Implication of small GTPases Rho in endothelial response to high dose of ionizing radiation.

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Microvasculature plays an important role in normal and tumoral tissue responses to high dose of irradiation (IR) as endothelial cells apoptotic death is a pre-requisite to deleterious effets of IR on surrounding tissues. Molecular mechanisms involved in this apoptotic pathway, despite being clearly independant of DNA damage, are still poorly understood. Small GTPases of the Rho family are crucial membrane-linked signalling proteins involved in many cellular functions, especially in actin cytoskeleton organisation but also in control of migration, proliferation and cell death. Their involvement in cellular response to ionizing radiation remains unclear, particularly in the endothelial compartment. Our study aims at studying 1) the regulation of activity of RhoA and Rac1, two main small Rho G proteins expressed in endothelial cells and 2) the possible role of these proteins in endothelial cellular functions critically affected by ionizing radiation like cytoskeleton reorganisation, cell death and migration. Using the microvascular endothelial cell line HMEC1 irradiated at 15 Gy, we show a rapid activation of RhoA concomitantly to an inactivation of Rac1. Analysis of actin cytoskeleton by confocal microscopy in HMEC1 cells indicate that 15 Gy-irradiation induces deep reorganisation of HMEC1 cell morphology, characterized by induction of stress fibers and decrease of lamellipodia, structures of polymerized actin respectively induced by RhoA and Rac1. We are currenly investigating the role of RhoA and Rac1 in induction of apoptotic cell death and in regulation of migration in 15 Gy-irradiated HMEC1, by the use of pharmacological specific inhibitors (Y-27632 for the RhoA pathway and NSC23766 for Rac1) and by invalidation of RhoA and Rac1 expression by stable RNA interference. Identifying Rho proteins as potential actors in endothelium damage to IR will permit a better understanding of molecular pathways involved and may lead to development of new strategies to modulate radiosensitization of this cellular compartment.

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