Gynecologic Oncology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Cytidine deaminase *Apobec3a* induction in fallopian epithelium after exposure to follicular fluid

Pavla Brachova [a], Nehemiah S. Alvarez [b], Bradley J. Van Voorhis [c], Lane K. Christenson [a,*]

[a] Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, United States
[b] Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160, United States
[c] Department of Obstetrics and Gynecology, University of Iowa, Iowa City, IA 52242, United States

HIGHLIGHTS

- Follicular fluid induces DNA damage accumulation in fallopian epithelial cells.
- Follicular fluid induces *Apobec3a* mRNA and protein expression in fallopian cells.
- APOBEC3A overexpression is sufficient to induce double strand DNA breaks.

ARTICLE INFO

Article history:
Received 19 September 2016
Received in revised form 7 February 2017
Accepted 8 February 2017
Available online xxxx

Keywords:
Follicular fluid
Fallopian tube
Apobec
Ovulation
DNA damage
Ovarian cancer

ABSTRACT

Objective. Ovarian carcinomas that originate from fallopian epithelial cells are suggested to arise due to repeated exposure to ovulatory follicular fluid (FF). Mechanistic explanation(s) for how this occurs are unknown. Here, we sought to understand if FF exposure to fallopian epithelial cells could induce DNA damage and expression of a known family of DNA mutators, apolipoprotein B mRNA editing enzyme, catalytic polypeptide (APOBEC) cytidine deaminases.

Methods. Follicular fluid and matched patient plasma samples were obtained from donors. Fallopian epithelial cells (FT33-TAg, FT189, FT190, and FT194) were cultured with FF or plasma for 24 h, and cell proliferation and DNA damage were assessed. Effects of FF on *Apobec* gene expression were determined by qRT-PCR and western blot analyses. Fallopian epithelial cells were transfected with an APOBEC3A expression vector and DNA damage was assessed.

Results. Follicular fluid exposure increased epithelial cell proliferation as measured by three independent methods, and DNA damage accumulation as assessed using three independent measures. This effect was specific to FF, as matched patient plasma did not have the same effects. Increased expression of *Apobec3a* was observed in fallopian epithelial cells following exposure to 5 of 8 patient FF samples, and transient overexpression of APOBEC3A was sufficient to induce double strand DNA breaks.

Conclusions. Follicular fluid can induce cell proliferation and DNA damage accumulation in cultured fallopian epithelial cells. Increased expression of APOBEC3A, a known DNA mutator, may explain the high incidence of DNA damage after FF exposure. The role of *Apobec3a* in ovulation-induced inflammation warrants further investigation.

1. Introduction

High-grade serous carcinomas make up the greatest proportion of ovarian neoplasms and have the worst prognosis, with long-term survival rates of <30% [1]. Non-invasive precursor lesions have been identified at the distal ends of the fallopian tube fimbria, leading to the hypothesis that the fallopian tube serves as the origin of a majority of high-grade serous carcinomas, rather than the ovarian surface epithelium [2–4]. Additionally, precursor lesions in fallopian fimbria frequently contain mutations in the tumor suppressor gene *TP53* identical to the *TP53* mutations occurring in the carcinoma, supporting a clonal relationship [5–7]. The non-genetic risk factor most positively associated with ovarian cancer is ovulation, although the causative factors of this association are unknown [8,9]. During ovulation, rupture of the

* Corresponding author at: 3075 HLSIC, 3901 Rainbow Blvd., Kansas City, KS 66160, United States.
E-mail addresses: pbrachova@kumc.edu (P. Brachova), nalvarez@kumc.edu (N.S. Alvarez), brad-van-voorhis@uiowa.edu (B.J. Van Voorhis), lchristenson@kumc.edu (L.K. Christenson).

http://dx.doi.org/10.1016/j.ygyno.2017.02.017
0090-8258/

preovulatory ovarian follicle releases the cumulus-oocyte-complex and the follicular fluid (FF). These materials are immediately juxtaposed to the fimbria of the fallopian tube and are actively funneled into the infundibulum in order to facilitate fertilization of the egg. It is hypothesized that the continual repetitive exposure to certain factors within FF may have detrimental effects on adjacent epithelial cells [10].

The physiological process of ovulation is inflammatory, and coincides with dramatic fluctuations in steroidal estrogen and progesterone secretion from the maturing ovulatory follicle following the surge of LH [11]. Inflammation is known to drive carcinogenesis processes [12], whereas hormonal signaling has been implicated in tumor progression [13]. Inflammatory factors increase in FF as ovulation approaches [14]. Previous studies have shown that human FF can induce an inflammatory signature in bovine oviductal epithelial cells [15]. This has led to the idea that FF may be tumorigenic, and that repetitive exposure of fallopian epithelial cells to this complex fluid may cause mutations and alterations leading to neoplastic transformation [10]. Three studies support the hypothesis that FF can cause DNA damage marks and cell proliferation in cultured fallopian epithelial cells [16–18]. In a mouse model, ovulation induced γH2A.X foci in oviductal epithelial cells in vivo after gonadotropin stimulation in a proliferation independent manner [16]. In another study, incubation of fallopian epithelial cells with FF for 24 h significantly increased γH2A.X foci compared with culture medium alone [17]. The third study used human FF and immortalized human fallopian epithelial cells to show that individual FF stratified into two groups: one with high reactive oxygen species (ROS) that increased the number of cells positive for γH2A.X, and one with low ROS that did not increase γH2A.X [18]. Although these studies suggest FF is responsible for inducing DNA damage, studies designed to control for the plasma constituents in FF, and a hypothesis driving the DNA-damaging properties of FF are essential to understand early molecular changes that occur during fallopian carcinogenesis related to ovulation.

