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# Effect of different dose rates of ionizing radiation on ciliogenesis in hTERT-RPE1 cells

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**Abstract**

Ionizing radiation (IR) induces genome instability and chromosome aberration in mammalian cells. IR exposure generates DNA damage, which is repaired by several DNA repair pathways. Meanwhile, IR also induces ciliogenesis and centrosome overduplication associated with cell cycle checkpoint mechanism. Centrosome number is strictly regulated, since overduplicated centrosomes cause aneuploidy, leading to tumorigenesis. Primary cilia play a sensory role in several signaling pathways during development and cellular homeostasis. In this study, to address how IR affects ciliogenesis, we irradiated telomerase reverse transcriptase-immortalized retina pigmentation epithelial cell, hTERT-RPE1, with γ-ray at different dose rates, that is 2 mGy/s (low dose rate) and 100 mGy/s (high dose rate). Centrosome and primary cilia were detected by immunofluorescence using γ-tubulin and acetylated-α-tubulin antibodies, respectively. After IR exposure, we saw an increase in cells with primary cilia and the combined treatment of IR exposure with serum starvation stimulation showed an additive effect. This study provides a new insight into radiation effect on the extracellular response.


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*Keywords:* centrosome, ciliogenesis, cell cycle checkpoint, genome instability

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## 1. Introduction

In mammalian cells, genome DNA is strictly maintained by several molecular mechanisms such as DNA repair, cell cycle checkpoint and chromosome segregation. Disruption of these mechanisms leads to genome instability, resulting in chromosome aneuploidy, which is considered one of the causes of tumorigenesis. Ionizing radiation (IR) is powerful inducer of genome instability through DNA damage including base damage, DNA single strand and double strand breaks and centrosome overduplication [1]. Centrosome consists of two centrioles, which is surrounded by pericentriolar material (PCM), is indispensable for proper cell division. Centrosome duplicates once per one cell cycle during DNA synthesis. Centrosome duplication cycle is regulated by centrosome consisting factors and cell cycle factors. Meanwhile, many DNA repair proteins localize to the centrosome. We previously reported that NBS1, the protein responsible for Nijmegen breakage syndrome (NBS) and Ataxia telangiectasia and RAD3-related protein (ATR) localize to the centrosome to regulate centrosome duplication by Breast Cancer 1 protein (BRCA1) dependent γ-tubulin mono-ubiquitination [2]. This result suggested DNA damage response proteins might be involved in whole genome maintenance system including DNA repair and centrosome maintenance.

Primary cilia are microtubule-based organelle that functions as biosensor of extracellular environment during development and cellular homeostasis [3]. Ciliogenesis is stimulated by cell cycle dependent manner in epithelial cells. Defect of cilia sensory proteins causes developmental disease including organ dependent inherited disease. ATR is one of master regulator of genome DNA damage signaling. Defects of *ATR* gene cause Seckel syndrome characterized by microcephaly and dwarfism. Recent report showed depletion of ATR reveals short length primary cilia and developmental defect [4]. Furthermore, mutation of centrosome protein Cep164 causes nephronophthisis-related ciliopathies (NPHP-RC), which are autosomal recessive diseases affecting kidney, retina and brain. Knockdown experiments using siRNA show Cep164 is involved in the DNA damage response [5]. These previous reports suggest that DNA damage response might have an important role in ciliogenesis. Indeed, IR exposure to the cells stimulates ciliogenesis [6, 7]. However, effect of different dose rate of IR exposure on the cilognesis is still unknown. In this study, we addressed here how different dose rate of IR exposure affects ciliogenesis in mammalian cells.

## 2. Materials and Methods

### 2-1 Cell culture

Telomerase reverse transcriptase-immortalized retina pigmentation epithelial cells (hTERT-RPE1, Clontech) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin at 37℃ with 5% $CO_2$. DMEM without FBS was used for serum starvation culture.

### 2-2 Immunofluorescence

Cells were cultured on slide glass (Matsunami) and irradiated with γ-ray. Cells were fixed with 100% cold methanol for 10 min and permeabilized with 0.5% tritonX-100 in phosphate-buffered saline (PBS) at 4℃ for 5min. Cells were then blocked with 1% bovine serum albumin (BSA) and incubated with primary antibodies, acetylated-α-tubulin (SIGMA: T7451) and γ-tubulin (SIGMA: T3559), for 4h to overnight. After primary antibodies incubation, cells were incubated with secondary antibodies, Alexa-488 conjugated anti-rabbit IgG (Molecular Probes) and Alexa-594 conjugated anti-mouse IgG (Molecular probes), for 1h at room temperature. Cells were mounted with fluorescence mounting medium (DAKO) containing 4',6-diamidino-2-phenylidole (DAPI). Represented images were captured using an inverted fluorescence microscope (Olympus).

### 2-3 Irradiation

Cells were irradiated with $^{60}$Co γ-ray source (Tokyo Institute of Technology).

### 2-4 Statistical analysis