group B while ALEX1 and Bax protein expression levels were significantly lower than those of group B. Differences in pair-wise comparison of USP39, CyclinD1, ALEX1 and Bax protein expression in lesions were statistically significant among three groups of patients (P<0.05).

Analysis of proliferation genes USP39, CyclinD1, ALEX1 and Bax expression in invasive breast cancer tissue with different ADC values was as follows: USP39 and CyclinD1 protein expression levels in invasive breast cancer tissue with high ADC value were significantly lower than those in invasive breast cancer tissue with low ADC value while ALEX1 and Bax protein expression levels were significantly higher than those in invasive breast cancer tissue with low ADC value. Differences were statistically significant in USP39, CyclinD1, ALEX1 and Bax protein expression in invasive breast cancer tissue with different ADC values (P<0.05).

Table 1.
Comparison of proliferation gene expression in lesions among three groups of patients.

Groups	n	USP39	CyclinD1	ALEX1	Bax
Group A	38	231.45±33.26$^{*\#}$	4.40±0.62$^{*\#}$	103.55±12.67$^{*\#}$	0.89±0.11$^{*\#}$
Group B	23	142.37±20.35*	2.73±0.36*	189.42±22.49*	2.26±0.37*
Group C	35	80.31±9.57	1.45±0.19	373.64±52.15	5.21±0.78

*: compared with gene expression in group C, P<0.05; $^{\#}$: compared with gene expression in group B, P<0.05.

Table 2.
Comparison of proliferation gene expression in invasive breast cancer tissue with different ADC values.

ADC value	n	USP39	CyclinD1	ALEX1	Bax
High ADC value	19	179.35±23.52	3.48±0.49	149.33±17.82	1.31±0.16
Low ADC value	19	298.42±37.21	5.51±0.78	61.22±8.48	0.44±0.07
T		8.298	9.215	12.139	19.487
P		<0.05	<0.05	<0.05	<0.05

Table 3.
Comparison of angiogenesis gene expression in lesions among three groups of patients.

Groups	*n*	VEGF	bFGF	Angplt-2	Angplt-3	Angplt-4
Group A	38	7.72±0.93*#	132.31±14.95*#	3.41±0.47*#	273.63±33.52*#	236.51±30.46*#
Group B	23	4.03±0.52*	78.79±9.35*	1.98±0.24*	165.62±19.54*	142.15±18.69*
Group C	35	1.78±0.22	40.35±5.62	0.77±0.09	98.52±10.39	79.87±9.35

*: compared with gene expression in group C, $P<0.05$; #: compared with gene expression in group B, $P<0.05$.

Table 4.
Comparison of angiogenesis gene expression in invasive breast cancer tissue with different ADC values.

ADC value	*n*	VEGF	bFGF	Angplt-2	Angplt-3	Angplt-4
High ADC value	19	5.57±0.78	98.52±10.38	2.59±0.36	203.42±25.83	172.41±20.35
Low ADC value	19	9.91±1.14	175.21±21.39	4.47±0.59	347.29±49.52	301.25±35.59
T		9.298	8.588	9.104	8.289	8.652
P		<0.05	<0.05	<0.05	<0.05	<0.05

3.3 Angiogenesis gene expression

Analysis of angiogenesis genes VEGF (ng/mL), bFGF (pg/mL), Angplt-2 (ng/mL), Angplt-3 (pg/mL) and Angplt-4 (pg/mL) expression in lesions among three groups of patients was as follows: VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression levels in lesions of group A and group B were significantly higher than those of group C; VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression levels in lesions of group A were significantly higher than those of group B. Differences in pair-wise comparison of VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression in lesions were statistically significant among three groups of patients ($P<0.05$).

Analysis of angiogenesis genes VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 expression in invasive breast cancer tissue with different ADC values was as follows: VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression levels in invasive breast cancer tissue with high ADC value were significantly lower than those in invasive breast cancer tissue with low ADC value. Differences were statistically significant in VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression in invasive breast cancer tissue with different ADC values ($P<0.05$).

