Development of highly sensitive genotoxicity test method using TK6 and its DNA repair mutants

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Abstract

TK assays were adopted as OECD-TG490 test guideline in 2015. The guideline operates on the MLA and TK assay using the TK6 cells. TK6 cells are human origin and express wild-type p53 protein. Some DNA repair deficient TK6 mutant cells have been developed by the genome editing technologies and used for studies of mutagenesis. Here, we created TK6 double-knockout mutants (TK6 mutants) for XPA and XRCC1, which are involved in the nucleotide excision repair and base excision repair, respectively. We examined auramine, a diarylmethane dye, which had showed positive in the Ames assay, but negative in the MLA. The spontaneous mutant frequency of the TK6 mutants was same as that of the wild type. However, the treatment of auramine remarkably increased the mutant frequency in TK6 mutants, but not in wild-type cells, indicating that TK assay using the TK6 mutants are useful for follow-up to Ames positive chemicals to confirm mutagenicity.

Generation of TK6 Double Mutant

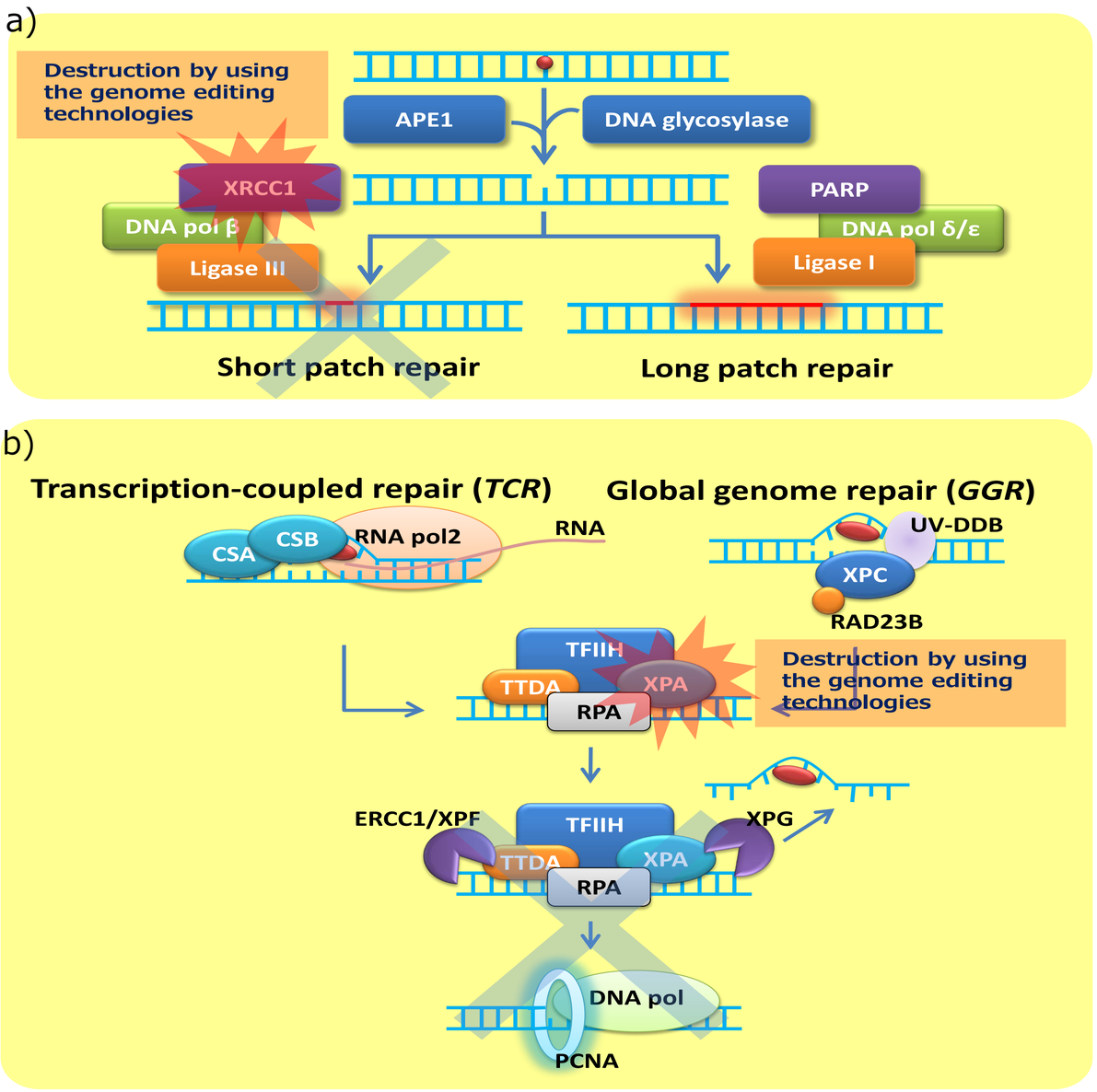


Fig. 1 TK6 mutant ($XPA^{-/-}/XRCC1^{-/-}$) generated by the genome editing technology and its phenotypic characterization.

The model for BER and NER pathways. The genes of a) XRCC1 and b) XPA in TK6 mutant were destructed by genome editing technologies. The jagged mark (red) indicates a target protein for the genome editing in the BER or NER pathway. c) The survival assays of cisplatin were performed by the colony formation assay using TK6 cells and its mutant cells.

Material and Method

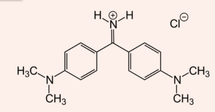
Structure	
Use	a diarylmethane dye, a fluorescent stain
Ames	**Positive**
MLA	**Negative**
Carcinogenicity	2B (IARC)

Fig. 2 Property of Auramine.

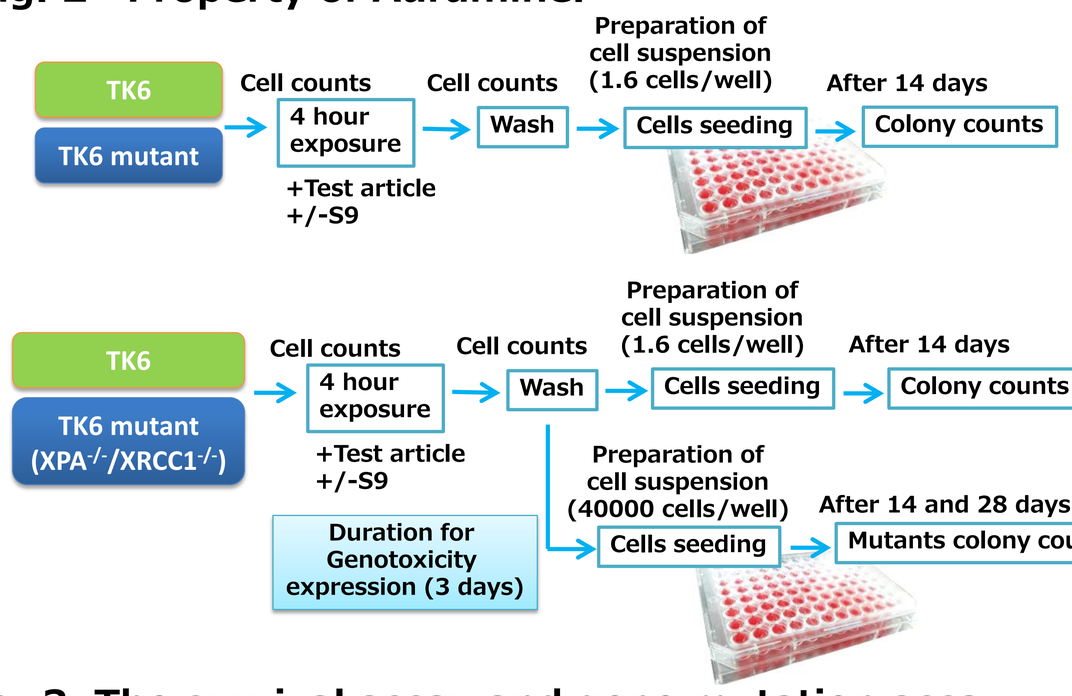


Fig. 3 The survival assay and gene mutation assay.

