Expression of AP-1 Subunits in Skin Tumor

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Abstract **Objective** To investigate the expression of AP-1 subunits in skin tumor and the adjacent tissue and its roles in the occurrence and development of skin tumor. **Methods** ABC immunohistochemistry was applied to detect c-jun, JunB, JunD, c-fos, Fra-1, Fra-2 expression in skin basal cell carcinoma, squamous cell carcinoma, Bowens disease and keratacanthoma. **Results** The expression of c-Jun and P-c-Jun increased dramatically in basal cell carcinoma, squamous cell carcinoma, Bowens disease and keratoacanthoma and JunB decreased sharply in basal cell carcinoma. **Conclusion** c-Jun is a positive regulator of cell proliferation and tumorigenesrs in skin tumor, and JunB had an antagonistic function to c-Jun.

Key words c-jun; JunB; JunD; c-fos; Fra-1; Fra-2; immunohistochemistry; skin tumor

AP-1 (Activator protein -1)is contained in many kinds of cell, which is closely related to the cell's differentiation, proliferation, apoptosis and the transformation of tumor. AP-1 consists of many subunits with different function .These subunits are both in coordination with and antagonist to each other in function. Much studies have been done in transgenic animal and cell model to make sure their functions. But, there are less reports about AP-1 in human body especially in human cutaneous tumor. This study take ABC immunohistochemical method to research the expression of subunits of AP-1 in cutaneous tumor and the adjacent tissue, in order to explore the relationship between AP-1 and occurence and development of cutaneous tumor.

MATERIALS AND METHODS

Clinic data

Thirteen cases of basal cell carcinoma, 5 cases of squamous cell carcinoma, 2 cases of Bowens disease and 4 cases of keratoacanthoma, were all from the operation of cutaneous tumor in the department of surgery and dermatology, Cancer hospital of guilin city during 2002 and 2003. The adjacent tissue was referred to the tissues which is within 1~2cm around the tumor. Fresh tissue specimen are embeded in OCT as soon as possible, and then to be preserved in liquid nitrogen.

Agentia

The first antibodies for immunohistochemistry is in the table 1.

Dyeing method of immunohistochemistry

Frozen sections with 6 um thick were fixed with 2% paraformaldehyde, 0.03% hydrogen dioxide was used to remove endogenous peroxidase, and sections were incubated with 5% normal animal blood serum to block; the first antibody was incubated in 4℃ overnight, AEC coloration,campeachy after stain. The immune globulin with same concentration and from the same animal was used to substitute the first antibody to serve as negative comparison. Above all steps (beside protein block) are washed by phosphate buffer.

Assessment of the results

Qualitation: the cells with red nucleus or brown substance were regard as positive expression.

Quantitation: (-)=no color, (±)=suspect of color, (+)=light color, (++)=mid-range color, (+++)= deep color.

RESULTS

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Table 1 the sources, concentration and manufactory of the antibodies

The name of the antibodies	sources	concentration	manufactory
Jun monoclonal antibody	mouse	1:100	BD Transduction Laboratories
phosphorylation C−Jun (KM−1)(P−C−Jun), monoclonal antibody	mouse	1:800	Santa Cruz Biotechnology
JunB(C−11) monoclonal antibody	mouse	1:200	Santa Cruz Biotechnology
JunD (329) polyclonal antibody	rabbit	1:200	Santa Cruz Biotechnology
c−fos(Ab−1) monoclonal antibody	mouse	1:200	Oncogen Research product
Fra−1(R−20) polyclonal antibody	rabbit	1:100	Oncogen Research product
Fra−2(Q−20) polyclonal antibody	rabbit	1:200	Santa Cruz Biotechnology

Over expression of c–jun

appeared in cell nucleus in basal cell carcinoma (13/13 cases) (figure 1), squamous cell carcinoma (2/5 cases), Bowens disease (2/2 cases) and keratoacanthoma (3/4 cases), a mid−expression could be seen in some epidermis basal cell close to the tumor, and light expression appeared in interstitial cell around some tumor.

