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A STUDY OF MULTI-GENE EXPRESSION IN THE HIGHLY METASTASIZING HUMAN OVARIAN CANCER CELL LINE HO-8910PM AND ITS MOTHER CELL LINE HO-8910*

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Objective: To investigate multi-gene expression in the highly metastasizing human ovarian cancer cell line HO-8910PM and its mother cell line HO-8910. Method: The expression of 9 kinds of gene products in HO-8910PM and its mother cell line HO-8910 was detected by S–P immunohistochemical method. Result: Eight kinds oncogene products showed various degrees of positive expression in both HO-8910PM and HO-8910 cell lines except gene bax. The expression of P53, Cyclin D_1, CD_{44} V6 and EGFR in HO-8910PM was stronger than that in HO-8910. However, the expression of P16, nm23 in HO-8910PM was weaker than that in HO-8910. There was no significant difference on the expression of C-*erb*B-2 and *bcl*-2 between the two cell lines. Conclusion: Stronger invasive and metastatic patential is found in HO-8910PM than that in HO-8910. Carcinogenesis is a result of multi-oncogene and multiple step process cooperation.

Key words: Ovarian cancer, Oncogene, Tumor suppresser gene, Immunohistochemistry.

Researches of tumor molecular biology in recent years have proved that proto-oncogene and tumor supressor gene play very important role in the process of carcinogenesis and its progress. To explore the role of some oncogenes and their relativities in the process of ovarian carcinogenesis, progression and metastasis, we took a comparatine study of multi-gene expressions in the highly metastatic human ovarian cancer cell line HO-8910PM and its mother cell line HO-8910 by S-P immunohistochemical methods.

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METERIALS AND MTHODS

The Highly Metastatic Human Ovarian Cancer Cell Line HO-8910PM

Human poorly differentiated ovarian serous cyst-adenocarcinoma cell line (HO-8910)[1] were transplanted into subcutis of nude mice to establish the model of highly metastatic human ovarian cancer transplanted subcutaneously into nude mice (NSMO).[2] Tissue from the tumor mass of a nude mouse of 7th subtransplantation of the highly metastasizing human ovarian cancer model (NSMO) was cultured *in vitro* and the cell line HO-8910PM was established. Cells of the subcultures grown on coverslips for 4 days were fixed in 95% alcohol, at last HE and immunohistochemistry staining were performed.

Human Ovarian Cancer Cell Line HO-8910

The process of its coverslips for HE and immunohistochemistry staining was the same as HO-8910PM.

Immunohistochemistry

Gene antibodies, conceatration and sources is

shown in Table 1. SP kit from Coullter-Immunotech Company. All samples before staining were treated in a microwave oven for antigen retrieval. Sections known to be the corresponding antibody positive expression was used as positive control, and negative control was obtained by adding TBS solution instead of the primary antibody.

Results Assessment

According to variable expression characters of different gene products, the positive staining might be located in cell membrane, cytoplasm or nuclear. Its staining presented yellow granules. Expression degree in include staining intensity and the proportion of positive cells were classified as follow:

"–" no staining positive cell.

"+" weakly staining or random staining cells and positive cells proportion <10%.

"++" moderate staining or positive cells proportion between 11–30%.

"+++" strong staining, positive cell proportion >30%.

RESULTS

Morphology under Light Microscope

Morphology under light microscope of both two cell lines had no significant difference. The cells were all of malignant epithelioid type and were mostly of polygonal shape. In some cells the cytoplasm was vacuolated. The enlarged and deeply stained nuclei were adherent to the cytoplasmic membrane. The nucleoli were enlarged and heterochromatin formed masses. Mitotic figures could be found easily. The epithelioid cells were in pavement arrangement. A few spindle-shaped and round-shaped cells were seen. Occasionally multinuclear giant cells with nuclei in wreath form were seen and the nucleus arranged like garland or cluster.

Table 1. Polygene products expressions in highly metastatic human ovarian carcinoma cell line HO-8910PM and its mother cell line HO-8910

Gene (products)	Source	Concentration	Positive intensity		Positive location
			HO-8910	HO-8910PM	
P53 (Do-7)	DAKO	1:50	+	++	Mainly in nuclear occasionally cytoplasm
P16 (F12)	Santa Cruz	1:100	+++	+	Mainly in cytoplasm occasionally nuclear
Cyclin D1 (HD_{11})	Santa Cruz	1:50	+	+++	Cytoplasm and nuclear, especially in multi-nuclear giant cell
bcl-2 (124)	DAKO	1:80	+	+	Cytoplasm
nm23 (NM-301)	Santa Cruz	1:50	+++	++	Mainly cytoplasm, especially arround nuclear
CD44 v6 ($2F_{10}$)	Zymed	1:50	++	+++	Mainly in cell membrane occasionally cytoplasm
C-*erb*B-2	DAKO	1:200	+++	+++	Cell membrane or cytoplasm
EGFR	Santa Cruz	1:200	++	+++	Cell membrane or cytoplasm
Bax ($4F_{11}$)	Coulter-Immunotech	1:50	–	–	

