ROBUST ESTABLISHMENT AND EXPANSION OF MULTILINEAGE HUMAN FALLOPIAN TUBE ORGANOIDS IN SERUM-FREE MEDIUM

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INTRODUCTION

High-grade serous ovarian cancer (HGSOC) is the most prevalent subtype of epithelial ovarian cancer. Despite its name, HGSOC originates in the fallopian tube (FT), but metastasizes quickly in the adnexal region and is the most lethal gynecological cancer worldwide. Organoid culture is emerging as a powerful model for studying normal FT cell biology and ovarian cancer. To standardize primary human FT and HGSOC organoid culture, we have developed GyneCult Fallopian Tube Organoid Medium (FTOM), an optimized serum-free medium and a workflow that supports robust and representative FT organoid culture from freshly isolated or cryopreserved primary human FT cells. FT cultures were initiated by seeding 4000 dissociated single cells directly into 20 µL Corning® Matrigel® domes and overlaying them with FT Organoid Medium. After seeding, cultures were maintained with full-medium changes every 3 - 4 days and were split either in a passage ratio of 1:3, or at 4,000 cells per 20 µL of Matrigel®, as single cells every 8 - 14 days. Cultures were analyzed by immunocytochemistry (ICC) to detect secretory markers keratin 7 (KRT7), oviductal glycoprotein 1 (OVGP1), and PAX8, as well as ciliated cell markers acetylated alpha tubulin (TUBA1A) and FOXJ1. Across all donor samples, approximately 16 ± 8% (mean ± SD) of dissociated EpCAM$^+$ FT cells formed 50 - 300 µm diameter cystic organoids within 14 days (n = 8). Cultures can be maintained for at least 5 passages with 14 - 20 cumulative population doublings, at 3 - 4 doublings per passage. ICC analysis confirmed that organoids contain both polarized KRT7$^+$ OVGP1$^+$ PAX8$^+$ secretory cells and acetylated TUBA1A$^+$ FOXJ1$^+$ ciliated cells, indicating multilineage capacity (n = 5). We also tested the compatibility of GyneCult FTOM for growing HGSOC samples, and we observed that 1 of the 3 tumor samples tested generated organoids with a solid and irregular shaped morphology. These cells could undergo a minimum of 5 cumulative population doublings over 5 passages, at 0.5 - 2 doublings per passage; this performance is in line with recently published HGSOC organoid culture methods. These results demonstrate that GyneCult Fallopian Tube Organoid Medium is a robust medium for initiating and culturing FT epithelium as organoids and is a valuable tool for studying FT biology, with the capacity to support HGSOC organoid culture.

METHODS

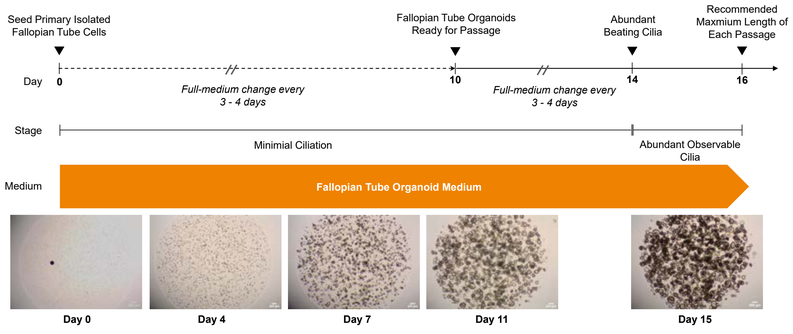

FIGURE 1. GyneCult Fallopian Tube Organoid Medium Workflow

Freshly isolated or cryopreserved human FT cells are embedded at 4000 cells per 20 µL Matrigel® dome into 24-well non-tissue culture plates. Pre-culturing of cells in adherent culture or epithelial cell purification prior organoid culture is not necessary. Once the domes gel, 500 µL of GyneCult FTOM (basal medium with 10X, 100X, and 5000X supplements) is added to the cultures, and the medium is changed every 3 - 4 days thereafter. Cultures are ready to passage at 10 days, but can be maintained with regular medium changes for up to 16 days. Robust cilia beating can be observed in live cultures using light microscopy on day 14. Scale bar = 200 µm.

