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■論 文■

ATM-induced Radiosensitization in Vitro and in Vivo

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Abstract - It has been known that ATM plays a central role in response of cells to ionizing radiation by enhancing DNA repair. We have investigated the feasibility of increasing radiosensitivity of tumor cells with the use of ATM inhibitors such as caffeine, pentoxifylline and wortmannin. Human colorectal cancer RKO.C cells and RKO-ATM cells (RKO cells overexpressing ATM) were used in the present study. The clonogenic cell survival in vitro indicated that RKO-ATM cells were markdely radioresistant than RKO.C cells. Treatment with 3 mM of caffeine significantly increased the radiosensitivity of cells, particulary the RKO-ATM cells, so that the radiosensitivity of RKO.C cells and RKO-ATM cells were almost similar. The radiation induced G2/M arrest in RKO-ATM cells was noticeably longer than that in RKO.C cells and caffeine treatment significantly reduced the length of the radiation induced G2/M arrest in both RKO.C and RKO-ATM cells. Pentoxifylline and wortmannin were also less effective than caffeine to radiosensitize RKO.C or RKO-ATM cells. However, wortmannin was more effective than caffeine against human lung adenocarcinoma A549 cells indicating the efficacy of ATM inhibitor to increase radiosensitivity is cell line dependent. For in vivo study, RKO.C cells were injected s.c. into the hind-leg of BALB/c-nuslc nude mice, and allowed to grow to 130mm3 tumor. The mice were i.p. injected with caffeine solution or saline and the tumors irradiated with 10 Gy of X-rays. The radiation induced growth delay was markedly increased by 1-2 mg/g of caffeine. It was concluded that caffeine increases radiosensitivity of tumor cells by inhibiting ATM kinase function , thereby inhibiting DNA repair, that occurs during the G2/M arrest after radiation.

Key words : ATM, Radiosensitization, G2 arrest

INTRODUCTION

It has been known that ATM plays a central role in response of cells to ionizing radiation by enhancing DNA repair. Based in large part on studies of the homologous proteins in yeast, it is predicted that ATM function as proximal signal transducers in G1, S, and G2 checkpoint pathways. With the exception of p53, the downstream components of these pathways remain largely undefined. We have investigated the feasibility of increasing radiosensitivity of tumor cells with the use of ATM inhibitors such as caffeine, pentoxifylline, and wortmannin. Also in an effort to examine and understand the molecular mechanism by which ATM might exert its cellular effects, we have expressed the full length wild type ATM in RKO cells.

MATERIALS and METHODS

Human colorectal cancer RKO.C cells, RKO-ATM cells (RKO cells over expressing ATM), and A549 human lung adenocarcinoma cells were used in the present study. The cells were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The cells were cultured in 25 ㎠ plastic tissue culture flasks at 37℃ in a humidified 5% CO2/95% air atmosphere. When the cells were in exponential growth phase at a cell density of 3×10^6 cells/25 ㎠ flasks, the medium was replaced with fresh medium that had been adjusted to the desired pH value using 30mM each of Tris, MOPS(3-(4-morpholino) propanesulfonic acid) and MES(morpholino ethanesulfonic acid) buffers. After the cells were conditioned to the new pH media for 30min at 37℃, they were irradiated with 12Gy of † r-rays at a dose rate of 0.9Gy/min with a 137Cs irradiator and then incubated at 37℃ incubator for varying lengths of time. The kinase activity of DNA-PK in RKO cells was measured using SignaTECT protein kinase assay systems(Promega). For ATM kinase assay ATM were immunoprecipitated from 0.2% Tween-20 extracts prepared from A549 cells. The kinase reaction mixtures(50mM Tris(pH7.4), 10mM MgCl2, 1mM DTT, 10 μM[-32P]ATP and 25ng/ul substrate) were added immunoprecipitates. The phophorylation reactions were incubated for 15min at 30℃. The reaction were spotted on filter paper and incorporation of ^{32}P was quantitated by β -counter. After irradiation, cells were fixed with 80% chilled ethanol, then washed with saline. Fixed pellets were stained with propidium iodide, scanned and analyzed with FACscan (Bector-Dickinson). For *in vivo* study, RKO cells were injected s.c. into the hind-leg of BALB/c-nuslc nude mice, and allowed to grow to 130㎣ tumor. The mice were i.p. injected with variable concentration of caffeine solution or saline and the tumors irradiated with 10Gy of X-rays.

