Absence of Radio-Sensitization mediated by Telomerase-inhibition

Hee Young Kim[1], Yeun-Jin Ju[1], Jeong-Eun Park[1], Kyoung-Mi Juhn[1], Miyong Yun[1], Young-Hoon Han[1], Chang-Mo Kang[2], Su-Jae Lee[3], Sang-Hoon Kim[4], Myung-Haing Cho[5], Gil Hong Park[6,*], and Kee-Ho Lee[1,*]
[1]Laboratory of Radiation Molecular Cancer, [2]Laboratory of Cytogenetics and Tissue Regeneration, Korea Institute of Radiological & Medical Sciences, Seoul, 139-706, Korea, [3]Department of Chemistry, Hanyang University College of Medicine, Seoul, 133-791, Korea, [4]Department of Biology, Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul, Korea, [5]Laboratory of Toxicology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea, [6]Department of Biochemistry, College of Medicine, Korea University, Seoul
[*]Corresponding author: Khlee@kcch.re.kr

1. Introduction

The radio-therapeutics's problem in tumor is the repeated return of radio-resistant tumor cells during radiotherapy. Therefore, many studies have been accomplished to develop many modulators regulating this mechanism [1, 2]. Besides, sensitizing agents have actively been exploited to enhance the radio-therapeutic efficacy for cancer. The combination anticancer radio-therapeutic cure with telomerase inhibition is useful to sensitize tumor cells to radiation, depending on telomere dysfunction and eventual genomic instability [3, 4]. In our studies, we showed that there was absence of radio-sensitization mediated by telomerase deficiency in clonal cell population.

2. Methods and Results

2.1 Estabilishment of telomerase-deficient clones

The Myc/Ras-transformed MEFs derived from mice carrying homozygous deletions of mTERC, and either the INK4a or p53 locus were obtained as described previously [5]. The single clones were picked, expanded 14 days after the transfection and passaged until 220 population doubling levels (PDL). Serial passages of individual clones obtained from late generation were performed at confluence with a split ratio of 1:16 (corresponding to four population doublings).

2.2 Determination of cell survival and viability.

The survival of transformed MEFs was determined by measuring the colony forming ability of single cells exposed to radiation. Cells were plated in 100 mm dishes at a density of 2,400 cells and then were exposed to γ-ray using a ^{137}Cs source, and then 8 days after the radiation, we stained with 0.1% crystal violet and measured colonies. The relative cell survivals were calculated as percentages of colonies counted in un-irradiated cells.

2.3 Radiosensitization of G5 mTERC$^{-/-}$INK4a$^{-/-}$

We performed clonal analysis using telomerase-deficient cell with or without extensive cell division. We confirmed the discrepancy of radiosensitivity between individual clone at an early passage and the clone at a late passage. Therefore, to determine the radiosensitization of clones, the measurement of cell survival to radiation was performed using colony-forming assay.

Long-term passages induced more radiosensitization of six of eight clones in G5 mTERC-/- INK4a-/- cultures, However, two clones of clones were unchanged at survival to radiation as compared to their corresponding early passaged clones. This result suggests that the clonal cell population without the radiosensitization via telomerase inhibition is occured. Also, when mTERC were rescued in clonal population, it produced two of four clones more resistant and the other two unchanged, as compared to their corresponding early passaged clones.

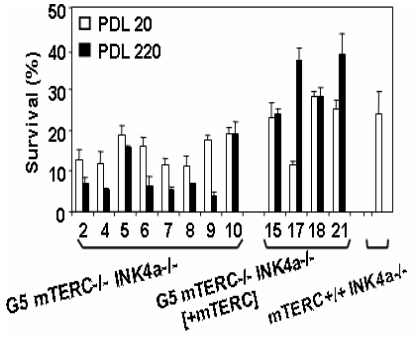

Fig.1. Occurrence of clones absense of radiosensitization mediated by telomerase deficiency. Survivals of individually isolated clones derived from Myc/Ras transformed G5 mTERC-/- INK4a-/- cultures with and without mTERC-reconstitution were measured after exposure to 3 Gy of radiation. Numbers of each panel indicate independently derived clones.

2.4 Radiosensitization of G6 mTERC$^{-/-}$p53$^{-/-}$

Next, we tested in p53-deficient cultures using G6 mTERC-/- p53-/- cells (data not shown). Also, we found that radiosensitizing efficiency was induced in the similar manner of G5 mTERC-/- INK4a-/- cultures at G6 mTERC-/- p53-/- cells. In result, eight of ten clones without mTERC-reconstitution also displayed a decrease of survival rate during the late passages, but, two did not changed in survival rate. It suggests that the

clones without the radiosensitization produce independent of telomerase.

3. Conclusions

Our present findings say that in most of clonal polulations, radio-sensitization was induced radiosensitization, dependent on telomerase inhibition, but late generation of telomerase-deficient clones occur mute of radiosensitization.

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