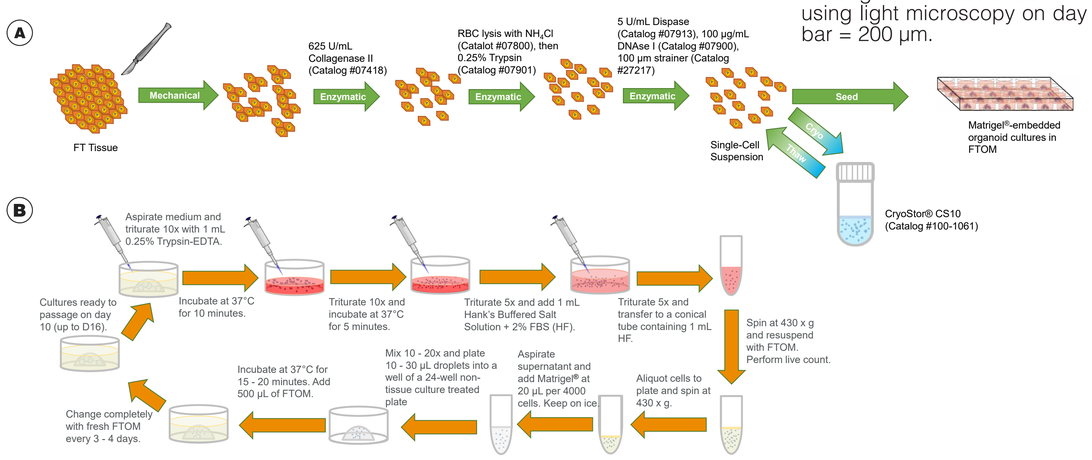

FIGURE 2. GyneCult FTOM Supports FT Organoid Initiation

(A) Human FT tissue is dissociated into single cells, which are amenable for direct seeding into organoid culture, cryopreservation in a biobank, and flow cytometric analysis. **(B)** Passaging protocol for the subculturing of FT organoids.

RESULTS

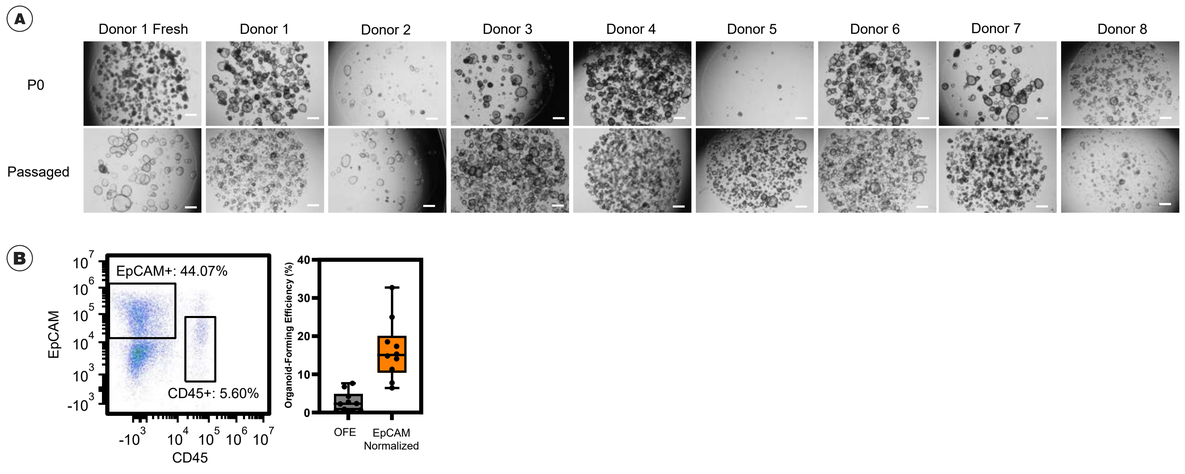

FIGURE 3. GyneCult FTOM Supports Long-Term Culture

(A) GyneCult FTOM supports the single-cell or clump passaging of FT organoids in 100% of the donors tested (n = 8); results from six donors are shown. Although there was donor-to-donor variability, FT organoids were typically cystic, with a prominent lumen and between 50 - 500 µm in diameter. Size distribution and morphology were maintained passaged to passage. All passaged organoid images are from P5 or later, except for donor 1 fresh and donor 2, which are P3 and P4, respectively. Scale bars = 500 µm. **(B)** Representative flow cytometric dot plot of a dissociated donor sample stained with EpCAM and CD45 antibodies (left panel). The organoid-forming efficiency (OFE; the fraction of single cells that form organoids in culture) of dissociated FT cells without and with correction for EpCAM$^+$ FT cells was 2.8 ± 2.6% and 16 ± 8%, respectively (mean ± SD, n = 10, includes 2 replicates of 2 donors, right panel).