The distribution of p−c−jun positive expression is similar to c−jun, much mid−coloration appeared in basal cell carcinoma (11/13 cases) (figure 2), squamous cell carcinoma (3/5 cases), Bowens disease (2/2 cases) and keratoacanthoma (3/4 cases).

Nucleus coloration of JunB were hardly seen in basal cell carcinoma (13/13 cases)(figure 3), but in squamous cell carcinoma (5/5 cases), Bowens disease (2/2 cases) and partly keratoacanthoma (2/4 cases) there were much nuclei mid−coloration.There are karyotin in heckle cell layer and granular cell layer of epidermis basal cell which around tumor. Basal cell layer coloration was deepest, there is karyotin in interstitial cell around some tumor.

JunD has middle quantitative nucleus coloration in all kinds of cutaneous tumors.The coloration in epidermis and interstitial cell around tumor is similar to JunB. There are a few or no nucleus coloration in basal cell carcinoma (10/13 cases)(figure 4). The middle nucleus coloration can be seen in squamous cell carcinoma (4/5 cases), Bowens disease (2/2 cases) and keratoacanthoma (3/4 cases).

There are a few nucleus coloration of c−fos in basal cell carcinoma (figure 5), squamous cell carcinoma, keratoacanthoma and Bowens disease. There are middle nucleus coloration in epidermis close to tumor which is distributed in basal cell and prickle cell layer.

There are a few nucleus coloration of Fra−1 in basal cell carcinoma (figure 6), squamous cell carcinoma, keratoacanthoma and Bowens disease. There are a few coloration in nucleus of epidermis close to the tumor.

There are a few nucleus coloration of Fra−2 in basal cell carcinoma (figure 7), squamous cell carcinoma, Bowens disease, and keratoacanthoma. There are also a few nucleus coloration in epidermis close to tumor.

This experimental result showed expression of un and p−c−jun were increased obviously in all kinds of cutaneous tumors, and the expression of JunB and c−fos decreased observably or even disappeared. Also JunD highly expressed in all kinds of cutaneous tumors and the epidermis around the tumor, while the expression of Fra−1 and Fra−2 were unconspicuous in all kinds of cutaneous tumors and the epidermis close to tumor (table 2). In epidermis around tumor, the expressiones of JunB and JunD were in basal cell layer, prickle cell layer, and granular cell layer, expression of c−fos was in basal cell layer and prickle cell layer, the other subunits of AP−1 only appeared in basal cell layer (table 3). the expressiones of c−jun, p−c−jun, JunB and JunD appeared also in interstitial cell around some tumor.

DISCUSSION

Activating transcription factor AP−1 is a kind of gene expression activating protein. It can combine some

special target gene at AP−1 combination point, according to the signals outside cells, in this way, AP−1 can regulate cell´s physiology and pathology process. AP−1 has two subunit called Jun and Fos. In Jun family, c−jun, JunB and JunD can compose homodimer, compose heterodimer with the numbers of Fos family. But in Fos family, c− fos, FosB, Fra−1 and Fra−2 can compose heterodimer only with the numbers of Jun family, which is the only way to express their activity. In this experiment a numbers of subunits appear co−expression in cutaneous tumor and epidermis close to the tumor, which correspond with intercoordination characteristic among each subunit protein.

c−jun is a positive control factor to promote development, it can induce cellular tumor transform in mammalian animals. But this transform need co−expression of some activated oncogenes such as Ras and Src [1], it also need serine phosphorylation in 63 and 73 point in amino terminus of c−jun [2]. Dominant negative mutant of c−jun can express in murid and squamous cell carcinoma of human, which can block infiltrative growth of tumor. Transgenic mice which contain c−jun dominant negative mutant can inhibit tumor accelerator DMBA−TPA. Contrary, JunB (JunD maybe) has the function of down−regulating cellular proliferation, and inhibit tumor growth. Overexpression of murine fibroblast from JunB has long G1 cycle time. Cell premature aging and low hyperplasia [3], Transgenic mice which lack of JunB in myeloid cell series develop a great quantity myeloblast and generous neutrophil, causing a kind of leukemia. Another reports show JunB also lack of the ability of cell transform.

