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ORIGINAL ARTICLE

Establishment of Primary Human Epithelial Ovarian Cancer Cells and Their Application to Cytotoxicity Assessment

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Background: Ovarian carcinomas are highly metastatic tumors and the most lethal gynecological malignancies in the world. Treatment of human epithelial ovarian cancer (hEOC) is based on the combination of surgery and chemotherapy. The acquisition of resistance is a major obstacle to the clinical use of platinum drugs for 75% of high-grade serous ovarian cancer treatment. Hence, the urgent strategy is to develop platform to predict the resistance to targeted therapy after surgery and to screen the potential monotherapy, including platinum, in clinical treatment. **Materials and Methods:** Our laboratory tried to take advantage of ascites from hEOC patients to understand the mechanism of platinum-based resistance and screen the potential monotherapy choice via the primary culture strategy. We first established the culture conditions for ascites of the ovarian cancer patients. **Results:** We showed that we could culture these primary cells up to 13th generation. We further treated these primary cells with various steroid hormones for the epithelial-to-mesenchymal transient and regular monotherapy, including cisplatin, docetaxel, and doxorubicin, for the cytotoxicity. Finally, we also demonstrated the responsiveness of this monotherapy with antibodies against cell cycle, autophagy, or other survival-related factors. **Conclusion:** Our screening platform will provide us to further examine the resistance mechanism of any monotherapy in a different individual patient. We hope that our primary culture system used for standard monotherapy screening might provide the alert to drug resistance and the better combinatory therapy for personalized therapy.

Key words: Human epithelial ovarian cancer, cisplatin, docetaxel, doxorubicin, drug resistance

INTRODUCTION

Ovarian carcinomas are highly metastatic tumors and the most lethal gynecological malignancies in the world.[1-4] Most of the ovarian cancer cells are believed to arise from the surface of the ovary and primarily invade the surrounding tissues and serosal cavities, and then, they are classified as "epithelial."[2,4,5] Due to the lack of effective screening programs, ovarian cancer is diagnosed at an early stage only in about 25% of the cases. Although there are many studies underlying the development and progression of ovarian cancer,[2,3,6,7] the actual tumorigenesis of ovarian cancer is not well defined.

Treatment of human epithelial ovarian cancer (hEOC) is based on the combination of surgery and chemotherapy.[1,8,9] Over the past three decades, the standard treatment for advanced ovarian cancer is surgical tumor debulking, followed by platinum-based chemotherapy. However, the 5-year survival of patients with hEOC remains only 45%. The

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acquisition of resistance is a major obstacle to the clinical use of platinum drugs for 75% of high-grade serous ovarian cancer treatment. The current standard treatment is a combination of paclitaxel and cisplatin (CDDP) which has shown the most survival benefits. A clinical classification of resistance based on the time between the end of the first treatment and recurrence was made: (1) refractory with progression during the first chemotherapy; (2) resistant to platinum (recurrence within 6 months); or (3) sensitive (recurrence after 6 months).[10] The current strategy for platinum-refractory and platinum-resistant tumors is to use other monotherapy, including gemcitabine, topotecan, liposomal doxorubicin (DXR) with or without trabectedin, or paclitaxel.[11,12] Hence, the urgent strategy is to develop platform to predict the resistant to target therapy after surgery and screen the potential monotherapy, including platinum, in clinical treatment.

Here, our laboratory tried to take advantage of ascites from hEOC patients to understand the mechanism of platinum-based resistance and screen the potential monotherapy choice via the primary culture strategy. The personalized hEOC cells might be primarily cultured before or after the targeted therapy. This methodology provides us the chance to dissect the drug-resistant mechanism and figure out the rationale to use monotherapy without platinum or multiple combination in the current clinical treatments.

