Role of Gap Junctional Intercellular Communication and NO Signaling in Low-Dose Radiation Hypersensitivity

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In the cells irradiated at low density, U87 cell line has shown a highly distinguishable HRS/IRR response (Chandna et al, Radiat Res, 157, 516-525, 2002), which was mitigated at higher density. In order to understand the basis of increased cellular lethality at low doses as well as the density-mediated suppression of HRS/IRR response, effect of low gamma radiation doses was studied using additional parameters including micronucleation, apoptosis induction and cell cycle progression delay in the absence and presence of inhibitors of gap junctional intercellular communication (GJIC) and NO signaling. Cells plated/ seeded at high cell multiplicity or density were pre-treated with phorbol ester PMA (phorbyl myristate acetate) and/or the NOS inhibitor LNMMA from 2h pre-irradiation, and treatment continued till 1h post-irradiation, after which cells were incubated in fresh growth medium. The low dose of 20cGy induced significant micronucleation in cells, and inhibition of GJIC by PMA (5nM) significantly enhanced this radiation-induced micronucleation in high density cultures. On the other hand, the NOS inhibitor LNMMA reduced micronucleation induced at 20cGy, and also significantly mitigated the PMA-induced enhancement of radiation-induced micronucleation. Interestingly, PMA could induce relatively larger number of binucleated cells, which indicates abrogation of G2/M checkpoint and might explain the higher induction of micronuclei in irradiated cells pre-treated with this agent. In contrast to PMA that enhanced micronucleation and reduced irradiated cell survival at low doses, LNMMA inhibited the micronucleation and enhanced survival in the irradiated cells, indicating the role of nitrosative stress in low-dose sensitivity of cells. A small but significant proportion of cells also showed nuclear fragmentation typical of apoptosis when pre-treated with PMA prior to irradiation at 20cGy. Further, a significant proportion of micronuclei induced at 20cGy were relatively larger in size, possibly indicating the induction of chromosome laggards due to spindle disturbances during the cell division. In this study, inhibition of GJIC significantly enhanced low dose lethality in the high density tumour cell cultures, whereas NOS inhibition reversed this effect. Indeed, the cell density mediated abrogation of HRS/IRR was significantly reversed in the presence of PMA, indicating protective role of gap junctional communication in HRS/IRR response. The study further indicated that PMA induced enhancement of radiation lethality could be effected through abrogation of G2/M cell cycle checkpoint.

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