Nitric oxide generated by ionizing radiation and EGF is implicated in EGF receptor phosphorylation in A549 lung carcinoma cells

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1. Introduction

Although it has been demonstrated that ionizing radiation (IR) control various cell functions in a different cell types, the mechanisms of its action via NO are not well understood. NO may potentially affect every type of mammalian cells, owing to its ubiquitous production and participate in the control of cell proliferation in a great variety of cell types.

The epidermal growth factor (EGF) receptor is a transmembrane glycoprotein of Mr 170,000. When EGF binds to its receptor, the receptor is dimerized and autophosphorylated at the carboxyl-terminal tyrosine 992, 1608, 1086, 1148 and 1173. This phosphorylated receptor initiates a series of signal tranduction events through interacting proteins of SH2 family including Shc, Grb2 and Sos, which in turn trigger ativation of MAPK cascades. Although the number of signaling events mediated by IR-induced NO is growing, it is still unclear how NO activate cellular signaling events. Thus, we examined the effect of NO on cellular phosphorylation and found that NO was produced by ionizing radiation in A549 lung adenocarcinoma cells and enhances the unique tyrosine phosphorylation on EGF receptor

2. Methods and Results

A549 lung adenocarcinoma cell was maintained in RPMI1640 containing 10% FBS. Intracellular NO level was determined by FASC using NO sensor dye, DAF-2 diacetate or modified Griess reagent. EGF receptor and Erk1/2 activation were investigated by Western blot analysis or immunoprecipitation Cell proliferation was examined using MTT assay.

2.1 NO production by IR

A549 cells were exposed to IR and performed by NO production using modified Griess reagent. IR-induced NO production was increased in time- and dose-dependent manner.

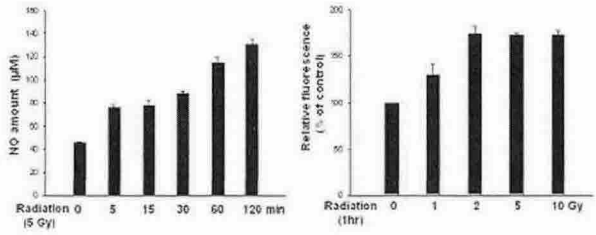

Figure 1. NO generation by ionizing radiation. Serum-starved A549 cells were exposed to ionizing radiation, and performed for NO quantitation

2.2 IR-induced proliferation of A549 cells

After exposure to IR, cell proliferation was determined using MTT method. At sub-lethal dose (2 Gy), cell proliferation was induced by IR. Furthermore, SNAP, NO donor similarly induced cell proliferation and PTIO, an NO scavenger, suppressed the IR- and SNAP-induced cell proliferation.

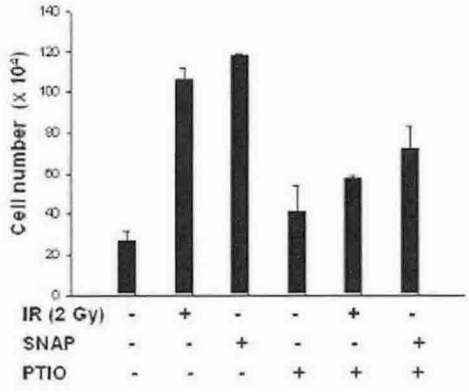

Figure 2. IR- and NO-induced proliferation of A549 cells. After pretreatment of PTIO, A549 cells were exposed to IR or SNAP and performed for proliferation using MTT assay.

2.3 IR-induced EGF receptor phosphorylation and Erk activation.

EGF receptor is intimately involved in cell proliferation signal events and overexpressed in A549 lung adenocarcinoma cells. Therefore, authors examined whether IR affected EGF receptor phosphorylation.

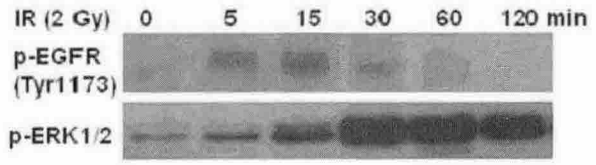

Figure 3. IR-induced phosphorylation of EGF receptor. Serum-starved A549 cells were exposed to IR and Western blot analysis was performed for pEGFR and pErk1/2.

IR induced EGF receptor phosphorylation (Tyr1173) and also phosphorylated Erk1/2, its downstream signal molecules.

3. Conclusion

IR induced NO generation in time- and dose-dependent manner in A549 lung carcinoma cells. And, it increased the proliferation at sub-lethal dose and SNAP, an NO donor mimicked IR effect. PTIO, an NO scavenger, suppressed the phosphorylation of EGF receptor and Ekr1/2 by IR and SNAP. Therefore, it is suggested that NO, in part, play a role in the IR-induced proliferation and EGF receptor phosphorylation in A549 cells.

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