Radiation induces p38-mediated endothelial cell death through ceramide generation and membrane remodeling

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Involvement of sphingolipid ceramide and its metabolic enzyme, the acid sphingomyelinase (ASM) in irradiated microvascular endothelial cells have been demonstrated in lung's, small intestines' and tumor's murine models. However, molecular mechanisms involved in the apoptotic pathway are poorly understood. Using the microvascular endothelial cell HMEC1, we showed that ceramide generation in the membrane appears within the five minutes following 15 Gy. Our recent work (Bonnaud, Cancer Research, 2007) showed that radiation-induced apoptosis detected within 24 hours is inhibited by treatment with pharmacological ASM inhibitors in HMEC1 cells and therefore prove the use of those cell line as a good in vitro model to study the ceramide mediated apoptosis after exposure to ionising radiation. High-dose irradiation is known to induce the death-pathway p38 in microvascular endothelial cells, other than HMEC-1. We also observed, in our cell model exposed to 15 Gy, a rapid p38 phosphorylation visualised by phosphoblot and immunofluorescence. p38 blockade by MAPK inhibitor III or shRNA decreased radiation induced death in HMEC-1. Link between ceramide generation and p38 activation has been made after studies of raft microdomain organisation into the cell membrane. In fact, ceramide is well-described to induce the coalescence of raft microdomains in several cell models after a large sprectrum of stresses, such as H2O2, cytokines, heat shock. We detected a raft-marker ganglioside GM1 relocalisation from a discrete pattern in the cell surface, to large and polarised areas, following irradiation. The two concomitant phenomena, i.e. ceramide-induced raft coalescence and p38 death-pathway activation, has been connected by use of drugs, such as nystatin, disorganizing raft formation, which inhibited radiation-induced raft coalescence, activation of p38 and the subsequent death-induction of microvascular cells. Molecular apoptotic pathway induced by radiation has been confirmed by addition of exogenous natural ceramide or bacterial bSM in HMEC-1, which induced membrane reorganisation, p38 activation and death. In the present work, a singular cell culture model allows to better describe the cascade of disconnected events leading to microvascular cell destruction in response to high dose of ionizing radiation.

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