

Acid Sphingomyelinase Secretion by Irradiated Endothelial Cells: A Role in Intestinal Epithelial Cell Damage?

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Endothelial cell (EC) response has been implicated in the induction of intestinal damage following high dose radiation exposure. Furthermore, acid sphingomyelinase (ASM), a lipid hydrolase, has been shown to contribute to the pathophysiology of lung, liver, and heart dysfunction through increased amount of extracellular ASM. However, the potential role of ASM release in radiation-induced intestinal damage has not yet been considered. Therefore, the aim of this study was to investigate whether ASM production by EC is modulated by irradiation and implicated in epithelial cell damage. First, we set up a non contact coculture model with 15 Gy-irradiated EC (HMVEC-L) and non-irradiated T84 epithelial cells to examine whether *in vitro* irradiation of EC resulted in the induction of epithelial cell damage via paracrine pathway. Twenty-four hours following irradiation of EC, a decreased cell number (29%) and percentage in mitosis (66%) as well as increased apoptosis (1.5-fold) and cell-surface area (1.5-fold) were shown in non-irradiated T84 cells. Next, we assessed whether ASM might be a potential mediator of this observed bystander effect. Twenty-four hours following EC radiation exposure, ASM was measured both in endothelial cell culture supernatant and lysate by ASM activity assay and Western blot. We showed for the first time that irradiation of EC induced a significant 1.4-fold increased ASM activity in culture supernatant. A 1.4-fold increased ASM was also observed in EC lysate. Then, we determined the effect of exogenous ASM on T84 cells. Human placental ASM induced a dose-dependent decreased epithelial cell number and increased apoptosis. A concentration of 100 mU/ml of exogenous ASM recapitulated the deleterious effects in T84 cells observed in bystander coculture conditions and correlated with the calculated ASM concentration in irradiated EC culture supernatant. RNA interference of ASM is currently used to determine if silencing ASM in irradiated EC reduced epithelial cell damage. Finally, we measured ASM activity in serum of 15-Gy total body irradiated mice. We showed for the first time a 1.6-fold increase in serum ASM activity from 30 min to 4 h following radiation exposure. Together our data suggest that endothelial ASM may be a key mediator in the pathogenesis of radiation-induced intestinal dysfunction.