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SUPEROXIDE DISMUTASES IN RADIORESISTANT PC-3 HUMAN PROSTATE CARCINOMA CELLS

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Abstract

The molecular mechanism of gamma-ionizing radiation (IR) resistance of human prostate cancer cells PC-3 is not known. Since low-LET-IR effects are primarily achieved through generation of reactive oxygen species (ROS), IR-induced expression of ROS-metabolizing antioxidant enzymes, Mn- and CuZn-superoxide dismutase (Mn- and CuZnSOD) and catalase (CAT), and their upstream regulator transcription factor NFκB was followed. Significant elevation of both SODs was found in cells irradiated with 10- and 20 Gy, while CAT and NFkB expression was unchanged. Since, such conditions lead to accumulation of H_2O_2, it is concluded that radioresistance of PC-3 cells may emerge from positive feed-forward vicious circle established between H_2O_2 activation of NFκB and elevated MnSOD activity.

Introduction

In the hormone refractory human prostate cancer cells metastatic to bone (PC-3) gamma-ionizing radiation (IR) exerts both antiproliferative and cytotoxic effects, with the former prevailing [1]. The IR dose which inhibits cell proliferation for 50 % (ID_{50}) is≈11 Gy, which is rather high compared with other epithelial carcinomas, while 50 % cell cytotoxicity (EC_{50}) is not attainable within the dose range of 2-20 Gy (V.Vučić-Ph.D. thesis). At the IR dose of 20 Gy only 27 % of PC-3 cells died. The molecular mechanism of this remarkable IR-resistance of PC-3 cells is not known. It may be ascribed to the lack of functional p53 protein which under normal conditions regulates cellular response to IR. As most of low-LET IR effects are achieved indirectly, *i.e.* through radiolysis of water *via* reactive oxygen species (ROS), it was of interest to investigate regulatory pathways activated by ROS. One such pathway involves action of antioxidant defence enzymes (AOE) that are subject of this study: mitochondrial Mn-superoxide dismutase (MnSOD), cytosolic CuZn-superoxide dismutase (CuZn-SOD), peroxisomal catalase (CAT) and their upstream regulator, transcription factor NFκB which might be responsible for the observed PC-3 cells' radioresistance [2].

Experimental

Cells were irradiated in the log phase of growth at 37 °C with 2, 10 or 20 Gy from ^{60}Co gamma-IR source, at the dose rate of 20 Gy/h. Cells were trypsinized, washed, and lysed by 1 % Triton X-100 in 10 mM TrisHCl buffer pH 7.4, containing 0.32 M sucrose and 5 mM $MgCl_2$. Total protein concentration (P in mg/mL) was determined by the method of Lowry. Cell lysates were denatured by sodium dodecyl-sulphate (SDS) containing La-

emmli buffer, boiled (100 °C, 3 min), separated by 10 % SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked by 1 % BSA in 10 mM Tris buffer pH 7.4, containing 150 mM NaCl and 0.1 % Tween-20. For Western blot analysis (WB) membranes were separately incubated with commercial polyclonal antibodies raised in rabbit: (a) anti-MnSOD (Stressgen-SOD110), (b) anti-CuZnSOD (Stressgen-SOD100), (c) anti-catalse (Calbiochem-219010), (d) anti-NFκB (NFκB -p65, Santa Cruz-SC-372) or (e) anti-actin antibody (CSA-400). A secondary goat anti-rabbit IgG-HRP conjugate (Stressgen-SAB-300) was used for colorimetric antibody-antigen detection. The quantification of specific bands was performed by PC Imager and expressed in arbitrary units (AU/mgP) of n=4 independent measurements (mean±S.D.). Analysis of variance (one-way ANOVA) followed by Tukey's posthoc test was used to estimate statistically significant differences (P<0.05).

Results and Discussion

Quantification of antioxidant enzymes (AOE) in IR-treated PC-3 cells revealed significant induction of both MnSOD and CuZnSOD *(Figure 1)* by 10- and 20 Gy. The expression level of CAT was unchanged by IR-treatment, as well as the level of AOE upstream regulator NFκB *(Table 1)*.

