

Proteomic Assessment of Human Ovarian Cancer Cell Lines Xenotransplanted into the Hamster Cheek Pouch

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Abstract: Ovarian cancer is the most lethal gynecological cancer in America. This study tested the feasibility of linking our hamster experimental system to the clinical diagnosis and treatment of ovarian cancer. In particular, I xenotransplanted established human tumor cell lines (donor sample) into the hamster cheek pouch (immunologically privileged host site) and then assessed transplant status. After generating formalin-fixed / paraffin-embedded (FFPE) histology specimens of viable xenotransplant masses from two cell lines (SKOV3p43 and A2780p4), I evaluated expression of specific proteins in the transplants (proteomic assessments) using immunoblot and immunohistochemical analysis. Our results so far provide Proof-Of-Principle for a new clinical testing service to assist the diagnosis, prognosis, and personalized treatment of ovarian cancer.

1. Introduction

According to the American Cancer Society and other sources [1, 2], endometrial cancer is the most common gynecological cancer and epithelial ovarian cancer remains the most lethal gynecological cancer in the USA. Most patients present with advanced disease and thus have a poor prognosis. Our involvement with the topic of gynecological pathophysiology began with the development of a novel animal experimental system [3] that was originally designed to model a historical medical treatment protocol that generated tragic consequences. I refer to the mistaken belief that administering high doses of the synthetic estrogen, diethylstilbestrol (DES), would prevent miscarriages [4]. That practice ceased in 1971 with the first report of reproductive tract anomalies including cancer in the offspring of DES-treated mothers [5]. Our model of that medical misadventure, known as the “DES Syndrome”, differs from most others in that it is based on the hamster. While our use of that animal experimental system provided a unique means to study perinatal DES-induced disruption throughout the female and male reproductive system, it also provided experience with use of the hamster cheek pouch as a very convenient transplantation site for both tissues and cells [7]. More specifically, the pouch represents an immunologically privileged site that will accept and support the growth and development of most normal and neoplastic tissues of both allogeneic and xenogeneic origin [6]. That capability prompted the current pilot project regarding testing the hamster cheek pouch for xenotransplantation of human ovarian cancer cells.

2. Experiment, Results, Discussion, and Significance

a. Cultured cells and tumor tissue processing: Our current collection of cell lines includes ES-2 (derived from a clear cell ovarian carcinoma); OVCAR-3 (derived from a serious ovarian adenocarcinoma); TOV-112D (derived from an ovarian endometrioid carcinoma); A278P4 (human ovarian cancer cell line); MCF-7 (breast cancer cell line); and SKOV3 (human ovarian carcinoma).

b. Hamster cheek pouch transplantation: For the surgical procedure, animals are anesthetized with an intraperitoneal injection (i.p.) of Xylazine and Ketamine. An incision is made (~2mm) in one epithelial layer of the cheek pouch. The cell suspension (Breast CA: MCF-7; Ovarian CA: OVCAR-3, ES-2, TOV-112D, A278P4 and SKOV3) is introduced into the pocket between the two pouch layers. The incision site is then sealed with a liquid suturing agent.

c. Post-transplantation assessments: Post-transplantation inspections are conducted at least weekly. Based upon the gross observation, transplant masses along with trunk blood are harvested. Viable ovarian transplant masses are covered in fixative on a paraffin plate. The enveloped viable ovarian transplant mass is excised and immersed in freshly made fixative (4%para-formaldehyde, pH 7.2).The fixed tissues are stored in 70% ethanol prior to histological processing and then analysis by immunohistochemistry (IHC).

d. Western blotting: Total protein extracts were prepared from a collection of six human tumor cell lines (MCF-7; OVCAR-3; ES-2; TOV-112D; A278P4 and SKOV3) and subjected to Western blot analysis. In the procedure, proteins are separated by size during the gel electrophoresis stage and then detected by a specifically directed antibody. The size separation prior to blotting allows the protein molecular weight to be gauged as compared with known molecular weight markers.

e. Immunohistochemistry: Immunohistochemical (IHC) analysis was performed on FFPE sections generated from xenotransplant masses grown in the hamster cheek pouch and they were established from two human ovarian tumor cell lines (SKOV3p43 and A2780p4). Serial sections of the collection of transplant masses embedded in a single paraffin block were probed with selected antibodies to investigate the presence or absence of a specific protein at the tissue and cell-specific level.

f. Results and discussion: Immunohistochemical analysis detected prominent expression of HSP-60, ER α , VEGF, EGFR, SP1, RAP1, NF κ B p65, p-c-JUN, Cox-2, P63, E-cadherin, P120, and Epac-1 proteins in some areas of the viable tumor tissue masses with correspondingly distinct and specific nuclear and extranuclear staining that was differentially located in the tumor masses and very strong in the basal squamous cells of the cheek pouch external surface in all sections. Also, the molecular weight of the specific protein bands for some of the proteins detected in the immunoblot analysis (HSP60, P-120, E-Cadherin, VEGF, ER- α , and β -actin) were within the range expected. These results demonstrate that: 1) The hamster cheek pouch (host site) will accept and support the development of viable tumor masses from human ovarian cancer cells (donor samples); and 2) We can assess specific gene expression at the proteomic level in such donor cells and tumor xenotransplant masses.

3. Conclusion

Our results so far provide Proof-of Principle that the hamster cheek pouch transplantation can be exploited for Translational Research on ways to improve the clinical diagnosis, prognosis, and treatment of ovarian cancer.

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5. References

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