Results

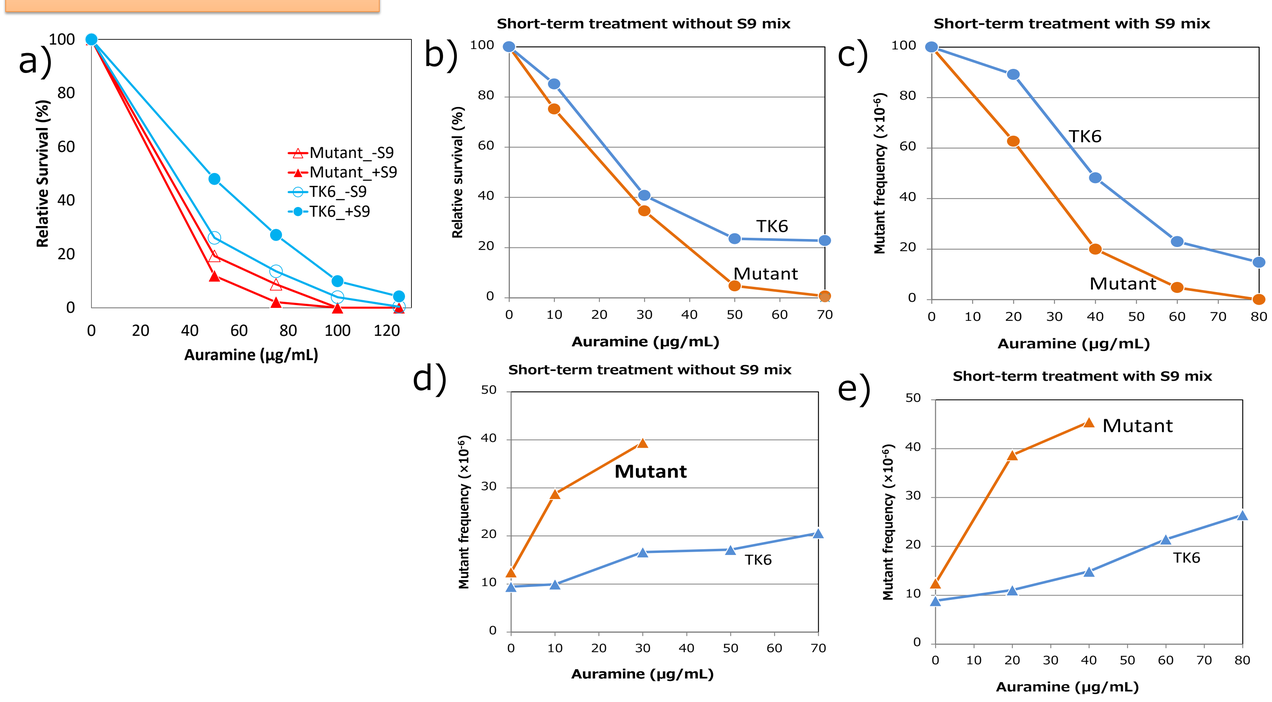


Fig. 4 The survival assay and gene mutation assay.

a) Dose range finding assay, b) survival assay without S9 mix, c) survival assay with S9 mix, d) mutation assay without S9 mix and e) mutation assay without S9 mix.

Conclusion

- **The spontaneous mutant frequency of the TK6 mutants was same as that of the wild type. However, the treatment of auramine remarkably increased the mutant frequency in TK6 mutants**
- **TK assay using the TK6 mutants is useful for follow-up to Ames positive chemicals to confirm mutagenicity.**

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