Table 2 Expression of AP−1 subunits in skin tumor

	basal cell carcinoma	squamous cell carcinoma	Bowens disease	keratoacanthoma
c−jun ↑	13/13	2/5	2/2	3/4
P−c−jun ↑	11/13	3/5	2/2	3/4
JunB ↓	13/13	0/5	0/2	2/4
JunD−−	10/13	4/5	2/2	3/4
C−fos ↓	13/13	1/5	0/2	1/4
Fra−1−−	9/13	3/5	0/2	4/4
Fra−2−−	10/13	5/5	2/2	4/4

Table 3 Expression of AP−1 subunits in the skin which close to the tumor

	basal cell layer	prickle cell layer	granular cell layer
c−jun	−~+		
P−c−jun	−~+		
JunB	+++	+	+
JunD−	+++	+	+
C−fos	++	+	
Fra−1−	−~+		
Fra−2	−~+		

In this study, expression of c−jun increased obviously in most cutaneous tumor, and expression mode of p−c−jun is almost coincide with c−jun, which means c−jun especially phosphorylation c−jun can promote tumorigenesis in cutaneous tumor of human.Meanwhile, we find that the expressions of c−jun and p−c−jun were not significant difference in the following tumor, such as squamous cell carcinoma which is malignant highly, Bowens disease which is low−malignant, basal cell carcinoma and keratoacanthoma which is a kind of precancerous lesion, which means AP−1 can not be used as marking to distinguish a tumor being malignant or not,it may just play a role in early stage of tumor generation, and is just a accidental element in the beginning of the tumorigenesis.

Up to date research on oral squamous carcinoma (SC)showed that c−jun was related with tumor´s differentiation, the lower differentiation, the lower expression of c−jun[5].

In this study, the number of cutaneous tumor was less, especially the low differentiation SC lack.So, we can not make sure the expression of c−jun has any connection to the differentiation of cutaneous tumor. Compared with the" normal" epidermis close to the tumor, the expression of JunB highly decreased even to disappeared in 13 basal cell carcinoma and 4 keratoacanthoma, which support that JunB is a inhibition factor to cellular proliferation. The decrease of its expression in skin has close connection to tumor´s development. The expression of JunB in squamous cell carcinoma and Bowens disease has no change, which showed there is

