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Characterization of centriole duplication in human epidermis, Bowen's disease, and squamous cell carcinoma

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ABSTRACT

Background: Centrosomes contain two centrioles: a pre-existing mature centriole and a newly formed immature centriole. Each centriole is duplicated once within a cell cycle, which is crucial for proper centrosome duplication and cell division.
Objective: To describe the centrosome duplication cycle in human epidermis, Bowen's disease (BD), and squamous cell carcinoma (SCC).
Methods: Immunofluorescent staining of centriolar proteins and Ki-67 was used to evaluate cell cycles and the number of centrioles. Centrobin and Outer dense fiber of sperm tails 2 (ODF2) were used as markers for immature and mature centrioles, respectively.
Results: Normal human primary epidermal keratinocytes in a monolayered culture have one centrobin^{+} centriole (CTRB^{1+} cells) supposed in G0/G1 phases or have two centrobin^{+} centrioles (CTRB^{2+} cells) supposed in S–G2 phase. In a three-dimensional culture and in vivo human epidermis, the majority of suprabasal cells were CTRB^{2+} cells, in spite of their non-proliferative Ki-67^{-} nature. The tumor mass of BD and SCC contained CTRB^{1+} cells and Ki-67^{+} proliferating and Ki-67^{-} non-proliferative CTRB^{2+} cells. Clumping cells in BD had increased numbers of centrioles, with an approximate 1:1 to 2:1 ratio of centrobin^{+} to ODF2^{+} centrioles.
Conclusions: The cell cycle arrest of suprabasal cells is distinct from the G0 arrest of monolayered epithelial cells. Tumor mass of BD and SCC contained non-proliferative cells with the characteristics of the suprabasal cells of normal epidermis. A constant ratio of the number of centrobin^{+} to ODF2^{+} centrioles indicates that multiple centrioles were induced by cell division failure rather than centriole overduplication in clumping cells.
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1. Introduction

The centrosome is the microtubule-organizing center of animal cells. Each cell has one centrosome, comprising a pair of centrioles and the surrounding pericentriolar material (PCM) [1]. Centrosomes are involved in spindle pole assembly, cell polarity, cell migration, and maintenance of cellular stemness [2–4]. Numerical and structural centrosome abnormalities have been observed in many human carcinomas including pre-invasive carcinomas. It remains unclear whether centrosome abnormalities cause, or are a result of, tumorigenesis [5].

Centrosome duplication occurs once in a cell cycle [6]. The centrosome duplication cycle is summarized in Fig. 1. There is one centrosome in the G0/G1 phase, which contains two centrioles: a fully matured centriole with distal appendages, and an immature centriole. These act as parental centrioles in the subsequent S phase. A procentriole is formed at the proximal end of each parental centriole. The two procentrioles then elongate to become centrioles, resulting in the formation of two pairs of centrioles in the G2 phase. At the G2/M transition, the two pairs of centrioles are separated by digestion of the linkage connecting the two parental centrioles. The immature parental

Abbreviations: BD, Bowen's disease; SCC, squamous cell carcinoma; ODF2, outer dense fiber of sperm tails 2; PCM, pericentriolar material; CTRB^{1+} cells, cells with one centrobin^{+} centriole; CTRB^{2+} cells, cells with two centrobin^{+} centrioles; HPEKs, human primary epidermal keratinocytes; GFP, green fluorescent protein; PBS, phosphate-buffered saline.

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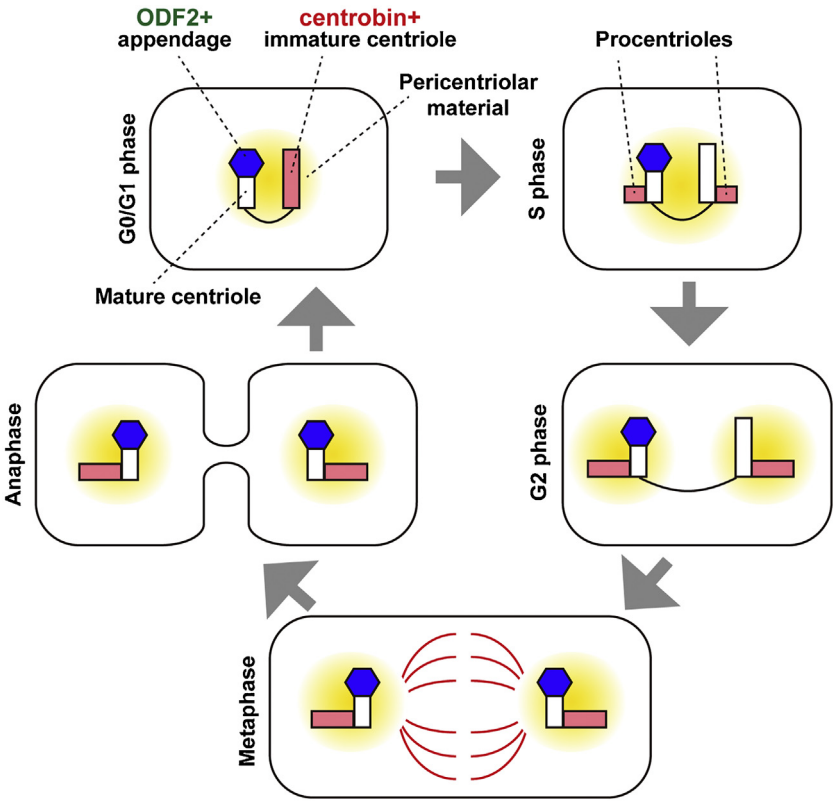