MATERIALS AND METHODS

Primary cultures of human epithelial ovarian cancer cells from ascitic fluids and chemicals

Ascitic fluids were collected from patients who underwent medical examinations at the National Defense Medical Center (Taipei, Taiwan, ROC). The Institutional Review Board of Tri-Service General Hospital, Taipei, Taiwan, approved the study protocol, and signed informed consent was obtained from all subjects before study participation (TSGHIRB No: 2-107-05-132). Ascites were aspirated directly from the patient into sterile suction bottles. 25 ml of ascitic fluid was mixed with 25 ml of culture medium (Dulbecco's modified Eagle's medium [DMEM] supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin) in T75 flasks (Corning, USA) and incubated at 37°C, 5% CO_2, and 95% humidified air. Medium was changed every 3 days until cells grew to 80% confluence for further verification. CDDP, estradiol (E2), dexamethasone (Dex), docetaxel (DXL), DXR, progesterone, retinoic acid (RA), thiazolyl blue tetrazolium bromide (MTT) and 3,5,3′-triiodo-L-thyronine (T3) were purchased from Sigma-Aldrich (USA).

Cell survival analysis

The cells were seeded in 96-well culture plates and allowed to grow for 1 day. The cells were then exposed to caffeine in fresh DMEM for the indicated periods. MTT solution (0.5 mg/ml in phosphate-buffered saline) was added to each well, and the plate was incubated for 2 h at 37°C. Dimethylsulfoxide (150 µl) was then added as a solubilizing agent, and the absorbance at 540 nm was measured using an ELISA plate reader (Multiskan EX, Thermo, USA). As a control, cells treated with media containing the vehicle were defined as 100% cell survival.

Immunoblot analysis

Cell lysates were prepared in lysis buffer (100 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1% SDS, and 1% Triton 100) at 4°C. Proteins were separated by SDS-PAGE, transferred to a polyvinylidene difluoride membrane (Millipore, USA), and detected using antibodies against alpha-actinin (Santa Cruz sc-17829), cytokeratin 7 (Abcam ab68459), DEC1 (Bethyl A300-649A), E-cadherin (Cell signaling #3195), LC3B (Cell Signaling #2775), p16 (Santa Cruz sc-377412), p21 (Santa Cruz sc-6246), p53 (Santa Cruz sc-126), Snail (Cell signaling #3895), Twist (Santa Cruz sc-81417), and vimentin (Cell signaling #5741).

RESULTS

The characterization of primary human epithelial ovarian cancer cells

We primarily cultured hEOC cells from the ascitic fluid of patients who were diagnosed with ovary cancer. Here, we showed the morphology of primary hEOC cells from two ascites [Figure 1a]. We further recorded the double time of indicated primary hEOC cells (#7 and #8), and their average double time was around 40 h [Figure 1b]. We collected 12 continuous passages of primary hEOC cells to examine three senescence markers, including DEC1, p16, and cytokeratin 7. Our data showed that p16 was an earlier sign than DEC1 and cytokeratin 7 in the primary hEOC cells [Figure 1c].

The effects of steroid hormones on the epithelial-to-mesenchymal transient in human epithelial ovarian cancer cells

One primary hEOC cells were treated with several steroid hormones, including Dex, E2, progesterone, RA, and T3, to examine their effects on the epithelial-to-mesenchymal transient factors. Differential declined trends were observed, such as p53 was suppressed by Dex, E2, and T3; p21 was suppressed by Dex, E2, and RA; N-cadherin, Snail, and Twist