RESULTS

The clonogenic cell survival in vitro indicated that RKO-ATM cells were markedly radioresistant than RKO.C cells. Treatment with 3 mM of caffeine significantly increased the radiosensitivity of cells, particularly the RKO-ATM cells, so that the radiosensitivity of RKO.C cells and RKO-ATM cells were almost similar (Fig. 1). The radiation induced G2/M arrest in RKO-ATM cells was noticeably longer than that in RKO.C cells and caffeine treatment significantly reduced the length of the radiation induced G2/M arrest in both RKO.C and RKO-ATM cells. Gene expression was examined using human 10k chip in RKO and RKO/ATM after irradiation. One hundred thirty five known genes and 15 unknown genes were differentially expressed between RKO and RKO/ATM after irradiation. Pentoxifylline and wortmannin were also less effective than caffeine to radiosensitize RKO.C or RKO-ATM cells. However, wortmannin was more effective than caffeine against human lung adenocarcinoma A549 cells (Fig. 2) indicating the efficacy of ATM inhibitor to increase radiosensitivity is cell line dependent. For in vivo study, RKO.C cells were injected s.c. into the hind-leg of BALB/c-nuslc nude mice, and allowed to grow to 130mm^3 tumor. The mice were i.p. injected with caffeine solution or saline and the tumors irradiated with 10 Gy of X-rays (Fig. 3). The radiation induced growth delay was markedly increased by 1-2 ㎎/g of caffeine.

ATM and DNA-PK kinase activity after 12 Gy irradiation in pH7.5, pH 6.6 medium showed that caffeine significantly inhibits DNA-PK and ATM kinase activities in RKO.C cells after irradiation (Fig. 4).

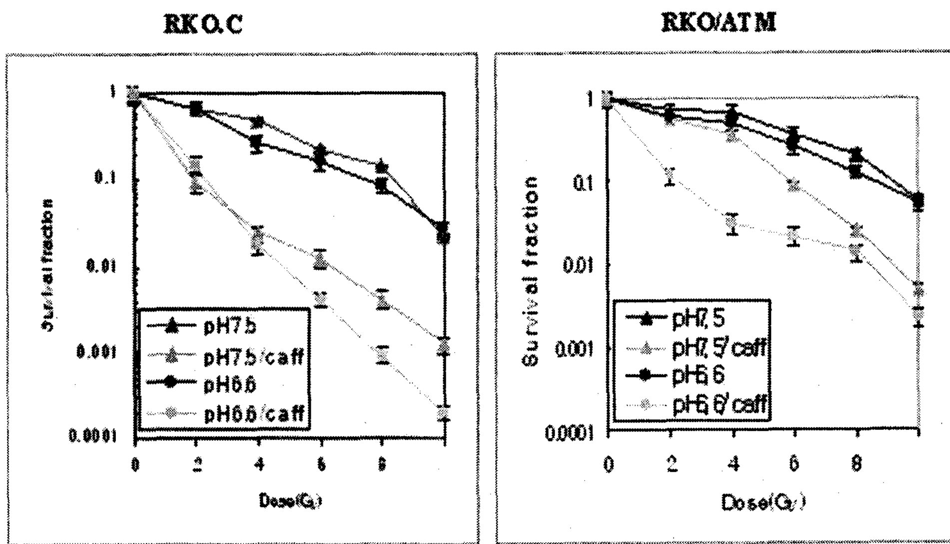


Fig 1. The clonogenic cell survival in vitro indicated that RKO-ATM cells were markedly radioresistant than RKO.C cells. Treatment with 3mM of caffeine significantly increased the radiosensitivity of cells, particulary the RKO-ATM cells, so that the radiosensitivity of RKO.C cells and RKO-ATM cells were almost similar.

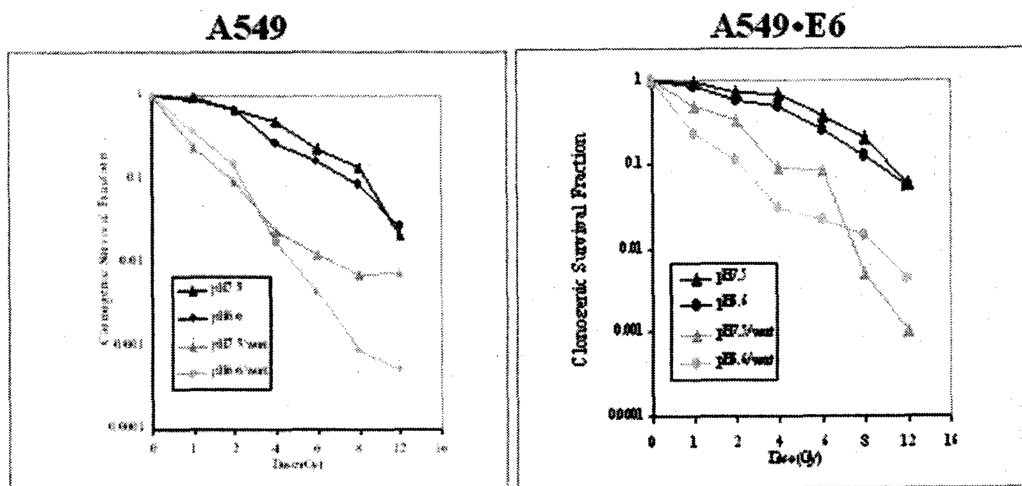
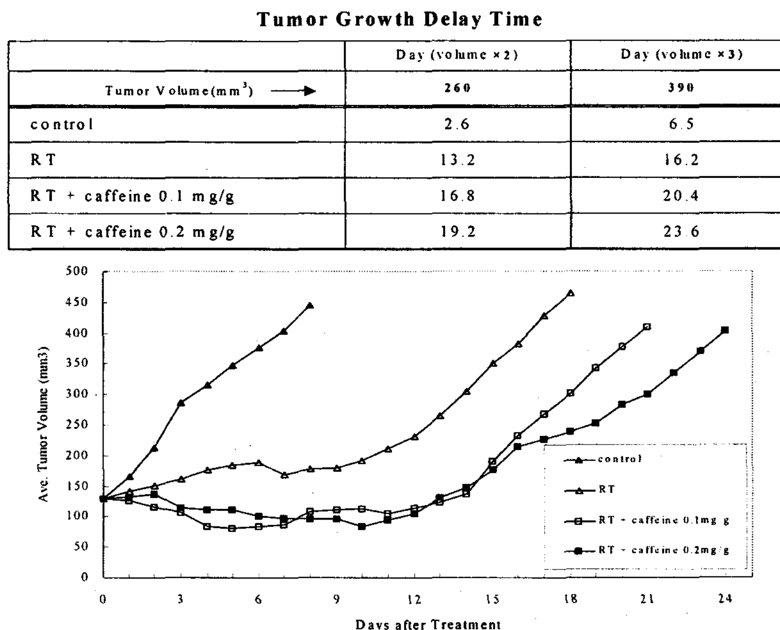


