Expressions of P-gp, p21 Kipl and p53 Proteins in Giant Cell Tumor of Bone and Their Significances

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Abstract **Objective** To investigate expressions of P-gp, p27 kipl and p53 proteins in giant cell tumor of bone and their clinical pathological significances. **Methods** P-gp, p27 kipl and p53 proteins in 36 cases of giant cell tumor(GCT) were examined with S-P immunohistochemistry technique. **Results** Twelve of 36 cases (33.3%) in this series were positive for P-gp. The positive rates of P-gp were 45.5%, 26.6%and 33.3% in grade I, II and III of the tumor, and were 21.4% and 75% in primary and recurrent tumors, respectively, with statistically significant difference (P<0.01)between them. The total positive rate of p27kipl in this series was 86.1% (31/36), with percentage of 100%, 94.7% and 33.3% in grade I, II and III, (P<0.01), and were 100% and 37.5% in primary and recurrent tumors, respectively (P<0.05). Only 3 cases (8.3%) were positive for p53 protein. **Conclusion** Overexpression of P-gp in giant cell tumor of bone may be one of the causes of insensitivity to chemotherapy, and there is no correlation between expression and histological grade of the tumor. The expression of p27kipl in the tumor is related to histological grade, recurrence and poor prognosis of the tumor, which suggested that the expression of P27kipl might be useful for prediction of the behavior and prognosis of the tumor. The expression of P53 may be one of the indexes of malignant tumor.

Key Words Bone neoplasm; P-gp; P27kipl; P53; Immunohistochemistry

Giant cell tumor (GCT) of bone is a osteolytic tumor originating form cancellous bone, and has strong local invasive and recurrent, and insensitivity to chemotherapy medicine. we used immunohistochemistry with S-P technique to examine P-gp, p27kipl and p53 proteins in 36 cases of GCT, to investigate the expressions of P-gp, p27kipl and p53 proteins in giant cell tumor of bone and their clinical pathological significances.

MATERIAL AND METHOD

Material 36 specimens of GCT were from the Hospital Attached to Zhejiang College of TCM, the Zhuji People´s Hospital of Zhejiang Province, Zhuji Red Cross Hospital Of Zhejiang Province. Among them, 28 cases were in primary tumors, and 8 cases were in recurrent tumors; 27 cases were male and 9 were female; By pathologic classify of Jeffe: 11 cases, 19 cases and 6 cases were in grade I, II and III respectively.

Method All specimens were fixed in 10% formalin, dehydrated routinely, embedded by wax, cut into slices at 4um, and made the P-gp, p27kipl and p53 proteins immunohistochemistry dyeing respectively. The monoclonal antibody of P-gp; p27kipl and p53 is producted by the Zymed company of United States. The SP reagent is producted by the Santa Craz company (purchased form zhongshan biotechnological company of Beijing), the first anti-body dilution density is 1:50. We used positive slice having known to make positive contrast, replacing the first anti-body with the PBS to make negative contrast, DAB demonstration, hematein redyeing.

Result judgment every slice choosed five high power objective visual field, counting 100 cells. Grades of positive cells were classified as negative (0~5%), low (6%~25%), medium (26%~50%), high (>50%). We made statistical treatment with Chi-squared test.

RESULTS

Twelve of 36 cases (33.3%) in this series were positive for P-gp(12/36), P-gp-positive cells locate in cell nucleus, and were stained buffy, the total positive rate of p27kipl in thes series was 86.1% (31/36), only 3 cases (8.3%) were positive for p53 protein, p27-positive cells and p53-positive cells locate in cytoplasm, and stained buffy also. Table 1 and table 2 shoco the histological grade of giant cell tumor of bone and the expressions of P-gp, p21kipl, p53 proteins in primary and recurrent tu-

Table 1 the relation of the expressions of P-gp, p21kipl, p53 proteins with the pathological grading of giant cell tumor of bone.