In our study, we compared the effects of exposure of FF and matched patient plasma on the induction DNA damage, and investigated a group of apolipoprotein B mRNA editing enzyme, catalytic polypeptide (APOBEC) cytidine deaminases as a possible mechanism for DNA damage accumulation. Activity of APOBEC enzymes can deaminate single stranded DNA during DNA replication, and ultimately induce double strand breaks via repair of the deoxyuridine by base excision repair [19,20]. One family member called activation-induced cytidine deaminase (AID) has been identified as increased following exposure to FF, however a comprehensive study of the APOBEC family has not yet been performed [21].

2. Materials and methods

2.1. Cell lines and cell cultures

Immortalized fallopian epithelial cell lines (FT33-TAg, FT189, FT190, and FT194) were cultured as previously described [22] and were generously provided by Dr. Ronny Drapkin (University of Pennsylvania, Philadelphia, PA). The FT33-TAg cell line was authenticated by short tandem repeat profiling (www.ddcmedical.com) and exhibited no evidence of cross-contamination with any known ATCC cell lines nor mycoplasma. Fallopian epithelial cells were cultured with 2.5% Ultraser G (2× serum, USG, Pall Corporation, Port Washington, NY). All media conditions were supplemented with 10 units/ml penicillin and 10 μg/ml streptomycin and cells were maintained in a humidified incubator with 5% CO_2 at 37 °C.

2.2. Follicular fluid and plasma patient material

Follicular fluid samples were obtained from eight patients undergoing in vitro fertilization (IVF) at the University of Iowa Hospitals and Clinics as previously described [23]. Patient material was obtained from women ≤38 years old, with male factor or tubal infertility. Patients demonstrated normal ovulatory function clinically and the etiology of infertility was not ovarian in nature. Data was collected on body mass index (BMI) in kg/m^2 and parity. Patients were stratified into a "Low BMI" group (n = 4; BMI < 25) and a "High BMI" group (n = 4; BMI > 25), however we did not observe significant differences between these groups in our results, therefore all patients were combined into one group. These two groups had the same numbers of live births.

All IVF cycles were preceded by one month of oral contraceptive pills and patients received the same gonadotropin formulations (follitropin beta, Follistim AQ: Merck & Co., Inc.; human menopausal gonadotropin/hMG, Menopur: Ferring Pharmaceuticals Inc., USA). Ovulation was triggered with 10,000 IU of chorionic gonadotropin (APP Pharmaceuticals, LLC, USA) when at least 2 follicles of ≥18 mm in diameter were achieved. Fluid was aspirated from the first follicle into a single sterile tube without culture medium and centrifuged at 3000 rpm for 15 min at 4 °C to eliminate potential cell contamination. The FF sample was flash-frozen in liquid nitrogen prior to storage at −80 °C. The remaining FF collected during the retrieval was pooled, centrifuged at 3000 rpm for 15 min at 4 °C, and stored at −80 °C. Plasma was collected from seven of the eight patients at the time of FF aspiration according to standard procedure into acid citrate dextrose tubes.

2.3. Protein analysis

Cells (20 μg protein) were lysed in SDS sample buffer with 5% β-mercaptoethanol, boiled for 5 min at 95 °C, and subjected to electrophoresis using 10% SDS-PAGE in running buffer at constant 120 V for 1 h. Proteins were electro-transferred onto nitrocellulose membranes, and blocked with 5% (w/v) skim milk in Tris-buffered saline with 0.05% (v/v) Tween-20 (TBST) for 1 h at room temperature. Membranes were then probed with primary anti-APOBEC3A (1:250; HPA043237, Sigma, St. Louis, MO) or anti-Actin (1:500; sc-1616 Santa Cruz, Dallas, TX) antibody overnight at 4 °C TBST/BSA (50 mM Tris, 150 mM NaCl, 0.05% Tween20, 1.5% BSA) followed by incubation with the secondary rabbit IgG HRP linked antibodies (GENA934, Sigma) for 2 h at room temperature in blocking buffer. Membranes were washed three times in TBST and detected by enhanced chemiluminescence (Thermo Fisher Scientific, Waltham, MA).

The Genotype-Tissue Expression (GTEx) Project database was utilized to identify *Apobec* and *AID* expression in normal fallopian tube epithelium (obtained from the GTEx Portal on 11/03/16). GTEx was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. RNA-Seq data from 6 fallopian tube epithelial cells are reported as average RPKM (reads per kilobase per million mapped reads).

2.4. RNA isolation and quantitative RT-PCR analysis

RNA was isolated using TRI Reagent Solution (Thermo Fisher Scientific), with a DNase I digestion step. Concentration and purity of RNA was evaluated using a Nanodrop spectrophotometer ND-1000 (Thermo Fisher Scientific), and absorbance ratios of A260/230 ratios of 2.0–2.2 and A260/280 ratios of 1.8–2.0 were considered pure. Total RNA was reverse-transcribed using SuperScript II Reverse Transcriptase (Life Technologies, Carlsbad, CA) with random hexamer primers. Quantitative PCR (qPCR) was performed with 1:5 dilution of cDNA on an Applied Biosystems HT7900 sequence detector. Primer sets used to detect Cyclin D1 (*Ccnd1*), APOBECs, tubulin binding protein (*TBP*) and 18S rRNA are shown is Supplemental Table 1. Samples were run in triplicate, and the ΔΔCt method was used to calculate the relative fold change between the samples after normalization with 18S rRNA or TBP [24]. The presence of a single dissociation curve confirmed the amplification of a single transcript and lack of primer dimers.

Please cite this article as: P. Brachova, et al., Cytidine deaminase *Apobec3a* induction in fallopian epithelium after exposure to follicular fluid, Gynecol Oncol (2017), http://dx.doi.org/10.1016/j.ygyno.2017.02.017