Immunostaining Results

The corresponding gene product expressions in HO-8910PM and its mother cell line HO-8910 is listed in Table 1. Table 1 showed that 8 kinds of gene products were positive immunostaining with various intensity in both two cell lines. The result suggested that both two cell lines had the same immunological

phenotype of these genes. The expression degrees of P53, Cyclin D1, CD44 v6, EGFR in HO-8910PM cell line were stronger than in its mother cell line HO-8910. But for nm23, P16, the expression degrees were weaker in HO-8910PM, for C-*erb*B-2 and *bcl*-2, there were no significant difference between expressions in two cell lines. The difference of expression degrees between two cell lines indicated that there existed some difference in proto/anti-oncogene, proto/anti-metastasis and proto/anti-apoptosis related genes between two cell lines. Also, the study showed different biological behaviors between two cell lines in the process of cancer cell growth, proliferation and differentiation. The results demonstrated that the highly metastatic cell line HO-8910PM had more invasive and metastatic potentiality than its mother cell line HO-8910.

DISCUSSION

Carcinogenesis, progression and differentiation are closely related to the cell cycle control. Molecular mechanism of the cell cycle control involved cyclin, cyclin-dependen kinases (CDKs) and CDKs suppressor protein. In the different phase of cell cycle, cyclin can binds to the corresponding CDKs, and the cyclin-CDKs complex can make CDK4 to catalysis Rb protein phosphorylation and regulate G1, S and G2 phase transition, thus promoting cell to mitoses. Wt P53 participates as a "molecular policeman" in G1, monitoring the integrity of cell genome. It induces P21 and inhibits CDKs, thus arresting the cell in G1 to give the cell time for repair of DNA damage. P16 can contends with cyclin D to bind to CDK4, thus inhibiting cell proliferation. It is proved that oncogene abnormally activated and anti-oncogene inactivated or the coding products overexpressed can make the cell cycle out of control and carcinogenesis. Metastasis of carcinoma is also controlled by various of genes or their products. Proto-metastasis related genes over-expressed or/and auti-metastasis related genes inactivated can make carcinoma metastasis happen. It has been demonstrated that there is heterogenesis between tumor cell groups and some of cell cubgroups have more metastatic potentiality.[5] We believe that it will be an important step in the research of metastatic mechanism to explore the characteristies of those cell subgroups with metastatic potentiality by some special gene labelling methods. In our study, expressions of P53, cyclin D1 in the highly metastatic cell line HO-8910PM were stronger than in its mother cell line HO-8910. But P16 expression was weaker in HO-8910PM.

NM23 has been proved to be a carcinoma cell metastatic supressor gene. It has a high homogenesis with NDPK in the cell, directly affecting the biological activity of cytoskeleton proteins such as microtube and microfilaments, then inhibiting cancer cell metastasis. In this study nm23 expression was weaker in HO-8910PM than in HO-8910.

CD44, as adhesion moleculer distributed extensively in the cell surface, is a kind of transmembrane glycoprotein, which can binds to stromatins or moleculars in ground substance such as hyaluronic acid, fibronectin, collagen, laminin, etc, and thus participating in cell moving. CD44 v6 is splice variants of CD44, which is closely related to carcinoma invasing and metastasizing. In this paper, CD44 v6 expression was stronger in HO-8910PM than in its mother cell line HO-8910.

C-*erb*B-2 and EGFR is a group of growth factor receptor transmembrane glycoprotein with highly homologous sequence and similar function. Its gene overexpression may be directly related to carcinoma progression, malignant degree and invasiveness, thus affecting the prognosis.

In our study, EGFR expression was stronger in HO-8910PM than in HO-8910.

Through comparative study of multi-gene products deleted in HO-8910PM and its mother cell line HO-8910, we demonstrated that the highly metastatic cell line HO-8910PM had cancer heterogenicity, thus becoming a cell subgroup with more metastatic potentiality compared with its mother cell line HO-8910. The results also indicates that carcinogenesis, progression and metastasis are a process of multi-gene parti-cipation and multiple steps process cooperation. A certain gene abnormal change may only affect some biological characteristics in the certain stage of carcinoma progression.

Apoptosis or genetic program coding cell death, is a phenomenon of physiological cell suicide to maintain the balance between cell proliferation and death in tissues and organs. In recent years, lots of researches proved that carcinogenesis in part involves the relative dysregulation between apoptosis related genes and proto-oncogenes,[8] such as *bcl*-2, which inhibits apoptosis induced by DNA damage, and bax, which inhibits *bcl*-2 and induces apoptosis.

In this study, *bcl*-2 positive expression was found

in both two cell lines, but bax expression was negative in both two cell lines. The results suggest that cell proliferation and differentiation of both two cell lines in part involves dysregulation between apoptosis related gene and proto-oncogenes.

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