102 Genetic Instability Induced by Cell Cycle Arrest at S Phase in V79 Cells

[1]Toshio MORI, [2]Mitsumasa HASHIMOTO, [2]Osamu NIKAIDO, [3]Keizo TANO, [4]Keiji SUZUKI and [5]Masami WATANABE.; [1]Nara Medical Univ., [2]Kanazawa Univ., [3]Kyoto Univ., [4]Yokohama City Univ. and [5]Nagasaki Univ.

To investigate a relationship between genetic instability and cell cycle arrest, V79 cells were treated with various concentrations of aphidicolin , an inhibitor of DNA synthesis, for 54 hours, then the emergence of methotrexate resistant (MTX^r, 150 nM) colonies were determined. Indeed, aphidicolin induced MTX^r cells in a concentration-dependent manner. Ten MTX^r cells were cloned and characterized in chromosome mode number, dihydrofolate reductase (DHFR) gene amplification and DHFR protein amount usng FITC-MTX and laser cytometry. Ten clones were divided into 3 groups. 1) No DHFR gene amplification, normal chromosome number. 2) 2 times DHFR gene amplification, normal chromosome number. 3) No DHFR amplification, 2 times increase in chromosome number. When clones from 3 groups were cultured with step-up selection of MTX for 3 months, 1st group obtained only 5,000 nM resistance, although 2nd and 3rd groups obtained more than 40,000 nM resistance. In highly resistant clones, 1st group did not show any DHFR gene amplification, whereas 2nd and 3rd groups showed considerable gene amplification. These results indicate that the cell cycle arrest at S phase can induce genetic instability like gene amplification in V79 cells.

103 Genetic Instability Induced by Cell Cycle Arrest at M Phase in Chinese Hamster V79 Cells

[1]Mitsumasa HASHIMOTO, [1]Osamu NIKAIDO, [2]Keizo TANO and [3]Toshio MORI.; [1]Kanazawa Univ., [2]Kyoto Univ. and [3]Nara Medical Univ.

To confirm that the cell cycle arrest can induce genetic instability like gene amplification, V79 cells were treated with various concentrations of colcemid, an inhibitor of cell division, for 48 hours, then the emergence of methotrexate resistant (MTX^r, 350nM) colonies were investigated. Colcemid increased cell killing and induced MTX^r cells in a concentration-dependent manner. Furthermore, flow cytometry showed that colcemid induced the formation of tetraploid cells with an 8C DNA content. Colony forming cells after colcemid treatment had also increased nuclear size in a concentration-dependent manner. These results suggested that the cell cycle arrest at M phase could induce genetic instability like gene amplification through tetraploidization, as observed by the arrest at S phase.

104 Analysis of human embryo cells transfected with antisense-p53 gene

Kenji MAEDA, Seiji KODAMA, Masami WATANABE; Fac.Pham.Sci.,Nagasaki Univ. Nagasaki 852

Mutations in p53 gene are the most common genetic alteration in human cancer.The p53 gene acts as a transcription activator that suppress abnormal cell proliferation by acting as a G_1 cell cycle checkpoint control for DNA damage. To study p53-dependent G_1 checkpoint, the p53 expression plasmid, pRc/RSV-p53 or the p53 suppressor plasmid,pRc/RSV-antisense-p53 were transfected into human embryo cells(HE7) and immortalized human diploid fibroblast cells(KMST-6).The life span (mean population doubling number) of HE7 cells transfected by pRc/RSV-antisense-p53 prolonged 10PDN over that of HE7 cells transfected by pRc/RSV-p53. This result suggests that p53 may be acting as a growth inhibitor in normal cell.