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Over-expression of nuclear NF-κB1 and c-Rel correlates with chemoresistance and prognosis of serous epithelial ovarian cancer

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ABSTRACT

Objective: This study aims to measure the expression and subcellular location of NF-κB1 and c-Rel protein in serous epithelial ovarian cancer (EOC), and to test the correlation between NF-κB1 and c-Rel expression and clinicopathological parameters, chemoresistance, and prognosis of serous EOC.
Methods: A total of 63 specimens of serous EOC patients meeting the inclusion criteria with complete follow-up data were enrolled in our study. The specimens were divided into chemo-resistant group and sensitive group. The expression and subcellular location of NF-κB1 and c-Rel were assessed in paraffin sections using immunohistochemistry. The relationship between NF-κB1 and c-Rel protein expression and pathological characteristics of serous EOC, chemoresistance, prognosis and survival time was analyzed.
Results: The positive nuclear staining of NF-κB1 and c-Rel were significantly higher in the chemo-resistant serous EOC specimens than that in chemo-sensitive group.
Lymph node metastasis and the nuclear expression of NF-κB1 and c-Rel were independent risk factors associated with chemotherapy resistance of serous EOC. Nucleus NF-κB1 and c-Rel expression along with FIGO stage were independent risk factors that strongly correlated with prognosis of serous EOC. Western blot result showed the protein level of NF-κB1 and c-Rel was significantly higher in chemoresistant group compared with in sensitive group.
Conclusions: Over-expression of nuclear NF-κB1 and c-Rel are strong risk factors associated with chemoresistance and the prognosis of serous epithelial ovarian cancer.

1. Introduction

Epithelial ovarian cancer (EOC) accounts for a majority of ovarian cancer and the mortality rate of which ranked the highest among all the gynecological malignancies (Siegel et al., 2013). Patients with EOC are often diagnosed with late-stage disease. The initial treatment is complete surgical debulking followed by chemotherapy which normally consists of paclitaxel and carboplatin (Aletti et al., 2007; Gubbels et al., 2010). However, 60 to 80% of the patients became recurrent between 6 months and 2 years after initial chemotherapy. As a result, the 5-year survival rate is still only 15 to 20% (McGuire and Markman, 2003). Thus searching for the potential biomarker for chemoresistance and to further study the mechanism is of great importance for serous EOC.

The nuclear factor κB (NF-κB) family of transcription factors could induce or repress gene expression by binding to discrete DNA sequences in promoters and enhancers. In mammalian, there are five subunits of NF-κB family including RelA (p65), RelB, c-Rel, p50 / p105 (NF-κB1) and p52 / p100 (NF-κB2). These subunits could form into homodimers and heterodimers (Ghosh et al., 1998; Gilmore et al., 1996; Karin, 1999). The NF-κB family of proteins have been reported to be involved in several key processes such as cell apoptotic and cell cycle and thereafter could contribute to cancer progression (Joyce et al., 2001; Karin and Lin, 2002).

To date, NF-κB family of transcription factors has been studied extensively in lymphoid development and lymphoid malignancies and NF-κB signaling also has been indicated in breast, colon, and lung carcinomas (Karin, 2006). More and more researchers are focusing on studying the importance of NF-κB family in ovarian cancer. For example, there is study about the NF-κB family in the propagation of ovarian cancer cell line (Lin et al., 2007). And researchers studied the expression of some subunits of NF-κB in ovarian cancer tissues (Annunziata et al., 2010; Giopanou et al., 2014; Guo et al., 2009). However until now, there is no report about the expression and the subcellular localization of NF-κB1 along with c-Rel and their relationship with chemoresistance of serous epithelial ovarian cancer.

Our study was the first to validate the differential expression and subcellular location of NF-κB1 and c-Rel between chemo-resistant serous EOC specimens and the chemo-sensitive group. And further indicate the association of these two subunits of NF-κB family factors to the chemoresistance and the prognosis of serous epithelial ovarian cancer.

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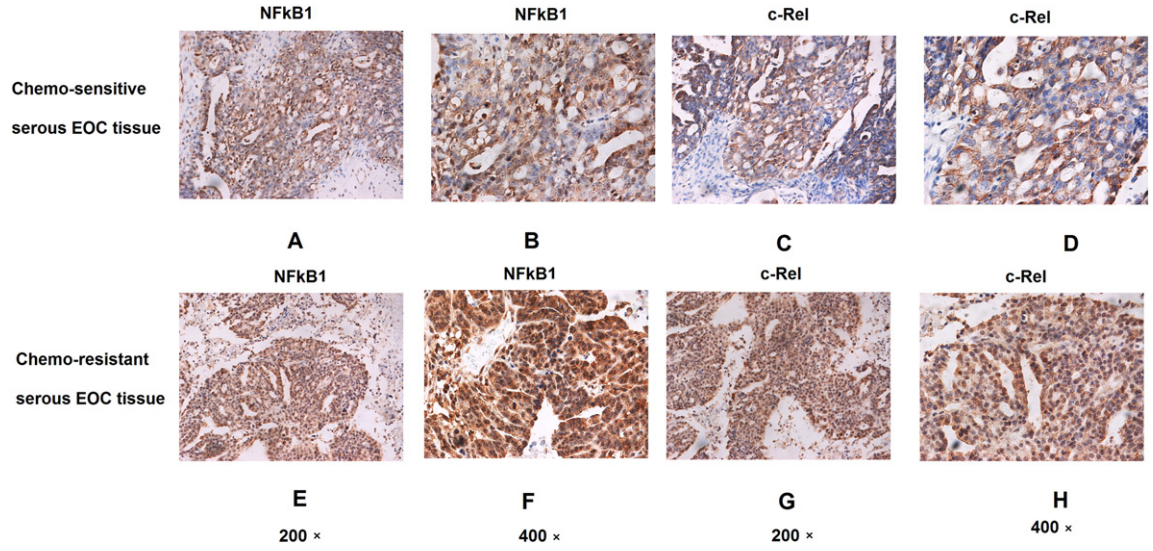

