PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2015, 42(2): 195~196
www.pibb.ac.cn

Chemoresistance to CDDP Induces EMT Through Activation of RAC1 in SKOV3 Human Ovarian Cancer Cells

Dear Editor,

The incidence rate of epithelial ovarian cancers ranks third among female reproductive system cancers, and its mortality rate stands first among the list of gynecologic tumors. The standard mode of treatment is cytoreductive surgery (debulking) plus the administration of platinum-based chemotherapy drugs. About 70% of advanced patients who receive standard treatments can achieve clinical remission. However, a certain percentage of patients with clinical remission will still experience tumor recurrence, metastasis, and drug resistance. Thus, an exploration on the common interrelated molecular mechanisms of epithelial ovarian cancer metastasis and drug resistance is the focus of our study. Epithelial-mesenchymal transition (EMT) is not only the basis of biological processes of embryonic development, but it also can lead to the enhancement of invasion and the metastasis ability of some epithelial tumor cells. At the same time, it can reduce the sensitivity of tumor cells to chemotherapy drugs[1–3]. RAC1, which is one of the members of the Rho subfamily, can mediate important signal transductions *via* an active form of GTP-RAC1, and play a role as a molecular switch in the regulation of cell biological behaviors, such as EMT[4–6]. And based on a large amount of basic research, RAC1, as one of the member of the Rho subfamily appears to play significant role in the proliferation, differentiation, apoptosis, movement, invasion, and transfer of various tumor cells as well as blood vessels[7]. Therefore, this study focuses on the occurrence process of EMT in SKOV3 cells, which is mediated by RAC1 activation, as well as the relationship between the process of cell sensitivity and cisplatin (CDDP), and the ability of invasion and metastasis. The Boyden chamber method was used to compare the migration and invasion ability between two kinds of cells, and the results showed that the adhesion and invasion abilities of SKOV3/DDP were significantly increased (Figure 1a, b). Meanwhile, we compared the expression level of GTP-RAC1 between two kinds of cells (SKOV3 and SKOV3/DDP). The results showed that the expression level of GTP-RAC1 in SKOV3/DDP was apparently higher than that in SKOV3 cells (Figure 1c). We compared the expression levels of EMT-related key proteins, such as E-cadherin (CDH1), Claudin (CLDN1), Vimentin (VIM), Snail1 (SNAI1) and Slug (SNAI2) cells, between two groups of cells using RT-PCR and Western blot methods. We found that in SKOV3/DDP cells, the expression levels of VIM and SNAI1 were increased, and the expression levels of CDH1 and CLDN1 were decreased (Figure 1d, e).

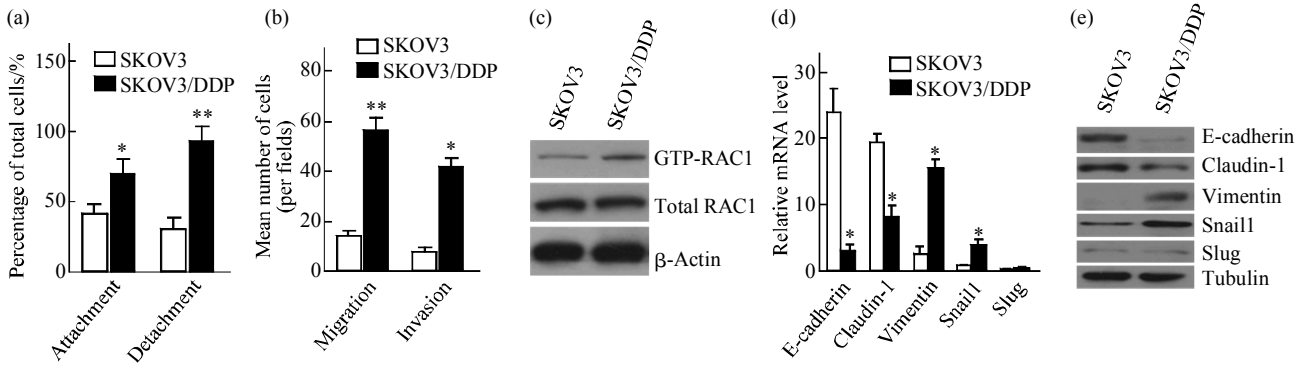

Fig. 1 Comparison between SKOV3 cisplatin-resistant cell line (SKOV3/DDP) and SKOV3 cells (SKOV3), the adhesion ability(a), the invasion and metastasis ability(b) and the expression level of activated RAC1 (GTP-RAC1)(c) in SKOV3/DDP cells were significantly higher than those in SKOV3 cells. Moreover, the SKOV3/DDP cells showed the gene(d) and protein(e) expressions of the mesenchymal-like cell associated markers

$^{*}P < 0.05$, $^{**}P < 0.01$.