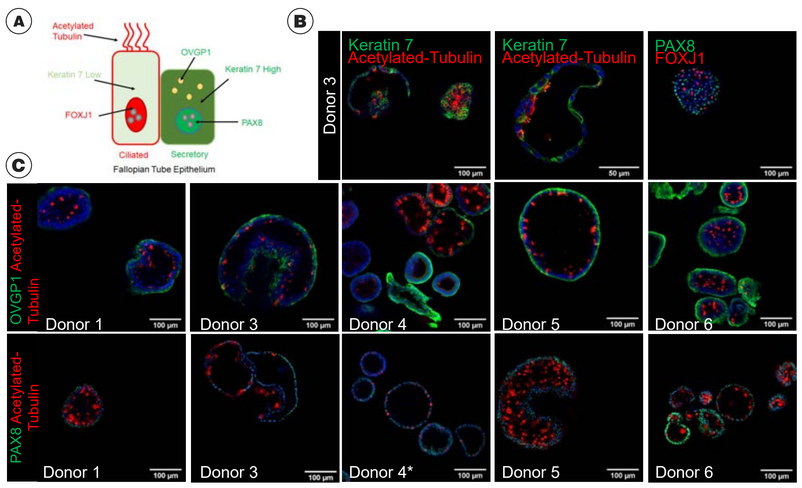

FIGURE 4. Fallopian Tube Organoids Recapitulate *in vivo* Epithelial Lineages

(A) Model of FT secretory (keratin 7$^+$, OVGP1$^+$, PAX8$^+$) and ciliated (FOXJ1$^+$, acetylated tubulin$^+$) cell lineages. **(B)** Keratin 7, acetylated tubulin, FOXJ1, and PAX8 staining in one donor. **(C)** OVGP1, acetylated tubulin, and PAX8 staining in 5 donors.
*Donor 4 PAX8 staining was co-stained with FOXJ1 while other PAX8 staining shown were co-stained with acetylated tubulin.

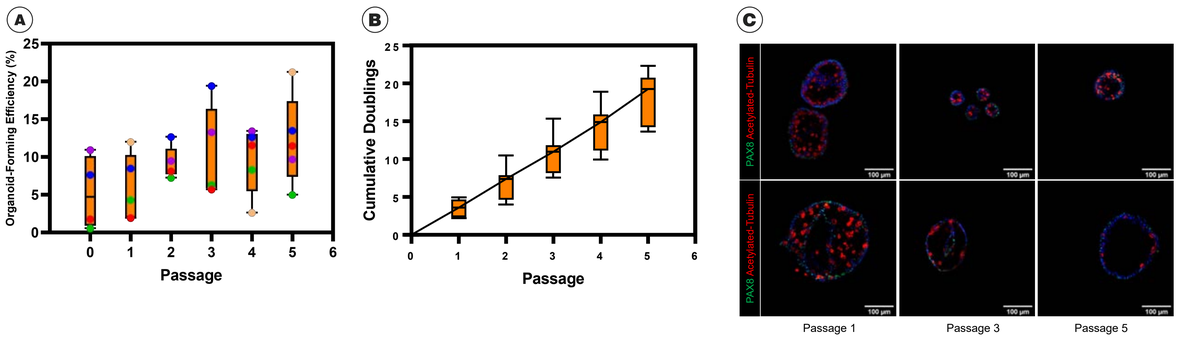

FIGURE 5. GyneCult FTOM Supports Efficient Organoid Formation, Robust Long-Term Proliferation, and Lineage Balance

(A) Box and whiskers plot showing OFE in 5 donors (different colors) cultured in FTOM through 5 passages. **(B)** Box and whiskers plot of cumulative doublings in 7 individual FT donors after 5 passages in FTOM. **(C)** PAX8 and acetylated tubulin staining at passages 1, 3, and 5 in FTOM of 2 donors. Scale bars = 100 µm.

