論文内容要旨

Evaluation of \textit{ATM} heterozygous mutations underlying individual differences in radiosensitivity using genome editing in human cultured cells

(ヒト培養細胞株におけるゲノム編集を用いた\textit{ATM}ヘテロ遺伝子変異による放射線感受性個人差への定量的評価)

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[Background] It has been suggested that there are individual differences in radiosensitivity within human populations, and that the variations in DNA repair genes might determine this heterogeneity. However, it is difficult to quantify the effect of genetic variants on the individual differences in radiosensitivity, since confounding factors within human populations affect radiosensitivity. It is therefore necessary to develop a highly sensitive system for detecting IR–induced DNA lesions in human cells with a candidate genetic variation in a uniform human genetic background.

[Methods] To precisely quantify the effect of a genetic variation on radiosensitivity, we generated ATM heterozygous knock-out (ATM+/−) cell clones as a carrier model of a radiation–hypersensitive autosomal–recessive disorder, ataxia–telangiectasia (A–T) using the CRISPR–ObLiGaRe method combined with the CRISPR/Cas9 system. This approach enabled the insertion of a drug–resistant gene cassette tagged with the genomic CRISPR/Cas9 recognition sequence into the specific ATM locus via NHEJ activity in the hTERT–RPE1 cell line from human normal retina pigmented cells. Cytokinesis–blocked micronucleus assay and chromosome aberration assay were used to access cell radiosensitivity. The dose–response curves were analysed using a linear–quadratic model using chromosomal aberration calculation (Cabas) software.

[Results] Based on the data of the semiautomatic radiosensitivity assays in ATM–edited cell clones, we concluded that A–T heterozygous null mutations had an effect of increasing cellular radiosensitivity by 2.6–fold, and suggested that they were indeed a genetic factor underlying individual differences in radiosensitivity within human populations. We demonstrated that a semi–automated CBMN assay in the CRISPR/ObLiGaRe–mediated model cells could quantify the effect of ATM heterozygous mutations on radiosensitivity.

[Discussion] We established an experimental flow combined with a semiautomatic CBMN assay and genome editing technology in a human cultured cell line as a unique system for evaluating genetic factors underlying individual differences in radiosensitivity. High throughput validation of the genomic alterations by reverse genetics might provide correct diagnosis and convincing genetic markers to generate a personal radiation protection system for practical and clinical situations including radiation disasters, radiation therapy and CT imaging. Taking these approaches together, further improvement of genome–editing technology in human cultured cell lines with a uniform genetic background might enable further exploration of the genetic mechanisms underlying individual differences in radiosensitivity within human populations.