From the perspective of EMT, the abovementioned study analyzed the common mechanism of decreased drug sensitivity and the increased invasion and metastasis ability in SKOV3 cells of epithelial ovarian cancers. This was to further confirm the impact of RAC1 activation on the abovementioned process. First, we chose to downregulate the expression of RAC1 in SKOV3/DDP cells with shRNA and observed a decreased RAC1 gene expression (SKOV3/DDP$^{RAC1\text{-}shRNA}$). We also compared the sensitivity of three kinds of cells, namely SKOV3, SKOV3/DDP, and SKOV3/DD$^{PRAC1\text{-}shRNA}$, to CDDP. The results showed that the drug sensitivity of SKOV3/DDP$^{RAC1\text{-}shRNA}$ was enhanced compared with SKOV3/DDP (Figure 2a). The downregulation of RAC1 in SKOV3/DDP cells may also decrease the adhesion and invasion ability (Figure 2b, c). In addition, compared with SKOV3, SKOV3/DDP$^{RAC1\text{-}shRNA}$ showed an obvious expression of epithelioid cell markers (Figure 2d, e).

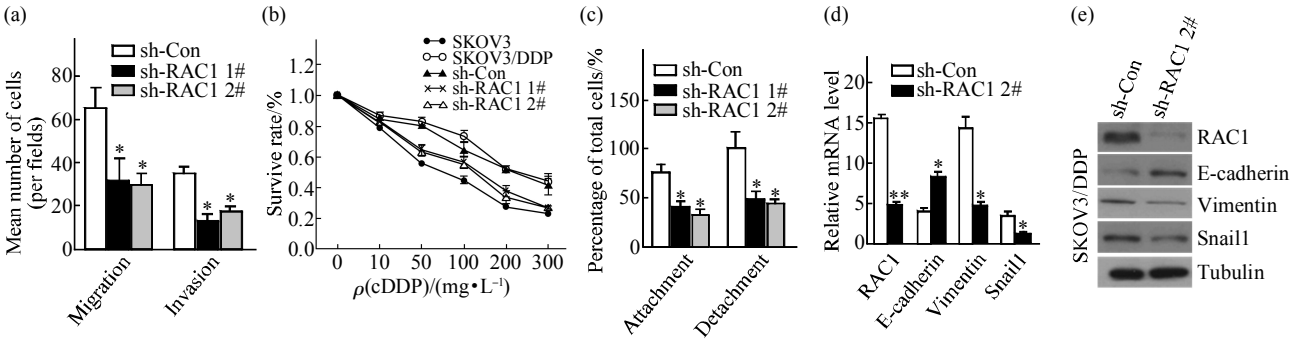

Fig. 2 After the RAC1 in SKOV3/DDP cells was interfered with sh-RNA technique, the drug sensitivity of SKOV3/DDP cells was increased(a). Meanwhile, the adhesion ability(b) and the invasion ability(c) of the cells were decreased, and the cells showed the gene(d) and protein(e) expressions of the epithelioid cell markers

*P < 0.05，**P < 0.01.

The study suggests that the expression and activation levels of RAC1 proteins in epithelial ovarian cancer cells can regulate the occurrence of change of EMT in cells, and the change is closely related to the drug sensitivities and the invasion and metastasis abilities of the cells. The process of occurrence of EMT in epithelial ovarian cancer cells maintains closer contact with the appearance and maintenance of tumor stem cells. Moreover, some studies showed that (CD44+/CD117+) ovarian cancer cells are closely related to the occurrence and development of tumors and the resistance to treatment [8−10]. The study on the "RAC1 activation-EMT process-activation or formation of ovarian cancer stem cells" is the current direction of our in-depth study.

Acknowledgements This work was supported by grants from The National Natural Science Foundation of China (81201730), China Postdoctoral Science Foundation Grant (2012M521565), National Basic Research Program of China (2012CB517902), National Natural Science Foundation of China (81330027).

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DOI: 10.16476/j.pibb.2015.0002

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