Regulatory Mechanisms of Annexin-Induced Chemotherapy Resistance in Cisplatin Resistant Lung Adenocarcinoma

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Abstract

Adenocarcinoma of lung has high incidence and a poor prognosis, with chemotherapy as the main therapeutic tool, most commonly with cisplatin. However, chemotherapy resistance develops in the majority of patients during clinical treatment. Mechanisms of resistance are complex and still unclear. Although annexin play important roles in various tumor resistance mechanisms, their actions in cisplatin-resistant lung adenocarcinoma remain unclear. Preliminary studies by our group found that in cisplatin-resistant lung cancer A549 cells and lung adenocarcinoma tissues, both mRNA and protein expression of annexins A1, A2 and A3 is increased. Using a library of annexin A1, A2 and A3 targeting combined molecules already established by ourselves we found that specific targeting decreased cisplatin-resistance. Taken together, the underlined effects of annexins A1, A2 and A3 on drug resistance and suggest molecular mechanisms in cisplatin-resistant A549 cells both in vivo and in vitro. Furthermore, the study points to improved research on occurrence and development of lung adenocarcinoma, with provision of effective targets and programmes for lung adenocarcinoma therapy in the clinic.

Keywords: Annexin - lung adenocarcinoma - chemotherapy - effective targets
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RT-PCR

Expression of Annexin A1, A2, A3 were analyzed by semiquantitative RT-PCR analysis. After the total RNA was prepared using a TRIZOL reagent (Life Technologies, Inc., 15596-026), the oligo (dT)-primed cDNA was synthesized using a RT-PCR kit (Agilent Technologies, 600182). The primers used in this study were summarized in Table 1.

Immunoblot analysis

After treatment with different conditions as described in the figure legends, cells were lysed in M2 buffer (20 mM Tris at pH 7.6, 0.5% NP-40, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, 2 mM DTT, 0.5 mM PMSF, 20 mM β-glycerol phosphate, 1 mM sodium vanadate, 1 mg/ml leupeptin). Fifty micrograms of the cell lysates were subjected to SDS-polyacrylamide gel and blotted onto PVDF membrane (Millipore, PVH00010). After blocking with 5% skim milk in TBS/T, the membrane was probed with the relevant antibody and visualized by enhanced chemiluminescence (ECL, RPN2106), according to the manufacturer’s instruction (Amersham).

Immunofluorescence analysis

HeLa cells were grown on glass coverslips and then infected with Ad-GFP-LC3. After 24h, the cells were treated as indicated in the figure legend and fixed with 4% paraformaldehyde for 10 min. Cells were permeabilized with PBS containing 0.2% Triton X-100 and 0.1 M glycine for 15 min, blocked with 10%BSA in PBS for 1 h and then incubated with rat anti-human

Statistical analysis

Data are expressed as the mean±SD from at least three separate experiments performed triplicate. The differences between groups were analyzed using Student’s t-test. *p<0.05 is considered statistically significant. Statistical analyses were performed using SPSS software ver. 13.0 (SPSS, Inc.).

Results

Firstly, in order to identify involvement of Annexins in cisplatin resistance, we constructed DDP resistant A549 lung adenocarcinoma stable cell line (A549/DDP), and compared expression of Annexin A1, A2 and A3 to A549 and A549/DDP in both mRNA and protein levels. Annexin A1, A2 and A3 highly expressed in A549/DDP cells (Figure 1 A, B), which suggested expression of Annexin A1, A2 and A3 closely related to cisplatin resistance. To further reveal the critical role of Annexin A1, A2 and A3 in cisplatin resistance in vivo, we observed the expression of Annexin A1, A2 and A3 in tumor tissues of lung adenocarcinoma patients. In consistent with in vitro data, expression of Annexin A1, A2 and A3 were up-regulated in cisplatin resistant patients’ tissues both in mRNA and protein levels (Figure 2). Taken together, Annexin A1, A2 and A3 overexpressed both in vitro and in vivo, suggested they are most likely to be key regulator of cisplatin resistance, thus pharmacological approaches targeting to Annexin A1, A2 and A3 are considered as effective therapy for cisplatin resistance. Therefore, screening through Crystallography and Molecular space simulation, we preliminarily constructed libraries of molecular compounds which could bind with Annexin A1, A2 and A3 respectively. Three compounds: compound 1, 2 and 3 which binding with Annexin A1, A2 and A3 respectively were selected and the structures are shown here (Figure 3). Annexin A1, A2 and A3 are all small molecule compounds, both A1 and A3 contain a benzo seven-membered-ring. A2 contains a benzo five-membered-ring, A1 and A3 contain an amide bond with α-amino. Treatment of A549/DDP cells with compound 1, 2 and 3, indeed, A549/DDP cells viability were dramatically decreased (Figure 4). The result confirmed effectiveness of...
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The annexin family is super family of calphobindin, that present in the intracellularly among major eucaryons, and play crucial physiologic functions, such as participating in cytoskeletal movement, regulating cell growth, forming ion channels, and participating in cellular signal transduction, among others (Mussunoor and Murray, 2008; Fatimathas and Moss, 2010). In recent years, studies have revealed that partial molecules enjoy close relationships with cancer emergence and development, as well as drug resistance (Gerke et al., 2005; Chen et al., 2012).