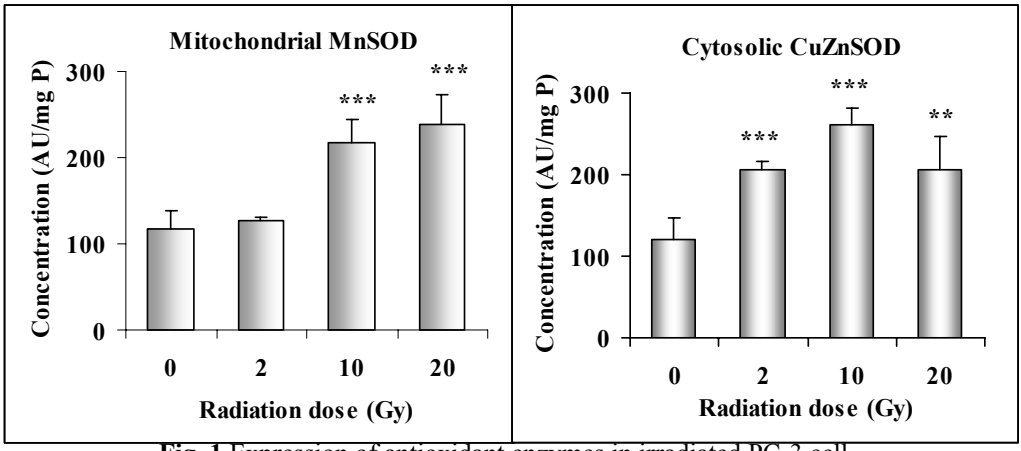

Fig. 1 Expression of antioxidant enzymes in irradiated PC-3 cell.
Results are presented as mean± S.D. (n=4)

Table 1 Expression of NFκB and catalase in irradiated PC-3 cells
Results are presented as mean± S.D. (n=4)

IR Dose / *Protein*	*0 Gy*	*2 Gy*	*10 Gy*	*20 Gy*
NFκB	110 ± 9	121 ± 16	124 ± 13	112 ± 9
CAT	92 ± 12	80 ± 26	103 ± 8	114 ± 11

Although the role of MnSOD in IR-response is highly dependent on the cell redox *milieu* in most cases its increased expression (*Figure 1*) enables protection against oxi-

dant injury which may be association with poor prognosis and resistance to radiation therapy [3]. This is specially the case in p53-deficient cells such as PC-3 which are constitutively adapted to prooxidative conditions and which respond to IR-treatment with further MnSOD induction. Scavenging of $O_2^{\bullet-}$ by MnSOD leads to an increase in H_2O_2, due to the lack of concomitant increase in the peroxide-scavenging enzymes CAT (*Table 1*). The IR-adaptation of PC-3 cells most probably occurs through a feed-forward regulation of MnSOD upstream regulator NFκB [4]. Although its expression is not enhanced by IR (*Table 1*) it may be activated by H_2O_2 through proteolytic cleavage of its inhibitory subunit IκB. In addition to that, enhanced of H_2O_2 probably leads to formation of hydroxyl radical ($OH^{\bullet}$), which is thought to be more toxic oxygen molecule *in vivo*. Inhibition of IR-treated PC-3 cell growth may be primarily attributed to decreased cell division rather than increased cell death, which was also the case in another metastatic human prostate carcinoma cell line DU-145 [5]. IR-induced adaptive responses mediated *via* NFκB activation involves genes such p21, Myc, 14-3-3 zeta, cyclin A, cyclin B1, and GADD153, regulating arrest of cell cycle progression, DNA repair, and apoptosis. All of them appear to be responsive to MnSOD expression. A pathway leading from NFκB to MnSOD to effector's genes (with antiapoptotic functions) is therefore a possible contributor to IR-induced resistance. CuZnSOD is another AOE responsible for cell growth and survival, which was induced by 10- and 20 Gy IR (*Figure 1*). Its enhanced expression may lead to further accumulation of H_2O_2 and potentiaton of a feed-forward circle with NFκB conferring PC-3 cell radioresistance.

Conclusion

High ionizing radiation resistance (IR) of human prostate cancer cells (PC-3) illustrated by $ID_{50} \approx 11$ Gy and $EC_{50} > 20$ Gy may be ascribed to the high constitutive and IR-induced expression of mitochondrial MnSOD and cytosolic CuZnSOD. In the absence of catalase expression enhancement of MnSOD and CuZnSOD may form positive feed-forward relation with the antiapoptotic NFκB gene regulator, which leads to relatively successful PC-3 cell adaptation to prooxidative conditions induced by IR.

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