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The Relationship Between Caveolin-1 and ERK1/2 in Urothelium Carcinoma

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ABSTRACT

To investigate the expression of Caveolin-1 in Urothelium carcinoma and normal urothelium, and preliminary discuss the relationship between Caveolin-1 and Extracellular signal regulated kinase (ERK.) Signal Pathway. Methods: (1) SP immunohistochemical and RT-PCR were used to detect the expression of Caveolin-1 in Urothelium carcinoma and normal urothelium. (2) Western blot analysis were used to detect the expression of Extracellular signal regulated kinase 1/2 (ERK1/2) and Phosphorylase-Extracellular signal regulated kinase 1/2(P-ERK1/2) in Caveolin-1 positive cases and negative cases. Results: The expression of Caveolin-1 in Urothelium carcinoma were significant higher than that in normal urothelium.($P<0.05$). Western blot revealed that The expression of Phosphorylase-Extracellular signal regulated kinase 1/2(P-ERK1/2) were significant higher in Caveolin-1 positive cases than that in negative cases. The relative expression levels of AKT in Caveolin-1 positive cases and negative cases were not statistically significant. Conclusion Up- regulated expression of cavelion-1 through activation of Extracellular signal regulated kinase 1/2 (ERK1/2) signaling pathway may play an important role in the process of Urothelium carcinoma, Cavelion-1 may be useful targets for prevent and treatment of Urothelium carcinoma.

KEYWORDS: Caveolin-1, ERK1/2, P-ERK1/2, Immunohistochemistry, Urothelium carcinoma, Western blot, RT-PCR.

INTRODUCTION

Urothelium carcinoma is a common malignant tumor of urinary system with high morbidity. It is proved that the generation and development of Urothelium carcinoma associated with a variety of carcinogenic factors. Its specific mechanism was not very clear at present. Caveolin-1 is the main structural protein of caveolae protein family, its function include cell membrane transport and signal transduction in the physiological state, tumor invasion and metastasis in pathologic state, the functions is based on the 50 ~ 100 nm utricle bubble structure, formed by the cell surface membrane retraction.

In many malignant tumors, the expression of Caveolin–1 were found abnormal, inferring it may be a tumor related genes. In different tumors, the specific mechanism of Caveolin-1 is not the same, and the research is less in bladder urothelial carcinoma. SP immunohistochemical and RT-PCR were used to detect the expression of Caveolin-1 in Urothelium carcinoma and normal urothelium in our study, the expression of ERK1/2 and P-ERK1/2 were further studied, , in order to probe into its specific mechanism.

MATERIALS AND METHODS

30 cases of urothelial carcinoma specimens from 2008 to 2013 in Chengde Traditional Chinese Medicine Hospital, were collected. Including 18 cases of male, 12 cases of female, 54~85 years old, average age (68.35 ± 6.64). Select 30 cases adjacent tissue (≥5 cm away from the carcinoma) as normal epithelium, all cases without radiotherapy, chemotherapy and immunotherapy. The corresponding paraffin embedding tissue block made into 4 μm thick serial section were used for immunohistochemical staining. Collect the corresponding fresh tissue at the same time, kept in liquid nitrogen for RT-PCR and Western blot.

Immunohistochemical

Rabbit Anti-human Caveolin-1 monoclonal antibody were bought from Chinese fir jinqiao biological technology co., LTD. SP kit and DAB chromogenic agent which were bought from Fujian new technology co., LTD. SP method were according to the kit instructions, Positive control groups were provided by reagent company and PBS was regarded as negative control groups.

RT-PCR analysis

AMV cDNA systhesis kit was bought from Takara co., LTD in Dalian. Primer was designed by Shanghai biological engineering company. The amplified fragment length of Caveolin-1 was 91bp, and β-actin was 250bp. The total RNA was prepared from 100 mg of fresh tissue stored in liquid nitrogen. The purity of the RNA was detected. The results showed that, if OD260/280 between 1.8-2.0, then without RNA degradation and protein pollution. cDNA were synthesized by reverse transcription of 0.3 μg total RNA. Reaction conditions as follows: 30℃, 10 min ; 42℃, 30 min ; 99℃, 5 min ; 5℃, 5 min。And the reverse transcription reaction system was 10 μL.. Caveolin-1 amplification reaction conditions as follows: 94 ℃, Pre degenerated 2 min, 94 ℃, degenerated, 30 s, 59℃, annealing 30 s, 72 ℃ for 1 min, 30 cycles, 10 ℃ for 10 min. 20 uL amplification products together with 6 uL DNA Ladder were used for 4% agarose gel electrophoresis (120 v, 45 min). Take photo with ZF type ultraviolet reflex analyzer. Using Quantity one 4.62 statistical software to count the integral optical density, integral optical density value ratio was used to analyze the differences between the groups.

