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Correlation of clinical data with fallopian tube specimen immune cells and tissue culture capacity

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ABSTRACT

Human fallopian tube fimbria secretory epithelial cells (hFTSECs) are considered an origin of ovarian cancer and methods for their culture from fallopian tube specimens have been reported. Our objective was to determine whether characteristics of the donors or surgeries were associated with the capacities of fimbria specimens to generate hFTSEC cultures or their immune profiles. There were no surgical complications attributable to fallopian tube removal. Attempts to establish primary hFTSEC cultures were successful in 37 of 55 specimens (67%). Success rates did not differ significantly between specimens grouped by patient or surgery characteristics. Established cultures could be revived after cryopreservation and none became contaminated with microorganisms. Two cultures evaluated for long term growth senesced between passages 10 and 15. M1 macrophages were the predominant cell type, while all other immune cells were present at much lower percentages. IL-10 and TGF-β exhibited opposing trends with M1 and M2 macrophages. Plasma IL-10 levels exhibited significant positive correlation with patient age. In conclusion, fallopian tube fimbria specimens exhibit a pro-inflammatory phenotype and can be used to provide a source of hFTSECs that can be cultured for a limited time regardless of the donor patient age or race, or the type of surgery performed.

1. Introduction

Epithelial ovarian cancer is often detected at late stage and is the most lethal of all gynecologic malignancies. Its etiology is poorly understood due to lack of early detection methods. The origin of the most common and lethal histology, high grade serous ovarian cancer, appears to include microscopic lesions that arise in the epithelial lining of the fimbria of the fallopian tube. This lining consists of proliferative secretory cells and non-proliferative ciliated cells (Goto et al., 2013). Pre-neoplastic regions defined as dysplastic regions of continuous secretory cells with no intervening ciliated cells (SCOUTs) (Piek et al., 2001; Chen et al., 2010) and serous tubal in situ carcinomas (STICs) have been found in BRCA mutant positive (BRCA +) women at high risk for ovarian cancer (Medeiros et al., 2006; Kindelberger et al., 2007). Systematic analysis of fallopian tubes identified STICs in 38% of BRCA + women (Medeiros et al., 2006). In a study of 55 consecutive cases of serous ovarian cancer not selected for BRCA status, 71% of the cancers involved the fallopian tube, and 48% of these cancer cases had a STIC which contained an identical p53 mutation to the cancer (Kindelberger et al., 2007).

Removal of fallopian tubes (bilateral salpingectomy) during surgery for benign gynecologic indications was shown to reduce the risk of ovarian cancer (Falconer et al., 2015). A population-based retrospective cohort study reported that bilateral salpingectomy at the time of benign gynecologic surgery did not significantly increase the surgical time or risks for hospital readmission or blood transfusions (McAlpine et al., 2014). These surgeries provide a translational research opportunity to collect and study fallopian tube specimens for their role in ovarian

Abbreviations: BS, bilateralsalpingectomy; BSO, bilateralsalpingo-oophorectomy; hFTSEC, human fallopian tube secretory epithelial cell; hTERT, human telomerase reverse transcriptase; IL-10, interleukin-10; IRB, institutional review board; MIS, minimally invasive surgery; NK, natural killer; OUHSC, University of Oklahoma Health Sciences Center; SCOUT, secretory outgrowth; STIC, serous tubal in situ carcinomas; TGFβ, transforming growth factor-β; Tregs, T regulatory cells

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carcinogenesis. Two reports have described growth of primary human fallopian tube secretory epithelial cell (hFTSEC) cultures from fallopian tube specimens, however the success rate of culture establishment attempts were not reported (Karst and Drapkin, 2012; Lawrenson et al., 2013). Therefore, it is not known if characteristics of the patient, such as age, menopausal status or body mass index (BMI) or the type of surgical procedure employed to collect the specimen, such as laparoscopic versus abdominal or salpingectomy versus salpingo-oophorectomy, are associated with the success rate of culture establishment. In most studies, establishment of primary cultures from surgical specimens fall well below 100% with varying success rates depending on the tissue of origin. The success rate of culture establishment is an important factor needed for appropriate study design.

Fallopian tube specimens could also be utilized to study how immune cell infiltrates contribute to tumorigenesis. Immune cells in fallopian tubes protect mucosal surfaces from infections, and also protect the sperm, embryo and fetus by controlling immune tolerance (Lee et al., 2015; Wira et al., 2014). Chronic inflammation caused by obesity however has been implicated in carcinogenesis (Wright and Simone, 2016). Incremental increases in macrophages, but not T lymphocytes, were observed in STIC and high grade serous ovarian cancer lesions (George et al., 2012). A mixture of innate and adaptive immune cells were observed in fallopian tube tissue, with significantly higher T-helper and B cells and significantly lower macrophages in specimens from post-menopausal compared to pre-menopausal women (Ardighieri et al., 2014). The immunohistochemical methods used in these studies however were only semi-quantitative and did not evaluate the immune cells differentiation or activation states. Circulating cytokines involved in immunosuppression and tissue repair, such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), are also increased with obesity and age, and regulate the differentiation state of macrophages in association with carcinogenesis (Brennan and Femdman, 2000; Fontana et al., 1992).

