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The presence of centrioles and centrosomes in ovarian mature cystic teratoma cells suggests human parthenotes developed *in vitro* can differentiate into mature cells without a sperm centriole

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ABSTRACT

In most animals, somatic cell centrosomes are inherited from the centriole of the fertilizing spermatozoa. The oocyte centriole degenerates during oogenesis, and completely disappears in metaphase II. Therefore, the embryos generated by *in vitro* parthenogenesis are supposed to develop without any centrioles. Exceptional acentriolar and/or acentrosomal developments are possible in mice and in some experimental cells; however, in most animals, the full developmental potential of parthenogenetic cells *in vitro* and the fate of their centrioles/centrosomes are not clearly understood. To predict the future of *in vitro* human parthenogenesis, we explored the centrioles/centrosomes in ovarian mature cystic teratoma cells by immunofluorescent staining and transmission electron microscopy. We confirmed the presence of centrioles and centrosomes in these well-known parthenogenetic ovarian tumor cells. Our findings clearly demonstrate that, even without a sperm centriole, parthenotes that develop from activated oocytes can produce their own centrioles/centrosomes, and can even develop into the well-differentiated mature tissue.

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1. Introduction

A centrosome consists of a pair of centrioles and surrounding pericentriolar material, and plays a pivotal role as a microtubule organizing center (MTOC) by nucleating microtubules in cell division. We now know that all centrioles in most animal cells, including those of humans, originated from the sperm centriole via the fertilization process. Centrosomes in oogonia degenerate during oogenesis and the centrioles completely disappear in oocytes in metaphase II (MII) [1]. A sperm centriole introduced into a fertilized ovum recruits pericentriolar proteins, such as γ tubulin, that are dispersed in the ooplasm, to form the zygote centrosome [2]. Therefore, the sperm centriole is the progenitor of all centrosomes in the somatic cells of most animals. There are some exceptions in rodents. For example in mice, sperm centrioles degenerate during fertilization, such that the embryos develop without any centrioles. The biosynthetic mechanisms of centriole formation during embryogenesis of these exceptional animals have not yet been elucidated. Currently, *in vitro* parthenogenetic cells are generated by artificial activation of mammalian oocytes. These *in vitro* parthenotes are also supposed to develop in the absence of a sperm centriole from a MII oocyte, which is devoid of a centriole, much like in mice. The fates of centrioles and centrosomes in *in vitro* parthenogenetic cells has never been clearly described. Recently, *in vitro* human parthenogenetic stem cells have been reported [3–5] and have drawn considerable attention, as an alternative to embryonic stem cells. Given the therapeutic purpose of *in vitro* human parthenogenetic stem cell studies, we have some questions regarding the medical eligibility of these cells in terms of their acentriolar development. Can these *in vitro* human parthenotes, which developed without any ancestral centriole, produce normal centrioles and centrosomes as in mice? Furthermore, recent reports have also described the importance of centrioles in cell differentiation as well as in cell division [6]. This being the case, can *in vitro* parthenotes differentiate into mature cells specific to their therapeutic purpose? In order to explore the outcome of acentriolar developments in human parthenotes, we sought to confirm whether centrioles and centrosomes are present, in the cells of ovarian mature cystic teratomas, which are well-known parthenogenetic tumors.

2. Material and methods

We determined the presence of centriole and centrosomes in parthenogenetic ovarian tumor cells with transmission electron microscopy (TEM) and immunofluorescence using γ-tubulin, as a centrosome specific marker.

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2.1. Tissue samples

All human tissue samples used in this study were collected after written informed consent was obtained from each patient. The study protocol was approved by the Institutional Review Board of Kyung Hee University Hospital. Tissue samples were collected from seven patients who underwent oophorectomy due to mature cystic teratomas. The samples were collected after confirming the benign natures of the tumors by immediate analysis of frozen sections. Resected tumors were incised and the liquefied component was evacuated and discarded and the inner surface of the teratoma cyst was flushed with normal saline. A few small fragments of teratoma tissue exhibiting characteristics of full maturation and differentiation were collected from the area of hair follicle in dermoid tissue. As a positive control of centriole/centrosome presence, fragments of non-teratoma tissue were collected simultaneously from the ovarian surface covering each teratoma cysts and/or from the adjacent fallopian tubes.

2.2. Immunofluorescence

Collected tissue samples were briefly washed with phosphate buffered saline solution (PBS), frozen with optimal cutting temperature (OCT) compound medium, and cut into 8 μm thick sections using a cryostat. Sections were prepared on glass slides and fixed in PBS with 10% paraformaldehyde and in 70% ethanol for 30 min each at room temperature (RT). Fixed samples were stored at 4 °C in PBS containing 1% bovine serum albumin (Sigma, 1% BSA–PBS) for less than four days before further procedures. The tissue samples on the slides were permeabilized in 1.5% BSA–PBS containing 0.25% Triton X-100 for 4 h at RT. Samples were then blocked in 1% BSA-PBS for 1 h at RT and incubated overnight at 4 °C with rabbit anti γ-tubulin (1:200, Sigma) as the primary antibody. Negative control samples were incubated in 1% BSA solution without primary antibody. After washing three times with 1% BSA–PBS, all samples were incubated with Alexa Fluor 488-labeled goat anti-rabbit IgG (1:200, Invitrogen, Molecular Probes Inc., USA) for 40 min at 37 °C in a humidified incubator. After washing three times in PBS for ten minutes each, samples were mounted with Vectashield Mounting Medium with propidium iodide (Vector Laboratories Inc., Burlingame, CA, USA).