Western blot

Rabbit Anti-human ERK1/2 and P-ERK1/2 monoclonal antibody were bought from Chinese fir jinqiao biological technology co., LTD. Fresh tissues were kept in- 80 ℃. Total protein concentration was determined by BCA working liquid. It was carried out with 12% Separation gel and 5% stacking gel. Loading quantity was according to the calculated result. Load 1×loading Buffer, 80V, control the voltage to 120 v when the marker reach the Separation gel. After the electrophoresis, cut according to the purpose, 130mA, voltage steady, transferred, 2h. After TBST liquid containing 5% skimmed milk closed. each membrane respectively incubated with anti-1. PVDF membrane were washed three times, 15,10,10min, dilute second antibody at 1:5000, add second antibody and keep 1h, developing and fixing. Readed the stripes width and grey value by Image J Image .The data was analyzed by statistical Microsoft.

Results of Evaluation

The positive staining for Caveolin-1 cells was expressed as dark brown granules, which were mainly located in Cytoplasm's under microscopy .The percentage of positive cells was divided into five grades (percentage cores): ≤ 5%=score 0; 6%-20%=score 1; 21%-50%=score 2; 51%-75%=score 3; and > 75%=score 4; the scores ≤ 1 was defined as negative, and > 1 as positive.

Statistical Analysis

SPSS18.0 statistical package was used to analyze data. The relationship between protein expression and clinical pathological indicators are determined by using chi-square test, $P < 0.05$ as statistically significant. RT-PCR and Western blot results were expressed with $\bar{x} \pm S$, with independent sample t test (Independent sample t text), $P<0.05$ as statistically significant.

RESULTS

Expression of Caveolin-1 in Urothelium carcinoma and normal urothelium

Immunohistochemical results show that, in 30 cases of Urothelium carcinoma, the expression rate of Caveolin-1 was 63.33% (19/30), in 30 cases of normal urothelium, the expression rate of Caveolin-1 was 36.36% (8/30), having a significant difference（$P<0.05$）. RT-PCR show that, the expression of Caveolin-1mRNA in normal urothelium was 0.54 ± 0.23, was lower than that in Urothelium carcinoma (1.25 ± 0.07), and the difference between the two groups with statistical significance.（$P<0.05$）(Fig 1, Table 1).

Expression of ERK1/2 and P-ERK1/2 in Caveolin-1 positive and negative group

Out of 30 cases of urothelial carcinoma, 19 cases with Caveolin-1 positive, and 22 negative. So we divided 30 cases into Caveolin-1-positive-group and Caveolin-1-negative-group. Western blot were used to detect the expression of ERK1/2 and P-ERK1/2 in each group. Results show that the expression of ERK1/2 was higher in Caveolin-1-positive-group（1.37 ± 0.14）than that in negative one (0.92 ± 0.09). The expression of P-ERK1/2 in Caveolin-1-positive-group was 1.27 ± 0.12, had no significant difference with negative one（1.21 ± 0.98）(Fig 2).

DISCUSSION

Caveolae genes were discovered by Palade in 1953, protein encoded by this gene named plasma membrane vesicles, also known as Caveolae protein. In Caveolae protein family, including Caveolin-1, Caveolin-2 and Caveolin-3. Caveolin is regarded as a marker protein[1]. Caveolin-1 gene located in 7q31.1, is a kind of integral protein in cell membrane, and the the relative molecular mass is 21 ~ 24 KD[2]. Caveolin-1 exists widely in a variety of human tissue. In tumor tissues, changes such as gene deletion, mutation or up-regulated, prompt the protein may act as both oncogenes and tumor suppressors. Immunohistochemical S-P method were used in gastric carcinoma and precancerous changes and chronic superficial gastritis[3] to detect the expression of Caveolin–1, confirmed that the expression of Caveolin-1 were higher in adenocarcinoma, intestinal metaplasia and hyperplasia tissue than in chronic superficial gastritis, and associated with lymph node. Flow cytometry immune method[4] were used to detect the quantitative expression of Caveolin-1 in ovarian tissue, the results showed that, the average fluorescence index of Caveolin-1was lower than that in normal ovarian tissue, prompting the low expression of Caveolin-1 in

Table 1: The expression of Caveolin-1 in Urothelium carcinoma and normal urothelium

	Normal urothelium				Urothelium carcinoma				P
IHC	N	positive	negative	Positive rate	N	positive	negative	Positive rate	0.02
	30	8	22	36.36%	30	19	11	63.33%	
RT-PCR	0.54±0.23				1.25±0.07				0.04

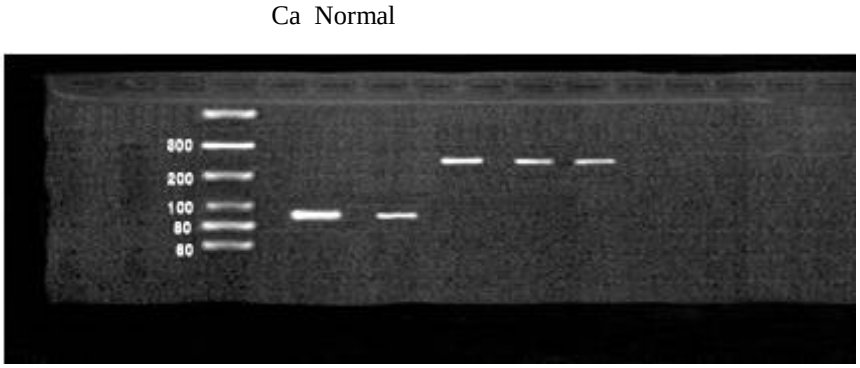

Fig 1: The expression of Caveolin-1 in Urothelium carcinoma and normal urothelium by RT-PCR

