Expression and Clinical Significance of Ras and Sos1 Proteins in Epithelial Ovarian Malignant Tumor Tissues: Postprint

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Abstract

Objective To detect the expression of Ras and Sos1 proteins in epithelial ovarian malignant tumors and analyze the correlation between the expression levels of these two proteins and clinicopathological indicators of epithelial ovarian malignant tumors.

Methods The expression of Ras and Sos1 proteins was detected using the immunohistochemical SABC method in 62 cases of epithelial ovarian malignant tumor tissues, 5 cases of borderline epithelial ovarian tumors, 15 cases of ovarian epithelial cystadenomas, and 18 cases of normal ovarian tissues, with statistical analysis of the correlation between the expression levels of the two proteins and indicators of tumor malignancy.

Results (1) The expression levels of Ras and Sos1 in epithelial ovarian malignant tumor tissues were significantly higher ($P<0.05$) than those in other tissues; (2) The expression sites of both proteins in epithelial ovarian malignant tumor cells were predominantly co-expression in the cell membrane and cytoplasm; (3) The expression level of Ras (optical density value) correlated with pathological classification ($P<0.05$): its expression in serous ovarian carcinoma was more significant than in other pathological types, whereas Sos1 protein expression showed no correlation with these pathological parameters; (4) Patients with high expression levels of both proteins had shorter disease-free survival times, but this was not statistically significant.

Conclusion Ras and Sos1 proteins may be intrinsically linked to the occurrence and development of epithelial ovarian malignant tumors; the expression of Ras protein shows tissue type differences, which can be used to support the specific diagnosis of ovarian serous adenocarcinoma; statistical analysis shows that patients with high expression levels of both proteins have shorter disease-free

survival times than those with low expression, but this result was not statistically significant, possibly due to the small number of cases, which requires further study with an expanded sample size.

Full Text

Preamble

Expressions of Ras and Sos1 in epithelial ovarian cancer tissues and their clinical significance

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Abstract

Objective: To detect the expression of Ras and Sos1 proteins in epithelial ovarian malignant tumors and analyze the correlation between their expression levels and clinicopathological parameters.
Methods: Immunohistochemical SABC method was used to detect Ras and Sos1 protein expression in 62 cases of epithelial ovarian malignant tumor tissue, 5 cases of borderline epithelial ovarian tumor, 15 cases of ovarian epithelial cystadenoma, and 18 cases of normal ovarian tissue. Statistical analysis was performed to examine the correlation between protein expression levels and tumor malignancy indicators.
Results: (1) The expression levels of both Ras and Sos1 in epithelial ovarian malignant tumor tissues were significantly higher than in other tissues ($P<0.05$). (2) Both proteins were predominantly co-expressed on the cell membrane and in the cytoplasm of epithelial ovarian malignant tumor cells. (3) Ras expression intensity (optical density value) correlated with pathological type ($P<0.05$), with expression being more pronounced in serous ovarian carcinoma than in other pathological types, whereas Sos1 protein expression showed no correlation with these pathological parameters. (4) Patients with high expression of both proteins had shorter disease-free survival, though this difference was not statistically significant.
Conclusion: Ras and Sos1 proteins may be intrinsically involved in the development and progression of epithelial ovarian malignant tumors. The tissue-specific variation in Ras expression can support the specific diagnosis of ovarian serous adenocarcinoma. Statistical analysis revealed that patients with high expression of both proteins had shorter disease-free survival than those with low expression, but this result was not significant, possibly due to the small sample size. Further studies with larger cohorts are needed.

Keywords: epithelial ovarian malignant tumor; immunohistochemistry; Ras;

Sos1

Introduction

Epithelial ovarian malignant tumor is the most lethal gynecological malignancy, with an overall 5-year survival rate not exceeding 40%, and both treatment protocols and prognostic outcomes have shown limited improvement over the past two decades [1-2]. Compared with other malignant tumors such as lung cancer and leukemia, targeted therapy for epithelial ovarian malignant tumors has yielded very limited survival benefits, making the search for specific therapeutic targets still potentially valuable.

Ras protein is a low-molecular-weight protein from the G protein family, composed of nearly 200 amino acids and located on the inner side of the cell membrane. It controls cell signal transduction through switching between activated and non-activated states and is a recognized proto-oncogene that inhibits apoptosis [3-4]. Point mutations in the Ras gene have been identified in various human tumors [5-7]. Sos1 (son of sevenless 1) is a guanine nucleotide exchange factor that promotes the conversion of Ras from its inactive to active state, serving as an activator of Ras [8]. Ras and Sos1 together constitute a signaling pathway that regulates cellular physiological processes [9]; key molecules in this pathway, both upstream and downstream, such as Grb2 and Raf-1, have been demonstrated to play important roles in the development, progression, and metastasis of epithelial ovarian malignant tumor cells [10-11]. Accumulating evidence suggests that Ras and the signaling pathway formed by Ras and Sos1 represent excellent targets for treating metastatic cancers [12-17].