Figure 2. Clonogenic survival of A549 and A549•E6 cells in pH 7.5 or pH 6.6 media after irradiation. Wortmannin was more effective than caffeine against human lung adenocarcinoma A549 cells indicating the efficacy of ATM inhibitor to increase radiosensitivity is cell line dependent.



Tumor Growth Delay Time

	Day (volume ×2)	Day (volume ×3)
Tumor Volume(mm^3) ⟶	260	390
control	2.6	6.5
RT	13.2	16.2
RT + caffeine 0.1 mg/g	16.8	20.4
RT + caffeine 0.2 mg/g	19.2	23.6

Fig. 3. The mice were i.p. injected with caffeine solution or saline and tumors irradiated with 10Gy of x-rays. The radiation induced growth delay was mrkedly increased by 0.1-0.2mg/g of caffeine.

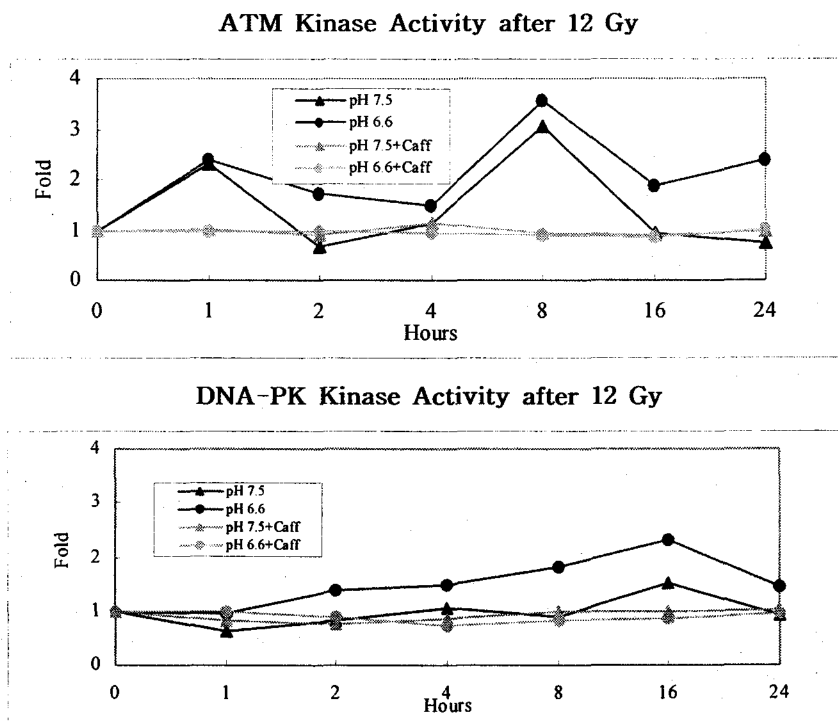


Fig. 4. ATM and DNA-PK kinase activity after 12 Gy irradiation in pH 7.5 or pH 6.6 medium. Caffeine significantly inhibits DNA-PK and ATM kinase activities in RKO.C cells after irradiation

CONCLUSIONS

Human colorectal cancer RKO.C cells, RKO-ATM cells (RKO cells over expressing ATM), and A549 human lung adenocarcinoma cells were used in the present study.

1. Radiation-induced DNA damage was repaired during the prolonged G2 arrest in RKO-ATM cells.
2. ATM modulates G2 arrest, so alteration of radiation-induced cell cycle arrest using ATM targeted inhibitors may significantly influence the response of tumors to radiotherapy.
3. Caffeine increases radiosensitivity of tumor cells by inhibiting ATM kinase function. There by inhibiting DNA repair: that occurs during the G2/M arrest after radiation.
4. In vivo study the radiation induced growth delay was markedly increased by 0.1-0.2mg/g of caffeine.

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