Contribution ID: **32** Type: **oral (15 minutes)**

TNFR2/p75 Signaling Induces Delayed Radiobiological Bystander Responses in BM-derived EPCs: Implications for Development of Mitigating Factors

Tuesday, 16 October 2012 18:00 (15 minutes)

Tumor necrosis factor(TNF) binds two receptors TNFR1/p55 and TNFR2/p75 and activates several signaling cascades. Ionizing radiation (IR) increases tissue levels of TNF. TNF signaling regulates numerous cytokines/chemokines that may mediate IR-induced non-targeted effects (NTE), a phenomenon where cells that are not directly "hit"by IR exhibit IR effects via signals received from distant IR cells. Little is known about the role of p55 or p75 in regulating NTE in bone marrow (BM)-derived endothelial progenitor cells (EPCs).
Medium transfer experiments (MTE) were performed ex-vivo with BM-derived EPCs from WT, p55 knockout(KO) and p75KO mice. EPCs were irradiated with 1Gy of γ-IR, then IR-conditioned medium (CM) was collected at 1, 5, 24 hrs, and 3, 5 days post-IR. Filtered (0.22μm) IR-CM was transferred to naïve non-IR EPCs of the same genotype. After 24 incubation, CM from IR EPCs was processed for ELISA profiling (16 genes) and IR EPCs from p55KO mice were processed for microarray profiling. CM-treated EPCs were processed for p-H2AX/p53BP1 staining for presence and decay of double strand breaks (DSB).
In WT EPCs the peak of mean p-H2AX foci/cell was at 24h, whereas in p55KOs the number of p-H2AX foci/cell were decreased twice on day 5 (9±0.8 vs 4.8±0.6, $p<0.01$, WT vs p55KOs). These finding indicate that altered TNF signaling inhibits early NTEs (hours) in BM-derived EPCs. Compared to WT, delayed (5 days) NTEs were increased in IR-CM-treated p55KO EPCs (3.8±0.4 vs 8.5±1, $p<0.02$, WT vs p55KO), suggesting significant role of TNF-TNFR/p75 signaling (the remaining active receptor in p55KO EPCs) in mediating delayed NTEs. ELISA profiling of 16 proteins in IR-CM over 5 days post-IR showed 200-1600% increases ($p<0.002$, p55KO vs WT) in cumulative levels of TNF, IFNr, IL1α, IL1β, IL6, EGF, MIP-1α, MCP-1, GM-CSF. Microarray profiling of γ-IR p55KO EPCs revealed 3999 significantly expressed genes (one-way ANOVA, $p<0.05$) with 1179 genes with false discovery rate (FDR) <0.05 (day 5: 371 genes were 2-fold up/down), and 194 at FDR<0.01 (day 5: 93 genes were 2-fold up/down), a significant increase in gene transcription 5 days post-IR.
We conclude that TNF ligand-receptor axis regulates NTEs in naïve EPCs and suggest that restoring TNF signaling balance could represent a preventative/mitigating measure for inhibition of delayed NTEs in tissues adjacent or distant from primary IR target.

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Session Classification: Non-Targeted Effects of Radiation

Track Classification: Non-Targeted Effects of Radiation