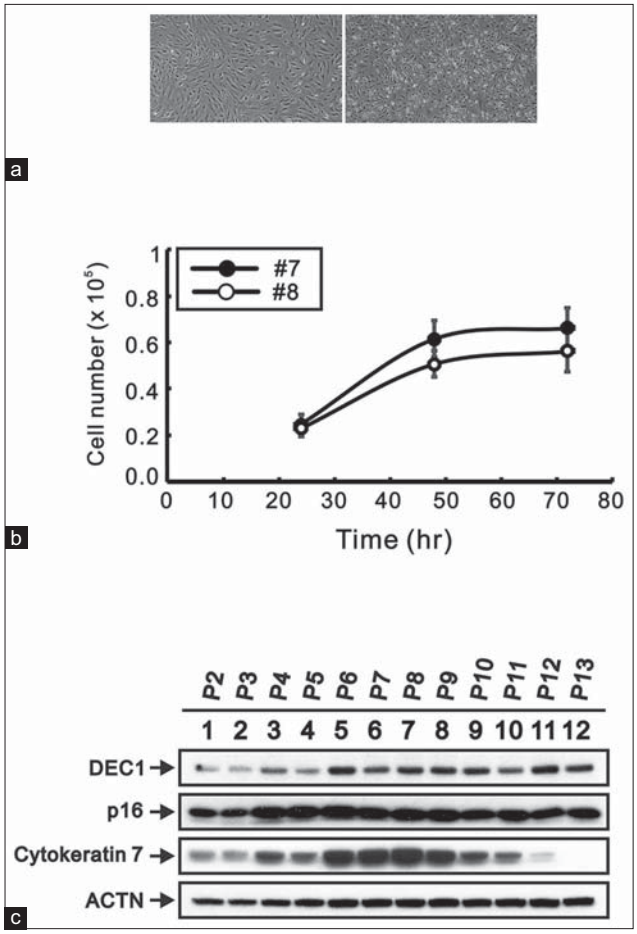

Figure 1: The morphology, double time, and senescence trend of primary human epithelial ovarian cancer cells. (a and b) Several populations of human epithelial ovarian cancer cells were collected for imaging and doubling time via light microscopy and thiazolyl blue tetrazolium bromide assay. (c) Primary human epithelial ovarian cancer cells were passaged for 12 serial generations and collected for the immunoblotting analysis with the selected senescence antibodies, including DEC1, p16, and cytokeratin 7. ACTN is a loading control. ACTN = Alpha-actinin

were suppressed by E2, RA, and T3. No apparent effect on E-cadherin was showed [Figure 2] in the primary hEOC cells.

The responsiveness and mechanisms of cisplatin, doxorubicin, and docetaxel in human epithelial ovarian cancer cells

We established the primary hEOC from patients' ascites for personalized target therapeutic strategy. Three common target therapeutic drugs, CDDP, DXR, and DXL, were treated primary hEOC cells for 24, 48, and 72 h. The cytotoxicity of CDDP on the primary hEOC cells was dependent on treatment concentration and time [Figure 3a]. The cytotoxicity of DXR was observed when primary hEOC cells were treated over 24 h [Figure 3b]. The cytotoxicity of DXL was

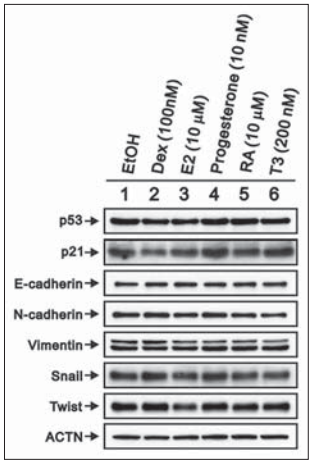

Figure 2: The effects of steroid hormones on the epithelial-to-mesenchymal transient in primary human epithelial ovarian cancer cells. Primary human epithelial ovarian cancer cells were treated with indicated steroid hormones, including, Dex, E2, RA, progesterone, and T3, for 24 h. Cell lysates were collected and subject to the immunoblotting analysis with antibodies against p53, p21, E-cadherin, N-cadherin, vimentin, Snail, and Twist. ACTN serves as a loading protein. E2 = Estradiol; Dex = Dexamethasone; ACTN = Alpha-actinin; RA = Retinoic acid; T3 = 3,5,3′-triiodo-L-thyronine

not apparent in the primary hEOC cells; however, the most efficiency was observed in 1000 nM for 72 h [Figure 3c]. We further analyzed the potential regulatory mechanisms of CDDP, DXL, and DXR in cell cycle and autophagy of primary hEOC cells. The increased p53 proteins were observed when primary hEOC cells were treated with CDDP, DXL, and DXR using the immunoblotting analysis [Figure 4]. In the parallel experiment, p21 proteins were increased by CDDP and DXR and decreased by DXL and the decrease of LC3B I was observed with the DXL and DXR treatment.