While the relationship between Ras, Sos1, and their signaling pathway with ovarian cancer has been extensively studied at the cellular and molecular levels, research on their association with clinical and pathological features remains limited, particularly regarding the relationship with disease-free survival time, for which no domestic or international reports have been found. This study aims to investigate the expression of Ras and Sos1 proteins in epithelial ovarian tumors and explore the correlation between their expression levels and clinicopathological malignancy indicators as well as disease-free survival time, thereby providing experimental evidence for understanding the role of the Sos1/Ras protein signaling pathway in the development of epithelial ovarian malignant tumors and offering insights for biological targeted therapy.

Methods

1.1 Tissue Samples

We selected 62 cases of epithelial ovarian malignant tumor, 5 cases of borderline epithelial ovarian tumor, 15 cases of ovarian epithelial cystadenoma, and 18 cases of normal ovarian tissue diagnosed between 2008 and 2013 at the First Affiliated Hospital of Chongqing Medical University and Yongchuan Hospital Affiliated to

Chongqing Medical University. Cancer patients had not received preoperative chemotherapy or radiotherapy and provided informed consent before the study. All specimens were promptly paraffin-embedded after resection. Clinicopathological data for epithelial ovarian malignant tumor patients are shown in Table 1 . For comparison purposes, patients were divided into two groups based on the 2014 FIGO surgical-pathological staging system. According to the 2014 NCCN Clinical Practice Guidelines for Ovarian Cancer, which state that "cytoreductive surgery should aim for residual tumor lesions <1 cm in diameter, with complete removal of all visible lesions being optimal," we defined surgical satisfaction as "residual tumor lesion diameter <1 cm" being "satisfactory" and otherwise "unsatisfactory."

1.2 Experimental Reagents

Immunohistochemistry kits, DAB chromogen, and citric acid antigen retrieval solution (pH 6.0) were purchased from Beijing Zhongshan Golden Bridge Co., Ltd. Monoclonal antibodies against Ras and Sos1 were purchased from Santa Cruz Biotechnology, USA.

1.3.1 Immunohistochemical Staining

Paraffin-embedded specimens were sectioned consecutively at 4 m thickness, followed by dewaxing, hydration, microwave antigen retrieval, serum blocking, and incubation with primary and secondary antibodies for immunohistochemical staining (SP method). The working concentration of antibodies was 1:250.

1.3.2 Qualitative and Quantitative Detection of Immunohistochemical Expression

Qualitative assessment [18]: At least two pathologists reviewed the slides in a double-blind manner. Five high-power fields were randomly selected under microscopy. Staining intensity was scored into four grades based on the presence of brownish-yellow granules in tumor cells: (1) no staining: -; (2) light yellow: +; (3) brownish-yellow: ++; (4) dark brown: +++.

Quantitative measurement: After immunohistochemical staining, photographs were taken under the same microscope using identical exposure values and white balance settings. The ipwin32 image analysis software was used for optical density measurement, with three fields measured per specimen and the average value recorded.

1.4 Statistical Analysis

The positive expression rates of Ras and Sos1 proteins in various ovarian tissues were calculated. Qualitative data were compared using the chi-square test with correction formulas for pairwise comparisons, with $P<0.05$ considered statistically significant. Using clinicopathological malignancy indicators of epithelial

ovarian malignant tumors (including pathological grade, surgical-pathological stage, age, pathological type, and surgical satisfaction) as independent variables and the optical density values of both proteins as dependent variables, correlation coefficients were calculated and significance tests performed, with $P<0.05$ considered statistically significant to analyze the correlation between protein expression levels and clinicopathology. Survival follow-up data from patients with advanced poorly differentiated adenocarcinoma and serous epithelial ovarian malignant tumors were analyzed using Kaplan-Meier methods, with treatment initiation time as the starting point and recurrence time as the endpoint for disease-free survival. Cases lost to follow-up or deaths before recurrence were treated as censored data. Patients were divided into high and low expression groups based on median optical density values. All data were processed using SPSS 19.0 software.

Results

2.1 Expression of Ras and Sos1 in Different Ovarian Tissues

The expression of both proteins in different ovarian tissues is shown in Tables 2 and 3 , and Figures 1 [Figure 1: see original paper] and 2 [Figure 2: see original paper].