Fig. 1. Immunohistochemical staining was applied to detect the expression of NF-κB1 and c-Rel in serous epithelial ovarian cancer tissues. (A–B) show the expression of NF-κB1 in chemo-sensitive tissue. A, 200×, B, 400×. (C–D) show the expression of c-Rel in chemo-sensitive tissue. C, 200×, D, 400×. (E–F) show the expression of NF-κB1 in chemo-resistant tissue. E, 200×, F, 400×. (G–H) show the expression of c-Rel in chemo-resistant tissue. G, 200×, H, 400×.

2. Material and methods

2.1. Patients and specimens

The study population consists of 63 patients with serous epithelial ovarian cancer (EOC) diagnosed at the Shengjing Hospital of China Medical University between 2008 and 2013. Patients were treated with debulking surgery, followed by 6–8 cycles of chemotherapy (paclitaxel along with carboplatin regimen). Clinical information was obtained from medical records. Information includes age, surgical stage, grade, pathological subtype and lymph node metastasis. Our study was approved by the Institutional Review Board of Shengjing Hospital of China Medical University (Ethical review approval documents number: 2012PS57K).

2.2. Assessment of chemotherapy response

According to the NCCN guideline, we divided the patients into chemoresistance group (n = 28) and chemosensitive group (n = 35).To be specific, the chemoresistance group were determined as response to the initial chemotherapy but relapsed in the late stage of chemotherapy or within 6 months after completion of chemotherapy. And after chemotherapy, the recurrence occurs between 6 and 12 months was partial sensitive group and recurrence beyond 12 months after the chemotherapy or with no recurrence as chemosensitive group.

2.3. Immunohistochemistry

The paraffin embedding histological section of each serous EOC tissue was 5 μm. Tissues were dewaxed, and antigen retrieval was performed using a low pH buffer (pH = 6.0) for 15 min at 100 °C. Staining of NF-κB1 was performed using NF-κB1 rabbit anti-human polyclonal antibody (diluted 1:50), and staining of c-Rel was performed using c-Rel rabbit anti-human monoclonal antibody (diluted 1:50). Both of the antibodies were purchased from Abcam (Abcam Co, Cambridge, UK). Nuclear expression of NF-κB1 and c-Rel was scored using the following system: negative = 0, weak/focal = 1, strong focal/widespread moderate staining = 2, and strong/widespread staining = 3. Tumors that scored 2 or 3 were considered nuclear positive for NF-κB1 and c-Rel. The cytoplasmic expression of NF-κB1 and c-Rel was scored as following: The color intensity was divided into 4 levels, to be specific, no staining scores 0, light yellow scores 1, brown yellow scores 2, and dark brown scores 3. On the other hand, positive cells were counted under 400× magnification and scored as following: a positive rate of less than 5% was 0, 5–25% was 1, 26–50% was 2, 51–75% was 3, and more than 75% was 4.The scores for immunoreactive intensity and positive cell rate were then multiplied and the ultimate result was: 0–2 was negative (−), 3–4 was weakly positive (+), 5–8 was mildly positive (++), and 9–12 was (+++) was strongly positive. The results were read by two independent observers and made the scoring decision to control for variability.

2.4. Multi-variable analysis

Binary logistic regression analysis (forward: conditional) was applied to analyze the risk factors associated with chemoresistance in serous EOC patients. The binary logistic regression analysis was performed using age, FIGO stage, grade, metastasis of lymph nodes and the expression of nuclear expression of NF-κB1 and c-Rel.

Table 1
Expression of nuclear cytoplasmic NFKB1 protein in serous epithelial ovarian cancers tissues.

	NFKB1 nuclear expression						NFKB1 cytoplasmic expression					
	Case	0	1	2–3	Positive ratio	P	Case	0–2	3–4	5–8	Positive ratio	P
Sensitive group (n)	35	3	19	13	37.14%		35	5	14	16	85.71%	
Resistancegroup (n)	28	0	5	23	82.14%	0.001*	28	3	9	16	89%	0.602**

* NF-κB1 nuclear expression (sensitive group vs resistance group, P = 0.001).
** NF-κB1 cytoplasmic expression (sensitive group vs resistance group, P = 0.602).