DISCUSSION

Despite all these improvements, hEOC survival remains low worldwide. The acquisition of resistance is a major obstacle to the clinical use of platinum-based drugs for 75% of high-grade serous ovarian cancer treatment.[11,12] In this study, our laboratory tried to take advantage of ascites from hEOC patients to understand the mechanism of platinum-based resistance and screen the potential monotherapy choice via the primary culture strategy. We first established the culture conditions for ascites (or tissues in the future) of the ovarian cancer patients. Our current data supported that we could culture these primary cells up to 13th generation. We further treated these primary cells with various steroid hormones for the morphology status and regular monotherapy, including CDDP, DXL, and DXR, for the cytotoxicity. Finally, we also demonstrated the

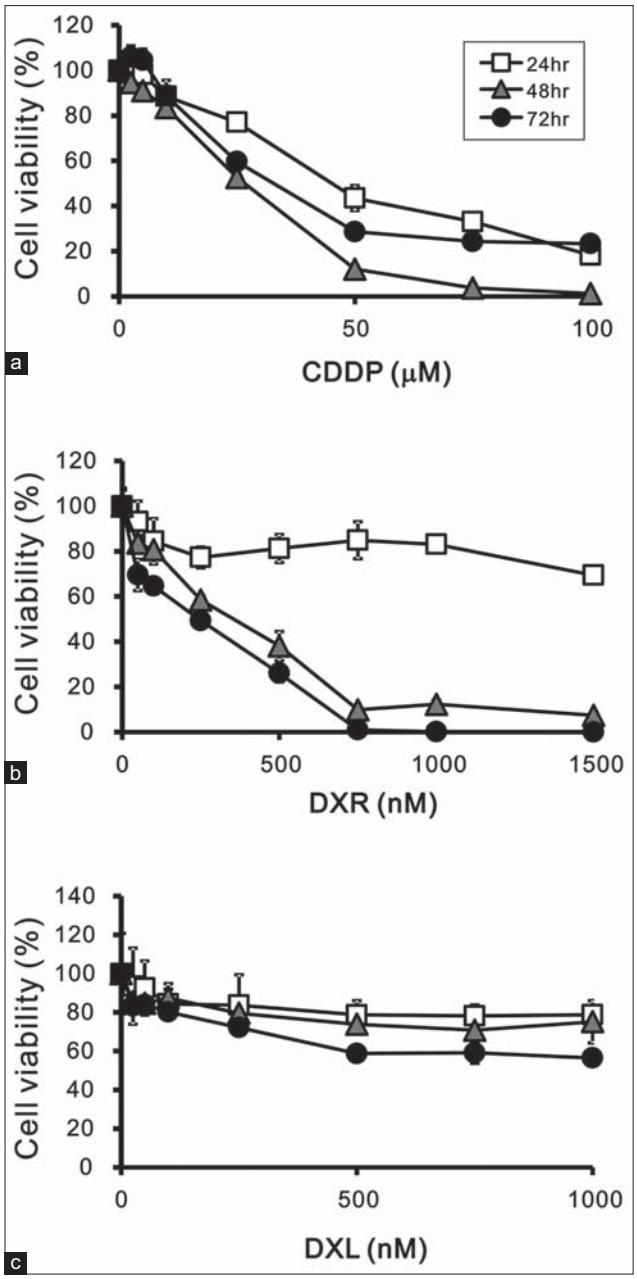

Figure 3: The responsiveness of primary human epithelial ovarian cancer cells for CDDP, DXR, and DXL. Primary human epithelial ovarian cancer cells were treated with indicated concentration of (a) CDDP, (b) DXR, and (c) DXL for 24, 48, and 72 h. The relative survival rate was measured by the thiazolyl blue tetrazolium bromide assay. CDDP = Cisplatin; DXL = Docetaxel; DXR = Doxorubicin