2.2 Expression Distribution of Ras and Sos1 Proteins in Epithelial Ovarian Malignant Tumor Cells

The expression distribution of Ras and Sos1 proteins in epithelial ovarian malignant tumor cells was observed in three patterns: expression on the cell membrane only, in the cytoplasm only, and in both locations simultaneously. As shown in Figure 3 [Figure 3: see original paper] and Table 4 , co-expression of Sos1 and Ras proteins on both the cell membrane and in the cytoplasm (71.19% and 82.26%, respectively) was significantly higher than expression on the membrane only (3.23% and 1.61%) or in the cytoplasm only (4.84% and 6.45%) ($P<0.005$), indicating that both proteins are predominantly co-expressed on the cell membrane and in the cytoplasm of epithelial ovarian malignant tumor cells.

2.3 Correlation Analysis of Ras and Sos1 Protein Expression with Clinicopathology in Epithelial Ovarian Cancer

Using SPSS 19.0 software, we analyzed the correlation between patient age, pathological type, surgical-pathological stage, pathological grade, and surgical satisfaction with the optical density values of Ras and Sos1 protein expression. The correlation coefficients and P values are shown in Table 5 . Ras expression intensity (optical density value) correlated with pathological type ($P<0.05$), while no other groups showed significant differences.

2.4 Analysis of Disease-Free Survival in Patients with Advanced Poorly Differentiated Adenocarcinoma and Serous Epithelial Ovarian Malignant Tumors

Thirty-two patients with advanced epithelial ovarian malignant tumors were included in the study. The median optical density values of Ras and Sos1 protein expression in tumor lesions were 0.12 and 0.88, respectively. Using these median values as cut-off points, patients were divided into high and low expression groups, with 17 patients in each high-expression group and 15 in each low-expression group.

Kaplan-Meier analysis was used to evaluate the impact of protein expression levels on disease-free survival. As shown in Figure 4 [Figure 4: see original paper], patients with higher expression of both proteins had shorter disease-free survival over time, though this difference was not statistically significant ($P>0.05$). The survival curves showed that the high-expression groups declined more steeply than the low-expression groups, but Log Rank tests revealed no significant difference ($P>0.05$).

Discussion

Since Ras was first described as an oncogene in 1982 [19-20], numerous studies targeting Ras and its downstream proteins have been conducted. The Ras network is vast and complex, with many interconnected pathways playing important roles [21]. According to existing reports, activated Ras genes have been found in various tumors including carcinomas, sarcomas, neuroblastomas, lymphosarcomas, and leukemias [22-23]. Bian Meilu et al. [24] found a Ras gene amplification rate of 43.8% in ovarian tumor tissues using Southern blot hybridization, with 89% of 9 well-differentiated patients showing Ras amplification. As a positive regulator of low-molecular-weight G proteins, Sos1 promotes Ras activation in the Ras signaling pathway. Studies have shown that Sos1 expression is high in epithelial ovarian cancer and positively correlates with Ras-GTP, the activated form of Ras protein [25]. These studies were based on laboratory research at the molecular level and achieved satisfactory results, but clinical applications remain limited.

Our immunohistochemical experiments examining Sos1 and Ras protein expression in various ovarian tissues demonstrated high expression in ovarian cancer tissues, consistent with findings from other tumor studies. We also investigated the correlation between their expression and clinicopathological indicators of tumor malignancy (patient age, pathological grade, surgical-pathological stage, surgical satisfaction, and pathological type). Although Sos1 expression intensity (optical density value) showed no correlation with these clinicopathological features ($P>0.05$), we found that Ras protein expression intensity was higher in serous ovarian carcinoma than in other types of epithelial ovarian malignant tumors. This suggests tissue-specific variation in Ras expression, which could support the specific diagnosis of ovarian serous adenocarcinoma: tissues with

higher optical density values are more likely to be serous adenocarcinoma.

Our survival analysis correlating disease-free survival time with different expression groups showed that survival curves for high-expression patients declined more steeply, consistent with theoretical predictions and potentially offering guidance for clinical prognosis. Although Log Rank, Breslow, and Tarone-Ware tests revealed no statistical significance, this likely reflects our small sample size (only 32 cases included in the disease-free survival analysis). Therefore, this study warrants further investigation with an expanded sample size to potentially apply Ras and Sos1 testing in clinical practice for ovarian cancer diagnosis, treatment, and prognostic assessment.