Compared with that of corresponding normal tissues, A1 enjoys significant differences in expression in precancerous lesions of various tissues and tumors. In addition, a large number of studies indicated that A1 does not consistent function in the development of drug-resistance in various tumors (Cao et al., 2008; Yu et al., 2008; Wang et al., 2010). High A1 expression increases drug resistance of breast adenocarcinoma; however, in NUGC cells, A1 deficiency is related to tumorigenesis and the development of resistance to the apoptosis induced by chemotherapeutic agents. Other studies have proven that A1 participates in the formation of intracellular multivesicle endosomes (Mussunoor and Murray, 2008). However, whether the multi-vesicle endosome participates in the transport of certain chemotherapeutic agents within tumor cells has not been proven thus far. Meanwhile, A2 is abnormally expressed in tumors, such as gastric cancer, colon cancer, prostate cancer, HCC, and pancreatic ductal adenocarcinoma, and is related to tumor invasion, metastasis, and prognosis. Moreover, previous studies reported that high A2 expression is related to the degree of malignancy, as well as invasion and metastasis (Emoto et al., 2001; Esposito et al., 2006; Sharma et al., 2006; Yee et al., 2007; Duncan et al., 2008). A2 is selectively expressed in lung adenocarcinoma cell lines. Furthermore, A2 promotes the invasion and metastasis of lung cancer cells. However, no statement has been made regarding its relationship with cisplatin-resistance in adenocarcinoma (Gillette et al., 2004; Huang et al., 2008). Meanwhile, recent research results indicated that A3 expression in the tissues of patients with cisplatin-resistant prostate cancer and ovarian cancer has increased significantly. In addition, the cycles of patients without tumors in the A3 high expression group are clearly shortened (Gillette et al., 2004; Huang et al., 2008). Related studies also prove that A3 produces drug resistance by reducing the platinum content, the platinum-DNA combining quantity within cells, and the p53 level in ovarian cancer cells (Yan et al., 2010). The tumorigenesis of cells with high A3 expression is significantly increased after cisplatin treatment, as demonstrated through an animal experiment. In addition, the resistance of cells with high A3 expression is clearly enhanced against platinum-based medicines; however, it does not induce cross-resistance with TAX and EPI (Yan et al., 2010).

Platinum resistance mechanisms, which are still unclear, mainly involve the following aspects: 1. Reduced drug accumulation within tumor cells, including reducing cellular drug intake and increasing active efflux; 2. Functions of tumor cells to withstand the transformation of cancer medicines, as well as enhanced detoxification; 3. Strengthened DNA repair by tumor cells; 4. In the induction of apoptosis by platinum-based medicines, the expression of regulatory proteins in the apoptosis pathway changes. Determining the mechanism underlying cisplatin resistance is of great significance to reverse the clinical tolerance and improve the 5-year survival rate of patients.

A1, A2, and A3 form intracellular membrane globules that participate in calcium-dependent endocytosis and exocytosis. By contrast, cisplatin resistance mainly reduces the drug intake of cells and increases active efflux. The experiment proves that the expression of A1, A2, and A3 increases significantly in cisplatin-resistant A549 cells, which indicates that these molecules participate in drug resistance in adenocarcinoma (Figure 1). If A1, A2, and A3 are specifically inhibited, the efflux and tolerance of cancer cells towards medications such as cisplatin may be reduced, thereby enabling their accumulation in cancer cells, reaching their effective concentrations, and ultimately inducing cancer apoptosis. Consequently, compound molecule libraries were simulated and established, and small molecules that combine with A1, A2, and A3 were specifically screened (Figure 2).

The paper studies the influence of A1, A2, and A3 expression on cisplatin resistance in lung adenocarcinoma in vitro and in vivo, and new content on the emergence and development drug resistance in lung adenocarcinoma is added. Meanwhile, A1, A2, and A3 were specifically targeted using small molecules to find new candidate...
compounds that effectively reverse drug resistance in adenocarcinoma, and provide more effective candidate treatment targets and drugs in the clinical treatment of lung adenocarcinoma.

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References