responsiveness of this monotherapy with antibodies again cell cycle, autophagy, or other survival-related factors. The current results revealed DXL and DXR increased LC3B II/I ratio which suggested the autophagy effect.[13] Hence, our screening platform will provide us to further examine the resistance mechanism of any monotherapy in a different individual patient. We hope that our primary culture system used for standard monotherapy screening might provide the alert to drug resistance and the better combinatory therapy for personalized therapy.

The standard treatment for patients is surgical cytoreduction followed by systemic platinum-based chemotherapy.[8,11,12] Platinum is an alkylating anticancer drug mediating through the cellular apoptosis.[14-16] It causes DNA damage by binding DNA and creating inter- or intra-strand cross-linkage lesions, resulting in activation of two different kinds of repair pathway: nucleotide excision repair and base excision repair systems for single-strand lesions and homologous recombination system for double-strand lesions. DNA damage cannot be repaired; replication and transcription processes will be stopped and induce cellular apoptosis. There are multiple factors that contribute to platinum resistance, but alterations of DNA repair processes, including checkpoint proteins, have been known for some time to be important in mediating resistance.[10]

Despite the activity of the first-line chemotherapy, which gives response rates up to 80% in the first-line treatment, the majority of patients die of their recurrent disease. Therefore, a large proportion of patients are candidates for the second-line treatment. Numerous mechanisms for monotherapy resistance have been reported. In clinical treatment, ovarian cancer needs new real-time monitoring for current monotherapy. Here, our laboratory tried to establish a real-time monitoring system from ascites or tissues in bedside. One study demonstrated that ascites from epithelial ovarian cancer contain high levels of functional decoy receptor 3 and are associated with platinum resistance.[17] Hence, we hope that we could clarify multiple factors involved in the platinum resistance in primary culture system, which provides earlier warnings and the information to choose better monotherapy for patients. In addition, patients with platinum-refractory and platinum-resistant tumors are good candidates for novel investigational approaches and studies of drug resistance.

CONCLUSION

In this study, our laboratory successfully took advantage of ascetic fluids from hEOC patients to be primarily cultured before or after the targeted therapy. The personalized hEOC cells might help us to elucidate the mechanism of drug resistance and screen the potential targeting choice. Hence, this methodology provides us the chance to dissect the risk of drug-resistant development and figure out the rationale for monotherapy without platinum or multiple combinations in the current clinical treatments.

