

Transient Glutathione Depletion before Irradiation triggers Apoptosis in Head and Neck Squamous Cell CarcinomaA. Boivin^a, C. Malesys^a, O. Chapet^b, C. Rodriguez-Lafrasse^a and D. Ardail^a^aLaboratoire de Radiobiologie EA3738, Faculté de médecine Lyon-Sud, 165 chemin du grand revoyet, 69921 oullins, France; ^bService de Radiothérapie, Centre Hospitalier Lyon-Sud, 69921 Oullins, France

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Glutathione, a major intra-cellular antioxidant, has been reported to play a fundamental role in the resistance of some cancer cells to radiotherapy so that this molecule can be considered as a potential clinical target for their radio-sensitization. The radio-resistant head and neck squamous carcinomas cell line SQ20B was shown to display a high endogenous level of reduced glutathione, up to 8-fold when compared to its radiosensitive counterpart SCC61 cell line. This result led us to consider the endogenous reduced glutathione as a key factor involved in the resistance of SQ20B cells to ionizing radiation. The purpose of this study was to experiment the incidence of a short and transient depletion of intracellular glutathione before irradiation of SQ20B cells. We therefore made use of the combination of dimethyl-fumarate (DMF), a glutathione-depleting agent in association with buthionine sulfoximine (BSO), a specific inhibitor of glutamate-cysteine ligase, the first enzyme of the glutathione biosynthesis pathway. In preliminary experiments, we have determined both the efficiency in lowering the endogenous glutathione level and the potential cyto-toxicity of these molecules in un-irradiated SQ20B cells. Although no toxicity was evidenced, a 95 % decrease of intracellular glutathione was obtained under our experimental conditions.

This treatment combined to irradiation led to the triggering of apoptosis of the radioresistant SQ20B cell line which significantly increased from 48 hours to longer times, as evidenced by an enhancement of 50 % of the activity of caspases and of the number of cells in the sub-G1 phase, up to 55 % 96h after irradiation. Triggering of apoptosis in SQ210B cells was found to involve the c-Jun N-terminal Kinase (JNK) pathway which was phosphorylated 2 h after irradiation. This activation of JNK was dependent upon the generation of radical oxygen species which resulted in the dissociation of thioredoxin-ASK 1 complex (MAPKKK of JNK pathway). The phosphorylated form of JNK led to the activation and translocation of Bax to mitochondria which further resulted in the alteration of the organelle, as evidenced by the loss of the mitochondrial transmembrane potential ($\Delta\Psi_m$) and the increase of the secondary radical species generated through the mitochondrial respiratory chain.

Taken altogether, our results demonstrate that a transient glutathione depletion before irradiation can trigger apoptosis of radio-resistant SQ20B cells through the activation of a JNK-dependent pathway. Moreover, the absence of a significant toxicity of this pharmacological treatment is of fundamental importance for further in vivo studies.