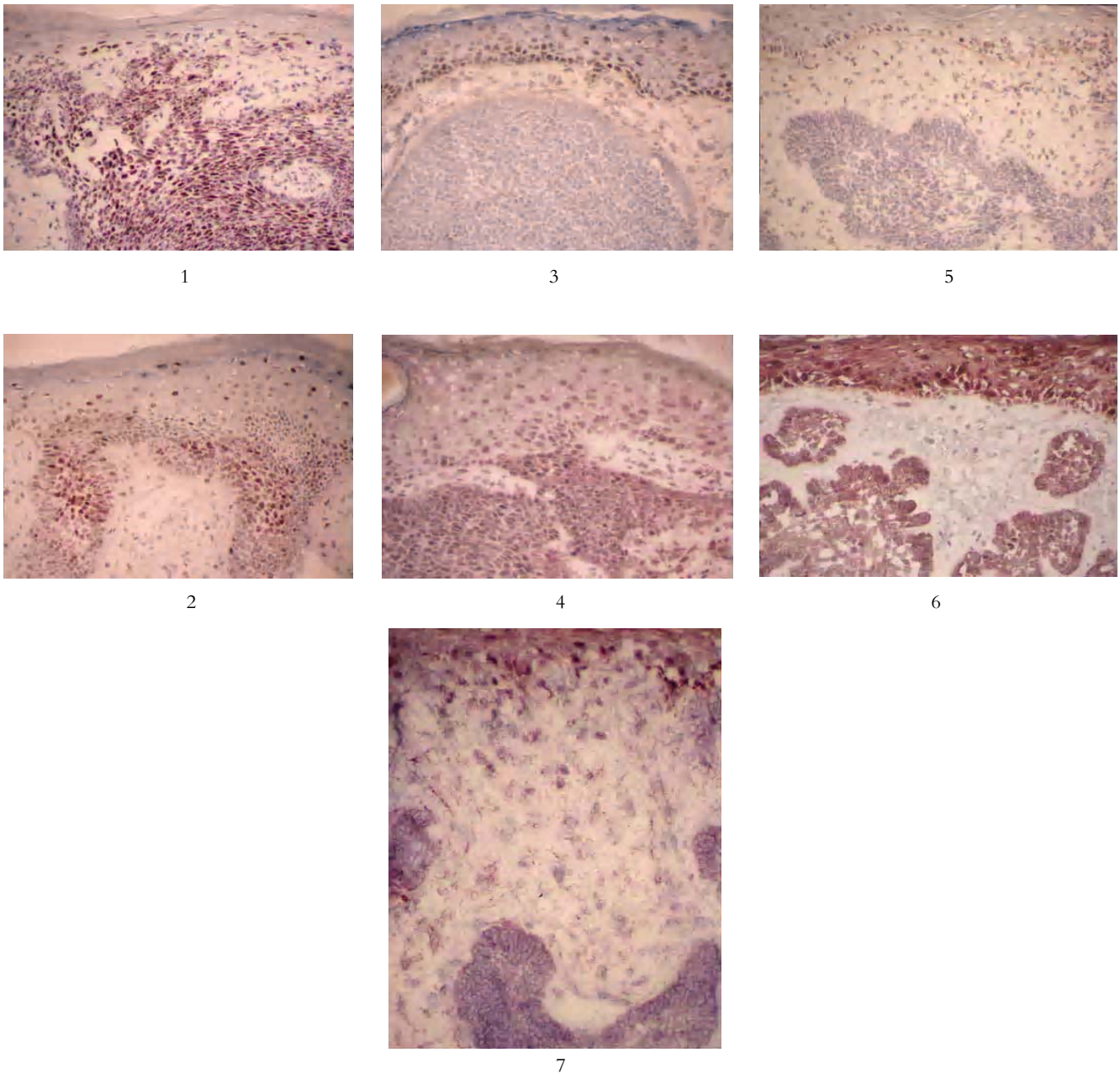

Fig. 1–7 The expression of AP−1 subunits in basal cell carcinoma
Immunohisochemistry showed that there is substantial number of BCC cell nuclei stained with anti−c−jun (figure 1), anti−p−c−jun (figure 2) and anti−JunD (figure 4) antibodies. There is few BCC cell nuclei stained with anti−JunB (figure 3), anti c−fos (figure 5), anti−Fra−1 (figure 6) and anti−Fra−2 (figure 7) antibodies. Original magnifications, ×200.

different mode of gene regulation in different cutaneous tumor development.

In transgenic mice, ectopic expression of c−fos can lead osteoblast transform to osteogenic sarcoma [6].In mouse that lack of c−fos, papillary epithelioma can not transform to aggressive squamous cell carcinoma. Fos−B can induce fibroblast transform in vitro[7], but Fra−1 and Fra−2 only have weak cell transform ability[8]. In spite of c− fos, FosB, Fra−1 and Fra−2 can be quickly induced by growth factor, but they are not essential to cell cycle, the fibroblast and embryonic stem cells without expression of these gene have no defect in hyperplasia [9]. In this experiment, expression of c−fos did not show increase markedly in this group cutaneous tumor,which surprised us a lot. Contrary its expression decreased in

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