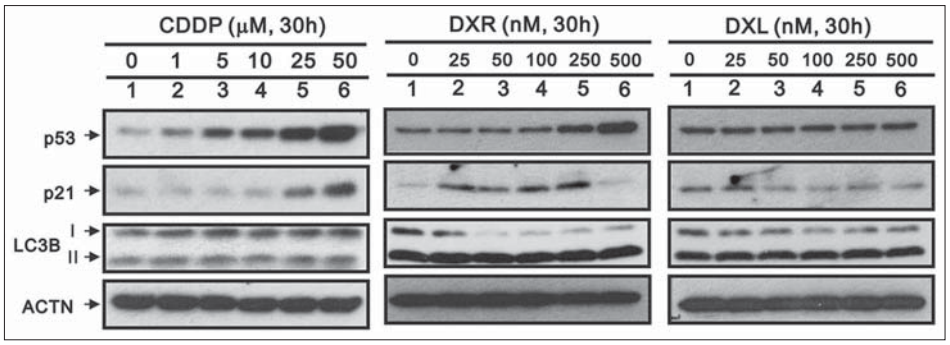

Figure 4: The effects of DXL and DXR on the cell cycle and autophagy in primary human epithelial ovarian cancer cells. Primary human epithelial ovarian cancer cells were treated with indicated amount of CDDP, DXL, and DXR for 30 h. Cell lysates were collected and subject to the immunoblotting analysis with antibodies against p53, p21, and LC3B. ACTN serves as a loading protein. CDDP = Cisplatin; E2 = Estradiol; Dex = Dexamethasone; DXL = Docetaxel; DXR = Doxorubicin; ACTN = Alpha-actinin

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ozols RF, Bookman MA, Connolly DC, Daly MB, Godwin AK, Schilder RJ, *et al.* Focus on epithelial ovarian cancer. Cancer Cell 2004;5:19-24.
2. Dubeau L. The cell of origin of ovarian epithelial tumours. Lancet Oncol 2008;9:1191-7.
3. Lengyel E. Ovarian cancer development and metastasis. Am J Pathol 2010;177:1053-64.
4. Garavaglia E, Sigismondi C, Ferrari S, Candiani M. The origin of endometriosis-associated ovarian cancer from uterine neoplastic lesions. Med Hypotheses 2018;110:80-2.
5. Bowtell DD. The genesis and evolution of high-grade serous ovarian cancer. Nat Rev Cancer 2010;10:803-8.
6. Dawson A, Fernandez ML, Anglesio M, Yong PJ, Carey MS. Endometriosis and endometriosis-associated cancers: New insights into the molecular mechanisms of ovarian cancer development. Ecancermedicalscience 2018;12:803.
7. Tingulstad S, Skjeldestad FE, Halvorsen TB, Hagen B. Survival and prognostic factors in patients with ovarian cancer. Obstet Gynecol 2003;101:885-91.
8. Kim A, Ueda Y, Naka T, Enomoto T. Therapeutic strategies in epithelial ovarian cancer. J Exp Clin Cancer Res 2012;31:14.
9. Pereira A, Pérez-Medina T, Magrina JF, Magtibay PM, Rodríguez-Tapia A, Cuesta-Guardiola T, *et al.* "The impact of debulking surgery in patients with node-positive epithelial ovarian cancer: Analysis of prognostic factors related to overall survival and progression-free survival after an extended long-term follow-up period". Surg Oncol 2016;25:49-59.
10. Martin LP, Hamilton TC, Schilder RJ. Platinum resistance: The role of DNA repair pathways. Clin Cancer Res 2008;14:1291-5.
11. Corrado G, Salutari V, Palluzzi E, Distefano MG, Scambia G, Ferrandina G, *et al.* Optimizing treatment in recurrent epithelial ovarian cancer. Expert Rev Anticancer Ther 2017;17:1147-58.
12. Pisano C, Bruni GS, Facchini G, Marchetti C, Pignata S. Treatment of recurrent epithelial ovarian cancer. Ther Clin Risk Manag 2009;5:421-6.
13. Tanida I, Ueno T, Kominami E. LC3 and autophagy. Methods Mol Biol 2008;445:77-88.
14. Konstantinopoulos PA, Matulonis UA. Targeting DNA damage response and repair as a therapeutic strategy for ovarian cancer. Hematol Oncol Clin North Am 2018;32:997-1010.
15. Abbotts R, Wilson DM 3rd. Coordination of DNA single strand break repair. Free Radic Biol Med 2017;107:228-44.
16. Iyama T, Wilson DM 3rd. DNA repair mechanisms in dividing and non-dividing cells. DNA Repair (Amst) 2013;12:620-36.
17. Connor JP, Felder M. Ascites from epithelial ovarian cancer contain high levels of functional decoy receptor 3 (DcR3) and is associated with platinum resistance. Gynecol Oncol 2008;111:330-5.