Gene Expression Analysis of Apoptosis and Oxidative Stress in Mouse Brain After Low-dose and Acute Radiation Exposure

Xiao Wen Mao, Tsehay Mekonnen and Daila S. Gridley
Department of Radiation Medicine, Radiation Research Laboratories
Loma Linda University & Medical Center, Loma Linda, CA, 92354 U.S.A.

Purpose: 1) To examine the induction of oxidative stress and apoptosis-associated gene expression profiles in brain after whole-body irradiation with low-dose/low-dose-rate (LDR) photons and acute exposure to photons 2) to compare these radiation-induced effects with those produced by LDR and acute exposure to protons.

Material and Methods: C57BL/6 mice were exposed to 2 Gy of photons or protons at 0.8 Gy/min and 0.9 Gy/min, respectively, both with and without pre-exposure to 0.01 Gy LDR γ-rays (^{57}Co) at 0.03 cGy/h. Brain tissues were harvested and quick-frozen for analyses by quantitative RT-PCR at 56 days post-irradiation (n = 3 mice/group).

Results: For apoptosis gene expression profile based on the 84 evaluated genes, pre-exposure to LDR and acute exposure to 2Gy of photon or proton significantly altered 12 and 29 genes, respectively ($p<0.05$). Several important genes involved in the initiation or activation of apoptotic signaling pathways were significantly up-/down-regulated following irradiation. This group included *fas* for LDR + photons; *fas*, *tnf* and *trp73* for LD + protons. Genes participating in the caspase cascade were also significantly regulated following irradiation which included *apaf*, *bcl2*, *bcl10*, *bax*, *casp2*, *casp8* and *casp14*. For oxidative stress gene profile, photons, protons, LDR + photons and LDR + protons significantly altered 11, 14, 9 and 25 genes, respectively, among 84 genes assessed ($p<0.05$). Many genes responsible for regulating the production of reactive oxygen species (ROS) were significantly up-regulated compared to controls, including: *gpx1*, *gpx5*, *gpx7*, *prdx4*, *noxa1* and *gsr*.

Conclusions: Acute whole-body exposure to either photons or protons modified expression of genes related to apoptosis and oxidative stress in brain tissue from mice nearly 2 months post-irradiation. The gene profiles were highly dependent upon radiation quality. Furthermore, proton radiation with pre-exposure to LDR photons triggered a more diverse gene expression profile compared to photon radiation with pre-exposure to LDR photons.

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[Note: Examples of data are presented below and on the next page.]

Table 1. Numbers of up-/down-regulated apoptosis and oxidative stress genes with at least 2-fold difference vs 0 Gy controls ($p<0.05$).

Groups	Numbers of genes	
	Apoptosis	Oxidative stress
2Gy (photons)	9	11
0.01 (LDR) + 2Gy (photons)	12	14
2Gy (protons)	15	9
0.01 (LDR) + 2Gy (protons)	29	25

Table 2. Fold change in apoptosis gene expression in brain vs 0 Gy controls ($p<0.05$).

	Photons		Protons	
Genes	**2Gy**	**LDR+2Gy**	**2Gy**	**LDR+2Gy**
Akt	-	-	-	-2.62
Apaf1	2.22	-	-	-
Atf5	-	-	-	-2.01
Bag3	2.35	2.45	2.23	-
Bcl2l10	-	-2.43	10.63	-
Bcl10	5.36	3.41	2.82	2.23
Birc2	-	-	-	-2.48
Birc5	2.95	2.30	3.43	3.22
Card6	-	-	21.03	3.87
Casp2	-	-	-	2.25
Casp8	-	-	2.14	-2.02
Casp14	-	-	9.19	2.09
Cd40	-	4.00	3.22	-
Cd40lg	-	-	5.41	2.54
Cd70	3.33	2.83	-	4.07
Cideb	-	-	-	2.10
Dad1	-	-	-	-27.97
Dapk1	-	-	-2.50	-2.77
Fas	2.34	3.13	-	8.48
Fasl	-	-	-	4.55
Il10	-	-	-	2.54
Lhx4	2.01	5.18	5.69	2.64
Ltbr	-	2.21	-	-
Naip1	-	-	-	2.46
Naip2	-	2.68	-	-
Nod1	3.50	2.16	2.51	2.31
Pak7	-	-	-	-2.32
RipK1	-	-	-	2.00
Rnf4	2.19	-	-	-
Rnf7	-	3.92	-	2.04
Tnf	-	-	2.19	2.54
Tnfrsf10b	-	-	-	-7.45
Tnfrsf11b	-	-	-	2.85
Tnfsf10	-	-	-	-7.72
Traf1	-	-	3.12	-
Trp63	-	-	5.77	-
Trp73	-	-	-	14.18
Tsc22d3	-	-	-	2.55

LDR: 0.01 Gy (^{57}Co γ-rays)

Strikingly different profiles were also noted among the four groups in expression of genes related to oxidative stress.