4. Discussion

Imageological examination is of great value in early screening of breast cancer, assessment of malignancy as well as judgment of efficacy and prognosis. MRI is a common means of imageological examination for breast cancer, conventional MRI can diagnose breast cancer by burr-like changes in mass margin, and dynamic enhanced MRI scanning can determine the mass properties through the time-signal intensity curve[4]. DW-MRI is the MRI method developed in recent years and has been proven by a growing body of researches to be able to be used to judge the breast mass properties, the examination method can determine ADC values to reflect the microscopic movement of water molecules in tissue, and the higher the ADC values, the more active the water molecule movement in tissue[5,6]. In the progression of malignant tumor, the proliferation of cancer cells causes cell density to increase and affects the activity of water molecules, thus reducing the activity of water molecules and ADC value[7,8]. In the study, analysis of the changes in ADC values of breast cancer showed that the ADC values of invasive breast cancer and intraductal carcinoma were significantly lower than that of benign breast mass, and the ADC value of invasive breast cancer was significantly lower than that of intraductal carcinoma. This shows that in the occurrence and infiltration of breast cancer, malignant cell proliferation can affect the activity of the water molecules in the tissue and decrease the activity of water molecules, which is shown as the decrease of ADC value.

Abnormal breast cancer cell proliferation is the important factor influencing the degree of water molecule activity in lesions, and the abnormal expression of USP39, CyclinD1 and other pro-proliferation genes as well as ALEX1, Bax and other anti-proliferation genes is associated with breast cancer cell proliferation. The coding products of the USP39 gene can regulate the ubiquitination process and enhance the process of deubiquitination to increase the CyclinD1 expression and accelerate the cell cycle progression, which in turn is conducive to cell proliferation[9,10]; ALEX1 belongs to the Arm family, and its encoding products can increase Bax expression, initiate mitochondrial cell apoptosis and inhibit cell proliferation[11,12]. In the study, analysis of the changes in the proliferation gene expression in breast mass showed that USP39 and CyclinD1 protein expression in invasive breast cancer and intraductal carcinoma were significantly higher than those in benign breast mass while ALEX1 and Bax protein expression were significantly lower than those in benign breast mass, and the USP39 and CyclinD1 protein expression in invasive breast cancer were significantly higher than those in intraductal carcinoma while ALEX1 and Bax protein expression were significantly lower than those in intraductal carcinoma. This shows that the high expression of the pro-proliferation genes and the low expression of anti-proliferation genes are closely related to the occurrence of breast cancer. Further analysis of the correlation between ADC values and

proliferation gene expression indicated that USP39 and CyclinD1 protein expression levels in invasive breast cancer tissue with high ADC value were significantly lower than those in invasive breast cancer tissue with low ADC value while ALEX1 and Bax protein expression levels were significantly higher than those in invasive breast cancer tissue with low ADC value. This indicates that the high expression of the pro-proliferation genes and the low expression of anti-proliferation genes can affect the proliferation of breast cancer cells, and thus decrease the ADC value.

The proliferation of cells in breast cancer lesions depends on the nutrients provided by the new blood vessels. VEGF, bFGF, Angplt-2, -3, -4 and other molecules are closely related to the angiogenesis in breast cancer. VEGF is the most powerful pro-angiogenesis molecule known at present, which can directly act on endothelial cells and promote their proliferation so as to form vascular structure and increase blood vessel density[13,14]; bFGF is a mitosis with extensive proliferation effect, which can promote endothelial cell proliferation and angiogenesis in tumor lesions; Angplt-2, -3 and -4 are the Angplt family members who are closely related to the angiogenesis of breast cancer, and can increase the number of new blood vessels through the various downstream signaling pathways[15,16]. In the study, analysis of the changes in the proliferation gene expression in breast mass showed that VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression levels in invasive breast cancer and intraductal carcinoma were significantly higher than those in benign breast mass, and VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression levels in invasive breast cancer were significantly higher than those in intraductal carcinoma. This indicates that the high expression of angiogenesis genes is closely related to the occurrence of breast cancer. Further analysis of the correlation between ADC values and angiogenesis gene expression showed that VEGF, bFGF, Angplt-2, Angplt-3 and Angplt-4 protein expression levels in invasive breast cancer tissue with high ADC value were significantly lower than those in invasive breast cancer tissue with low ADC value. This indicates that the high expression of angiogenesis genes is beneficial to the proliferation of breast cancer cells, which leads to the decrease of ADC value.

The analysis of ADC values and gene expression in this study confirmed that ADC value significantly decreased in invasive breast cancer, the cancer cell proliferation and angiogenesis in lesion were closely related to the decrease of ADC values, and it indicates that ADC value has evaluation value for the malignant degree of invasive breast cancer. The evaluation value of ADC value for the therapeutic effect of breast cancer can be further explored in the further study.

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