2.3. Fluorescent microscopy

Tissue samples prepared on glass slides were observed under an inverted microscope (TE 2000, Nikon) using Epi Fluorescent Equipment (NEE 540AC, Nikon). Images were taken with a cooled CCD digital camera (DMX 1200C, Nikon) and were merged using NIS Elements F 2.30 digital imaging software.

2.4. Transmission electron microscopy

Tissue samples fixed in 2.5% glutaraldehyde/0.1 M phosphate buffer (pH 7.2) were post-fixed in 1% osmium tetroxide and embedded in an Epon mixture. Thick sections (1 μm) were cut with an ultramicrotome and stained with toluidine blue. Areas of teratoma tissue suitable for electron microscopic examination were selected under a light microscope. Thin sections (70–80 nm) were stained with uranyl acetate and lead citrate and examined using TEM (Tecnai 20, FEI).

3. Results

All tumors in this study were pathologically confirmed to be ovarian mature (benign) cystic teratoma. Centrosomes were successfully observed as positively-stained γ-tubulin spots in samples from six of the seven cases (Fig. 1). The results were not definitive in the remaining case as one sample slide was negative and the other slide had many artifact spots. Dense intracytoplasmic centrioles were also observed in the examined dermoid cells under TEM (Fig. 2).

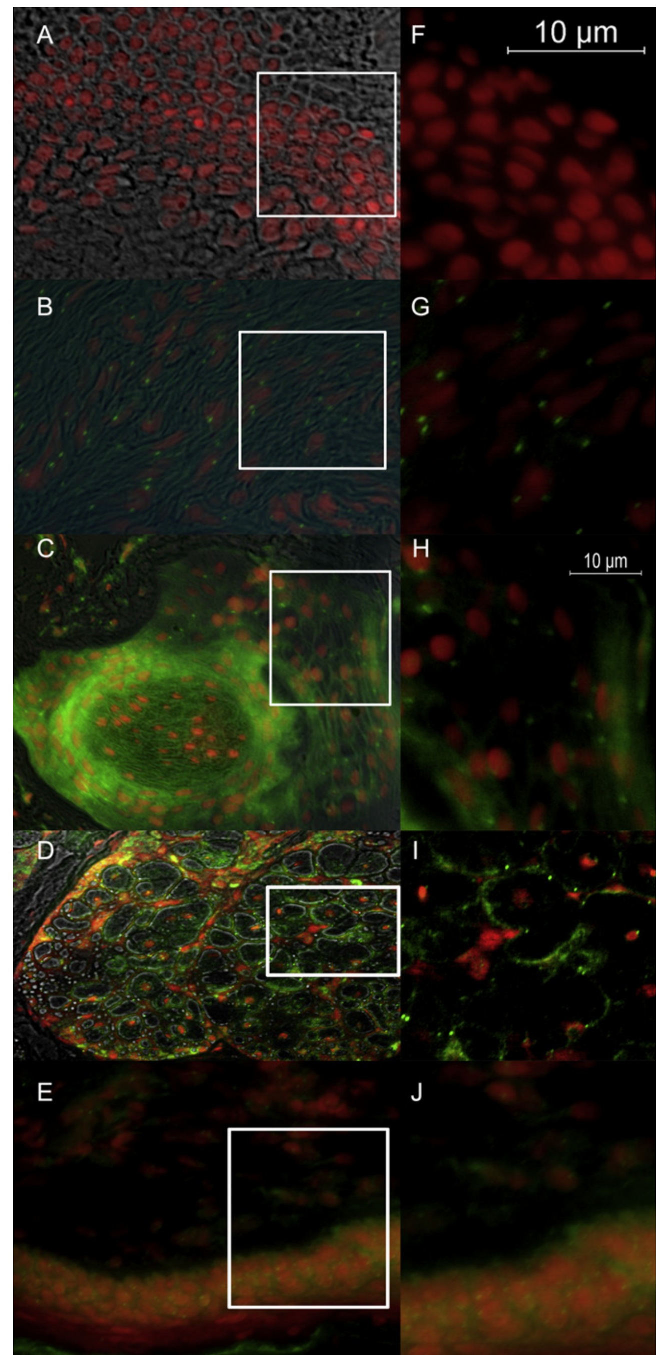

Fig. 1. Immunofluorescence staining of γ-tubulin in cells from ovarian mature cystic teratomas. The layers of γ-tubulin (green) staining, DNA (red) staining and phase contrast (A–E) were merged in each image. The cellular magnification images (F–J) were merged without the phase contrast layers. Normal ovarian cortex tissues (A and F) were stained as positive control. Dermoid cells from teratoma tissue were stained without primary antibody to γ-tubulin as a negative control (B and G). Green γ-tubulin spots are clearly visible around each red nuclei in the hair follicle (C and H), sebaceous gland (D and I) and skin epidermis (F and J) cells found in the teratoma. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

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