References

[1] Ferlay J, Shin H R, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008[J]. Int J Cancer, 2010, 127(12):
[2] Salehi F, Dunfield L, Phillips KP, et al. Risk factors for ovarian cancer: an overview with emphasis on hormonal factors[J]. J Toxicol Environ Health B Crit Rev, 2008, 11(3/4): 301-21.
[3] 朱一婧, 姜凤超. 以调控 Ras 信号传导为靶标的抗肿瘤药物研究进展 [J]. 药学学报, 2009, 44(1): 1-10.
[4] Zhao LH, Lobo S, Dong XW, et al. Erf4p and Erf2p form an endoplasmic Reticulum-associated complex involved in the plasma membrane localization of yeast Ras proteins[J]. J Biol Chem, 2002, 277(51): 49352-9.
[5] 于法常, 李胜, 牛作兴. K-Ras 基因与结直肠癌疗效 [J]. 国际肿瘤学杂志, 2014, 41(3): 214-6.
[6] Hollern DP, Yuwanita I, Andrechek ER. A mouse model with T58A mutations in Myc reduces the dependence on KRas mutations and has similarities to claudin-low human breast cancer[J]. Oncogene, 2013, 32(10): 1296-304.
[7] J nne PA, Shaw AT, Pereira JR, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: randomised, multicentre, placebo-controlled, phase 2 study[J]. Lancet Oncol, 2013, 14(1): 38-47.
[8] 陈慧君, 吴绪峰. 三重复合物 SOS1/EPS8/ABI1 的完整性决定上皮性卵巢恶性肿瘤细胞
[9] 宋艳, 周湘, 李慧芳, 等. 以 Ras 信号转导通路为靶点的抗肿瘤抑制剂的研究进展 [J]. 中南药学, 2009, 7(4): 293-6.
[10] Bourguignon, Y L, Zhu, et al. Hyaluronan promotes CD44v3-Vav2 interaction with Grb2-p185 HER2 and induces Rac1 and Ras signaling during ovarian tumor cell migration and growth[J]. J Biol Chem, 2001, 276(52): 48679-92.
[11] 毛甜甜. 上皮性卵巢恶性肿瘤中 EGFR, Raf-1, AKT 的表达及其意义 [D]. 南昌大学医学院, 2013.
[12] Lim K H, Counter C M. Reduction in the requirement of oncogenic Ras signaling to activation of PI3K/AKT pathway during tumor maintenance[J]. Cancer Cell, 2005, 8(5): 381-92.
[13] Brummelkamp TR, Bernards R, Agami R. Stable suppression of tumorigenicity by virus-mediated RNA interference[J]. Cancer Cell, 2002, 2(3): 243-7.
[14] Singh A, Greninger P, Rhodes D, et al. A gene expression signature as-

sociated with “K-Ras addiction’ ’ reveals regulators of EMT and tumor cell survival[J]. Cancer Cell, 2009, 15(6): 489-500.
[15] Chin L, Tam A, Pomerantz J, et al. Essential role for oncogenic Ras in tumour maintenance[J]. Nature, 1999, 400(6743): 468-72.
[16] Fisher GH, Wellen SL, Klimstra D, et al. Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes[J]. Genes Dev, 2001, 15(24): 3249-62.
[17] Collins MA, Brisset JC, Zhang Y, et al. Metastatic pancreatic can- cer is dependent on oncogenic Kras in mice[J]. Plos One, 2012, 7 (12): e49707-e49707.
[18] 商利. 食管鳞癌及癌前病变分子分型标志物研究 [D]. 北京协和医学院中国医学科学院/北京协和医学院/中国医学科学院/清华大学医学部, 2014.
[19] Der CJ, Krontiris TG, Cooper GM. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses[J]. Proc Natl Acad Sci USA, 1982, 79(11): 3637-40.
[20] Parada LF, Tabin CJ, Shih C, et al. Human EJ bladder carcinoma oncogene is homologue of harvey sarcoma virus ras gene[J]. Nature, 1982, 297(5866): 474-8.
[21] Barsagi D. RAS oncogenes: weaving a tumorigenic web[J]. Nat Rev Cancer, 2011, 11(11): 761-74.
[22] Mocoy MS. Charaeterizatin of human colon /lung cacrinoma oneogene[J]. Nature, 1983, 302(5903): 79-81.
[23] Pulciani S, Santos E, Lauver AV, et al. Oncogenes in solid human tumours[J]. Nature, 1982, 300(5892): 539-42.
[24] 卞美璐, 樊庆泊, 黄尚志, 等. 原癌基因 C-myc、C-N-ras、C-Ki-ras、C-erbB2 在卵巢癌组织中的扩增 [J]. 中华妇产科杂志, 1995(7):
[25] 彭慧娟. Crk 衔接蛋白介导之 DOCK180/Rac1、SOS1/Ras 信号通路对浆液性卵巢癌恶性程度的影响 [D]. 重庆: 重庆医科大学, 2013.

Note: Figure translations are in progress. See original paper for figures.