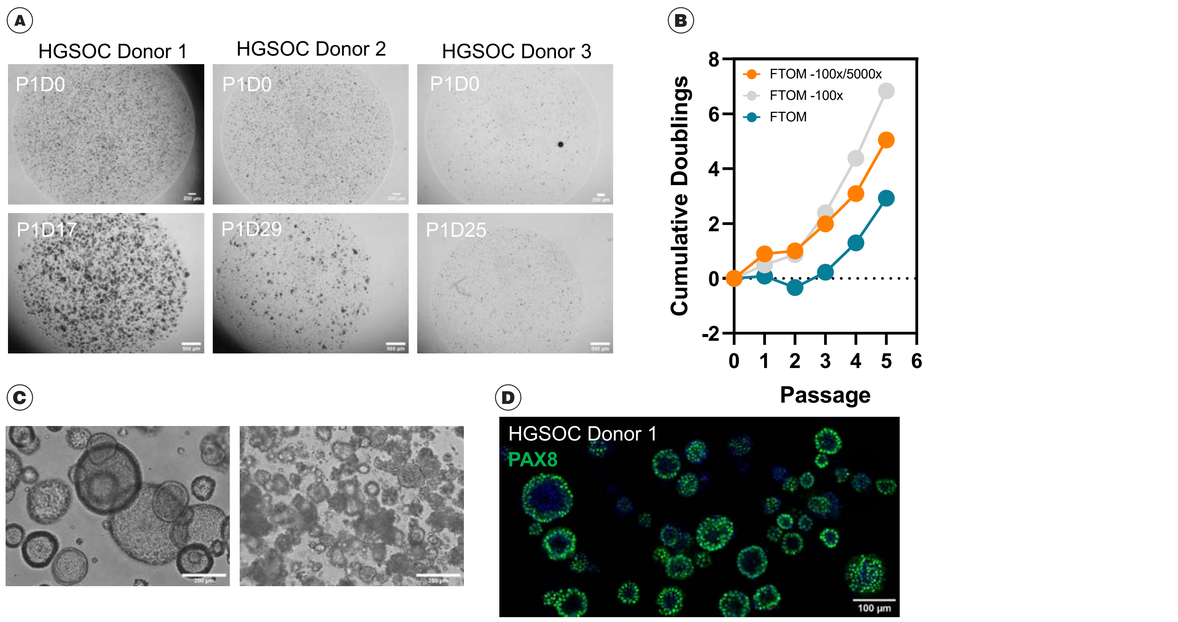

FIGURE 6. GyneCult FTOM Compatible with HGSOC Organoid Culture

(A) Passaged cultures of 3 HGSOC donors at day 0 (top row) and days indicated (bottom row). **(B)** Growth of HGSOC Donor 1 (recurrent sample from omentum) cultures in 5 passages in FTOM, FTOM without the 100X supplement, and FTOM without both the 100X and 5000X supplements. **(C)** Images of normal FT organoid (left panel) and HGSOC organoid (right panel) morphologies and sizes. Images are representative of different FT and HGSOC donors cultured. D. Passage 5 organoids from donor 1 stained with PAX8 (green) and DAPI (blue).

Summary

- STEMCELL's GyneCult Fallopian Tube Organoid Medium is a robust medium for initiating human FT organoid cultures that are passageable for a minimum of 5 passages (100% success, n = 8).
- Organoid cultures undergo an average of 18.4 ± 3.3 cumulative doublings (> 300,000-fold, n = 7), starting from 2000 - 12,000 unsorted cells from cryopreserved dissociated FT tissue within 5 passages (~50 - 60 days).
- Once organoid cultures are established, ~5 - 20% of seeded cells reliably form organoids at each passage, ensuring 600 - 1200 organoids per well per passage for downstream studies.
- Organoids cultured and passaged in GyneCult Fallopian Tube Organoid Medium stably recapitulate both of the major epithelial FT lineages as demonstrated by antigen expression (PAX8, keratin 7, and OVGP1 for secretory cells; FOXJ and acetylated tubulin for ciliated cells) and the clear presence of cilia-containing cells.
- GyneCult Fallopian Tube Organoid Medium supports a subset of HGSOC in organoid cultures.