Fig. 1. Schematic of centrosome duplication cycle.
Blue hexagon: centriolar appendage of mature centriole, where ODF2 is specifically accumulated; white rectangle: centrobin$^-$ mature centriole equipped with a centriolar appendage; pink rectangle: centrobin$^+$ immature centriole; yellow circle: pericentriolar material.

centriole fully matures at the G2/M transition [7]. Both pairs of centrioles then comprise one mature centriole and one newly formed immature centriole. Each pair of centrioles, along with the associated PCM, function as a spindle pole body in the M phase and are incorporated into each daughter cell [8]. The DNA replication cycle and the centrosome duplication cycle are differently controlled but tightly synchronized in the normal cell cycle [9].

Several centriolar proteins have been shown to localize asymmetrically to mature or immature centrioles. Centrobin is an important protein for centrosome duplication [10]. During the S phase, centrobin specifically localizes on the newly formed procentrioles. In the G2 and M phases, each centrosome has one newly formed centrobin$^+$ centriole and an older centrobin$^-$ centriole. After cell division, centrobin is present on the newer of the two centrioles in G1 phase cells [11]. Outer dense fiber of sperm tails 2 (ODF2), also known as cenexin, specifically localizes to the centriolar appendages of the fully matured centriole [12]. ODF2 is present only on the fully matured centriole throughout the G1, S, and G2 phases [13]. In a G2 phase cell with two centrosomes, one centrosome contains a newly formed centrobin$^+$/ODF2$^-$ centriole and a centrobin$^-$/ODF2$^+$ fully matured centriole, while the other contains a newly formed centrobin$^+$/ODF2$^-$ centriole and a centrobin$^-$/ODF2$^-$ parental centriole (Fig. 1). The centrobin$^-$/ODF2$^-$ centriole of a G2 phase cell matures and becomes a centrobin$^-$/ODF2$^+$ centriole in the M phase (Fig. 1) [11,13].

Relatively little is known about the centrosome duplication cycle in epidermal cells, especially human epidermis in vivo. This study investigated the centrosome duplication cycle of human epidermal keratinocytes and epidermal tumors by evaluating the subcellular localization of centrobin and ODF2.

2. Materials and methods

2.1. Study participants

Healthy skin samples from leftover skin collected during excisional surgery were obtained from seven patients with a mean age of 63.7 years (range: 12–89 years). Bowen's disease (BD) samples (seven patients; mean age, 66.1 years) and well-differentiated squamous cell carcinoma (SCC) samples (five patients; mean age, 73.8 years) were also obtained during excisional surgery. The list of samples is shown in Supp. Table S1. Sample collection was approved by the Ethics Committee of Keio University School of Medicine, Tokyo, Japan. Before use of preserved specimens, written informed consent was obtained from patients or the procedure to opt-out was posted on the website following the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. The study was conducted according to the principles of the Declaration of Helsinki. No identifying information was associated with samples and complete patient anonymity was maintained.

2.2. Cell culture

Normal human primary epidermal keratinocytes (HPEKs) were obtained from a single donor (CELLnTEC, Bern, Switzerland). HPEKs were cultured at 37 °C and 5% CO_2 in CnT-Prime epithelial culture medium (CELLnTEC). The medium was serum-free and low Ca (0.07 mM). The monolayered culture was prepared by seeding 1×10^5 cells in a 12 mm Transwell® with a 0.4 μm pore polyester membrane insert (Corning Inc., Corning, NY) and culturing for 2 days in CnT-Prime until cells became confluent. The culture medium was changed to CnT-Prime with low Ca (0.07 mM) or CnT-Prime 3D Barrier medium (CELLnTEC) with high Ca (1.27 mM).

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