The purpose of this study was to determine if patient and surgical parameters were associated with the success rate of hFTSEC establishment and immune cell infiltrates of fallopian tube fimbria specimens. No complications attributable to the removal of fallopian tubes during surgery for benign gynecologic conditions occurred. We had a 67% success rate in establishing primary hFTSEC cultures independent of the patient and surgery characteristics. The fimbria microenvironment was predominantly pro-inflammatory with 20% of the cell population consisting of macrophages exhibiting the M1 differentiation state. This is the first publication to document the presence of T regulatory cells (Tregs) in fallopian tube tissue, and opposing associations of IL-10 and TGF-β with M1 and M2 macrophages that are consistent with the roles of these cytokines in regulating the macrophage differentiation state.

2. Materials and methods

2.1. Patient recruitment and consent

This study was conducted under a University of Oklahoma Health Sciences Center (OUHSC) Institutional Review Board (IRB)-approved protocol (IRB #5563), which was approved on June 3rd, 2015. From June of 2015 until December of 2016, 91 healthy subjects were recruited from the OUHSC Gynecology and Urogynecology Clinics. Eligibility included patients scheduled for gynecologic surgery. Patients with cancer or who were less than 21 years of age were ineligible. Patients with only one remaining fallopian tube or indication for only unilateral salpingectomy were eligible for participation. Physicians in both clinics were introduced to trial, the inclusion and exclusion criteria, as well as the evidence and experience of removal of fallopian tubes in healthy subjects for ovarian cancer prevention at grand rounds and faculty meetings. Flyers describing the study, patient eligibility criteria and research personnel contact information were placed in the physician's workspace. Individual physicians informed the patients about the risks and benefits of salpingectomy as a part of the planned surgical procedure. Willing patients were then consented by research staff and asked to fill out a brief questionnaire regarding their menstrual history and use of hormone therapy. Research staff reviewed the operating room schedules weekly and reminded the surgeon and laboratory research personnel about the specimen collection. Pre-operative nursing staff were trained to review records for each patient and to draw a purple-top vial of whole blood at the time of the patient's pre-operative lab draw or IV placement and place it on ice.

2.2. Specimen processing

When the fallopian tubes were successfully removed, the operating room staff contacted the laboratory researcher using a phone number posted in each operating room for the duration of the study. When notified, the researcher on call would arrive at the surgical suite within 15 min, dress in surgical attire and enter the operating room where the fallopian tubes had been moved to a non-sterile field. The surgeon labeled the pad under the fallopian tubes to indicate the right and left side of the patient. Using scissors, the researcher made a longitudinal incision along the middle of the tube at the fimbriated end. Once the incision reached past the fimbria and the distal portion of the tube, a horizontal cut was made to separate a tissue sample consisting of half of the fimbria. For surgeries involving patients who had two tubes, one specimen was immediately placed in labeled containers containing DMEM/F-12 medium (Sigma Roche) containing antibiotic/antimycotic (Thermo Fisher, 1%) and fetal bovine serum (FBS, Serum Source International, 10%) on ice, and the other was placed in a cassette which was then filled with OCT cryopreservation medium (Thermo Fisher). The cassette and a paper label with the study subject de-identified code written in pencil was wrapped in aluminum foil and submerged in liquid nitrogen. The remaining fallopian tube specimen was sent to pathology. All specimens were immediately transported to the laboratory. The blood was processed into multiple plasma aliquots, which were labeled and stored at −80 °C.

2.3. Culturing of hFTSECs

Fresh fimbria specimens were processed for hFTSEC culturing in a biosafety cabinet under biosafety level 2 conditions using a published method (Karst and Drapkin, 2012) with the following modifications. The specimens were minced with a sterile scalpel blade to achieve pieces of ≤1 mm, or small enough to pass through 5 ml serological pipette for efficient isolation. The DMEM-F12 medium was supplemented with fetal bovine serum instead of the more expensive Ultroser G. A 0.2% of trypsin instead of regular 0.05% trypsin was used to release cells from collagen-coated tissue culture plates, which reduced the time needed for trypsinization from 15 to 3–5 min while increasing the percentages of live cells recovered. Inoculation density after trypsinization was $0.5–0.7 \times 10^5$ cells/ml in 6-well plate, at confluency cells density was $5–7 \times 10^5$ cells/ml.

The plate of cells was incubated at 37 °C with 5% CO_2 and inspected the next day with a microscope for attached cell colonies or any signs of contamination, and replenished with fresh medium to remove cell debris. The culture plate was microscopically examined every two days and replenished with fresh medium. Those cultures which did not grow to confluency by day 6 were sterilized and discarded. Cultures that achieved confluency after about 6 days of incubation were categorized as successful and trypsinized. A portion of the cells was placed onto a fresh culture plate, and the remaining portion was cryopreserved in DMEM/F-12 medium (60%), Antibiotic/Antimycotic (1%), FBS (36%) and DMSO (4%). After 5–6 passages, the hFTSECs could be cultured in a dish without the collagen coat and trypsinized using 1X trypsin.