Increased chemosensitivity of paclitaxel by telomeric fusion-induced genomic instability

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1. Introduction

A telomere is a region of repetive DNA at the end of chromosomes. They protect a cell's chromosomes from fusing with each other or rearranging and so cells are normally destroyed when their telomeres are consumed. Most normal somatic cells lose telomeric repeats after each cell division. Telomeric shortening in humans can induce replicative senescence which blocks cell division. This mechanism appears to prevent genomic instability by limiting the number of cell divisions [1]. Telomerase is an attractive molecular target, since its activity has been found in more than 85% of human cancers.

Combination therapy with chemotherapeutic agent is superior to single in overall response rate and progression free survival. In this study, we showed that telomerase null cells are more hypersensitive by paclitaxel treatment than at wild type cells.

2. Methods and Results

The Myc/Ras-transformed mouse embryonic fibroblast (MEF) derived from mice carrying homozygous deletions of mTERC and p53 locus were obtained as described previously [5,6,7]. mTERC$^{-/-}$p53$^{-/-}$ MEFs grown in DMEM containing 10% FBS, were co-transfected with 2ug each of expression constructs for Myc and H-Ras^{G12}, and either mTERC [8] or an empty Bluescript KS(+) (stratagene) vector.

Mouse TERC was subcloned into the retroviral vector pBabe-puro. The retroviruses were produced by transient transfection of pMFG-puro, mTERC/pBabe plasmids into H29D packaging cells. mTERC$^{-/-}$p53$^{-/-}$ MEF cells were infected with retrovirus containing 6 ㎍/㎖ of polybrene for 4 hrs.

2.1 Telomerase inhibition is required for the enhancement of paclitaxelsesitivity, which can occur irrespective of p53 status.

To assess whether telomerase inhibition may enhance chemo-therapeutic efficacy of paclixel, paclitaxel were exposed to Myc/Ras-transformed mTERC$^{-/-}$p53$^{-/-}$MEF cells derived from the later generation telomerase null mice which lacked telomerase RNA component. G6 mTERC$^{-/-}$p53$^{-/-}$ culture were more paclitaxel-sensitivity than those of the corresponding mTERC-rescued cells (Fig.1). Because the exposure of paclitaxel in G6 mTERC$^{-/-}$p53$^{-/-}$ cultures elicited severe decrease in cell survival. However, the mTERC-reconstitution did not affect the survival rates of early generation G2 mTERC$^{-/-}$p53$^{-/-}$ cultures which retain intact telomere function.

The lower survival rates found in G6 mTERC$^{-/-}$p53$^{-/-}$ cultures than their corresponding mTERC-reconstituted cultures clearly indicate that telomerase inhibition can sensitize telomerase-positive cells with critically short telomerases toward paclitaxel and that this sensitization could occur regardless of p53 status.

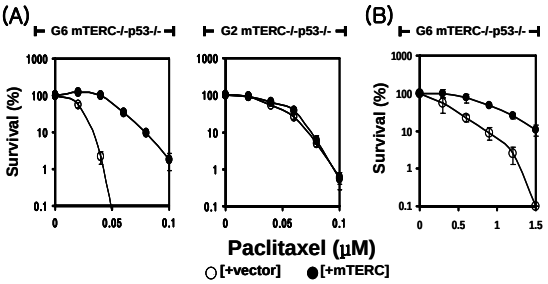

Fig. 1. paclitaxel sensitivity of Myc/Ras-transformed mTERC$^{-/-}$p53$^{-/-}$ MEFs with dysfunctional telomere.

2.2 Abundant mitotic death in telomere dysfunctional cells.

Mitotic arrest of paclitaxel-treated cells has been associated with apoptosis. Paclitaxel-induced apoptosis can occur either directly after a mitotic arrest or following an aberrant mitotic exit into a G1-like 'multinucleate state'.

The telomerase deficient MEFs in response to paclitaxel exposure induced an impaired cell cycle disruption with more accumulation of tetraploid population than telomerase-reconstituted MEFs (Data not shown).

3. Conclusions

Paclitaxel is one of the broadest-spectrum anticancer agents and it kills cancer cells more efficiently in cells

with p53 mutation than in those with wild-type p53. Besides, the exposure of paclitaxel in long term generation G6 $mTERC^{-/-}p53^{-/-}$ cultures elicited severe decrease in cell survival through induction of chromosomal end-to-end fusions.

These findings suggest that the increased genomic instability through telomere dysfunction might profoundly increase chemosensitization after paclitaxel treatment in tumore, which could be occurred in cells defective of p53 as well as wt-p53.

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