Pathological Grading	Case no.	Pgp			Positive no(%)	p27kip1			Positive no(%)	p53			Positive no(%)
		+	++	+++		+	++	+++		+	++	+++	
Ⅰ	11	4	1	0	5(45.5)	2	6	3	11(100)	0	0	0	0
Ⅱ	19	3	2	0	5(26.3)	3	11	4	18(94.7)	0	0	0	0
Ⅲ	6	1	1	0	2(33.3)	1	1	0	2(33.3)	2	1	0	3(50)
Total	36	8	4	0	12(33.3)	6	18	7	31(86.1)	2	1	0	3(8.3)

Table 2 The relation of the expressions of P-gp, p21kipl, p53 proteins with the primary and recurrent tumors of giant cell tumor of bone

Grading	Case no.	Pgp			Positive no(%)	p27kip1			Positive no(%)	p53			Positive no(%)
		+	++	+++		+	++	+++		+	++	+++	
Primary tumor	28	5	1	0	6(21.4)	4	17	7	28(100)	0	0	0	0
Recurrent tumor	8	4	2	0	6(75)	2	1	0	3(37.5)	0	1	0	3(37.5)
Total	36	9	3	0	12(33.3)	6	18	7	31(86.1)	2	1	0	3(8.3)

negative(-), low(+), medium(++), high (+++)

mors respectively.

DISCUSSION

Giant cell tumor of bone is a osteolytic and latent malignant tumor, and is insensitivity to chemotherapy. radiotherapy may trend sarcomatous change. Literature report [1], histological grades has no significance unless categorical sarcom, because 1%~20% tumor of innocuous histological form has metastasis [2] . Multi-drug tolerance (MDR) is the main cause of the failure of chemotherapy. The investigation demonstrated that P-glycoprotien was the background of MDR. Homo sapiens genome existence two kinds of MDR gene, is MDR-1 and MDR-2. The investigation had made clear that the expression level of MDR-1 has relation with the drug resistance of tumor cells. P-gp is one kind of products of MDR gene, its transmembrane potential has capacity dependence "drug pump" function. The expression of P-gp in this series detected that the expression was higher in recurrenr tumors than in primary tumors (P<0.01), overexpression of P-gp in giant cell tumor of bone may be one of the causes of insensitivity to chemotherapy, and there is no correlation between expression and histological grade of the tumor.

p27kipl protein is a heat stable proteins which was discovered by Polyak in 1994[3], The molecular weight of P27kipl protein is 27000, was discovered in transforming growth factor-b (TGF-B) and growth suppression cells that was disposed by cell contact. This proteins conjugate with cyclin E-CDK1 and cyclin D-CDK4 in vitro, suppressing the CDK´s activity coith relation to dosage. P27 protien has a function that can restrictly adjust cell cycle. In cell cycle, p27 protein represses the kinase compound of G1 phase, for instance, cyclin E-CDK2 and cyclin D-CDK4 etc. it makes cells can´t pass G1 phase, supressing the tumor´s occurrence and progression. The investigation made by Yasui et al[4] detected that the decrease in the expression of p27 has notable correlation with the advance of carcinoma of stomach, the depth of tumor infiltration and the lymph node metastasis. A investigation about 113 cases of carcinoma of prostate made by Tsihlias et al[5] detected that the low expression of p27 protein had relation with recurrence and poor prognosis of the tumor. In this study, the positive rates of p27 is 86.1%, higer than hanzhuang´s report [6], in which the positive rates of p27 protein in 40 cases of giant cell tumor of bone is 45%. The expression of p27 protein is descenting with the rising of pathological grades. The positive rates of p27 were 100%, 94.7% and 33.% in grade I, II and III, there had significant difference(P<0.01); the positive rates of p27 protein in recurrence tumors was lower than in primary tu-

mors. The distribution of p27 protein in grade I, grade II and primary tumors is in megakaryocyte and mononuclear phagocyte, and in grade III and recurrence tumors is in megakaryocyte. The difference of distribution indicated that p27kip1 for an anti-oncogene had important action in tumor's occurrence and development. This experimental result had made clear that the low expression of p27 in giant cell tumor of bone has relation to histological grades, recurrence and poor prognosis of the tumor. the detection of p27kip1 in giant cell tumor of bone might be useful for prediction of the behavior and prognoses of the tumor.

P53 gene is divided into two types, wild type and mutant. wild type of p53 is anti-oncogene, its half life is short, and is hard to detect. Using immunohistochemistical method we can detect mutant of p53, it has no action of restraining cancer[7,8] otherwise, it has action of promoting cancer. The investigation made by Massui et al[9] detected that the expression of p53 protein had relation to histological grades, recurrence and metastasis of the tumor. In this series, the positive rates of p53 in grade III of the tumor is 50%, in recurrence of the tumor is 37.5%, there is no expression in grade I, grade II and primary tumor. Which suggested that there is gene mutation of p53 in grade III and recurrence tumor, and the expression of P53 may be act as on index of malignant tumor.

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