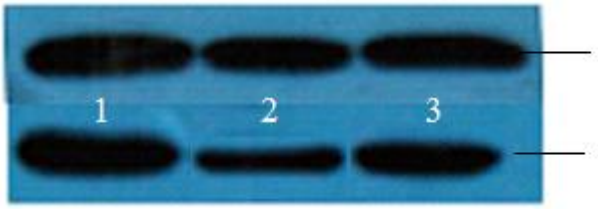

Fig 2: The expression of P-ERK1/2 in Caveolin-1 positive and negative group.

Ovarian cancer, and it maybe a tumor suppressor genes. Many studies found low expression of Caveolin-1 in cervical cancer, breast cancer, pancreatic cancer, lung and colon cancer, but high expression in prostate cancer, bladder cancer, esophageal cancer and osteosarcoma tissue.[5-8] Immunohistochemical results show that, in 30 cases of Urothelium carcinoma, the expression rate of Caveolin-1 was 63.33% (19/30), in 30 cases of normal urothelium, the expression rate of Caveolin-1 was 36.36% (8/30), having a significant difference （P<0.0）RT-PCR show that, the expression of Caveolin-1mRNA in normal urothelium was 0.54 ± 0.23, was lower than that in Urothelium carcinoma (1.25±0.07), and the difference between the two groups with statistical significance.（P<0.05）.

Ras[9] signaling pathway is also called Ras/Raf/MEK/ERK signaling pathway or ERK signaling pathway, which play a key role in many physiological and pathological regulation, ERK(Extracellular signal regulated kinase) is an important signal molecules in the pathway. p－ERK) is the activated state of ERK, and plays an important role in proliferation and cell cycle, which may be associated with urothelial carcinoma. Studies have shown that a variety of signal molecules in signaling pathways including ERK1/2 were decorated in Caveolin-1. In non-small cell lung cancer[10], Caveolin – 1 activated the receptor by combining with cell epidermal growth factor, and then active p42/44MAPK to promote the proliferation of tumor cells. Our experiment results show that the expression of ERK1/2 was higher in Caveolin-1-positive-group （1.37 ± 0.14） than that in negative one (0.92±0.09). The expression of P-ERK1/2 in Caveolin-1-positive-group was 1.27 ± 0.12, had no significant difference with negative one (1.21 ± 0.98). Caveolin-1 may play a role by Ras signaling pathways in Urothelial carcinoma.

The occurrence of urothelial carcinoma is the effect of multiple factors, Caveolin-1 is expected to be new targets for the prevention and treatment, and expand the new way of biological treatment.

REFERENCES

1. Anderson RG. The caveolae membrane system. Annu Rev Bichem, 1998, 67: 199- 225.

2. Zhao xing, Zhao Yuyang, Wang Jun. Expressions of Caveoli n- 1 in esophageal carcinoma and it's clinical significance. Chin J Health Care Med, 2014,14(3): 177~178.

3. XU Wen ji, ZHUANG Yingying, CHEN Yuli, et al. The expression ofCaveolin -1 in gastric adenocarcinoma and preneoplastic changes. Chin J Gastroentero lH epato, 2010,19(3): 209~212.

4. Du Xiaoli, Zhu Huifang. Expression of Caveolin-1 gene proteion in ovarian serous carcinoma and its clinical significance. Chinese Medical. 2015,12 (1): 35~38.

5. Fong A Garcia E Gwynn L et al. Expression of caveolin-1 and caveolin -2 in urothelial carcinoma of the urinary bladder correlates with tumor grade and squamous differentiation [J]. Am J Clin Pathol 2003 120 1 93-100.

6. Witkiewicz AK, Dasgupta A, Sotgia F, et al. An absence of stromal caveolin－1 expression predicts early tumor recurrence and poor clinical outcome in human breast cancers [J]. Am J Pathol, 2009, 174:2023-2034.

7. Songserm T Pongrakhananon V Chanvorachote P. Sub-toxic cisplatin mediates anoikis resistance through hydrogen peroxide induced Caveolin-1 up-regulation in non-small cell lung cancer cells [J]. Anticancer Res. 2012,32 (5): 1659-1669.

8. Ochoa-Hern ndez AB, Ju rez-V zquez CI, Reynoso MAR et al. WNT- -catenin signaling
pathway and its relationship with cancer [J]. Cir Cir, 2012, 80(4): 389~398.

9. McCubrey J A, Steelman L S, Chappell W H, et al. Roles of the Raf /MEK/ERK pathway in cell growth, malignant transformation and drug resistance [J[Biochim Biophys Acta, 2007, 1773(8):1263-1284.

10. Songserm T Pongrakhananon V Chanvorachote P. Sub-toxic cisplatin mediates anoikis resistance through hydrogen peroxideinduced Caveolin-1 up-regulation in non-small cell lung cancer cells [J]. Anticancer Res, 2012 32(5):1659-1669.

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