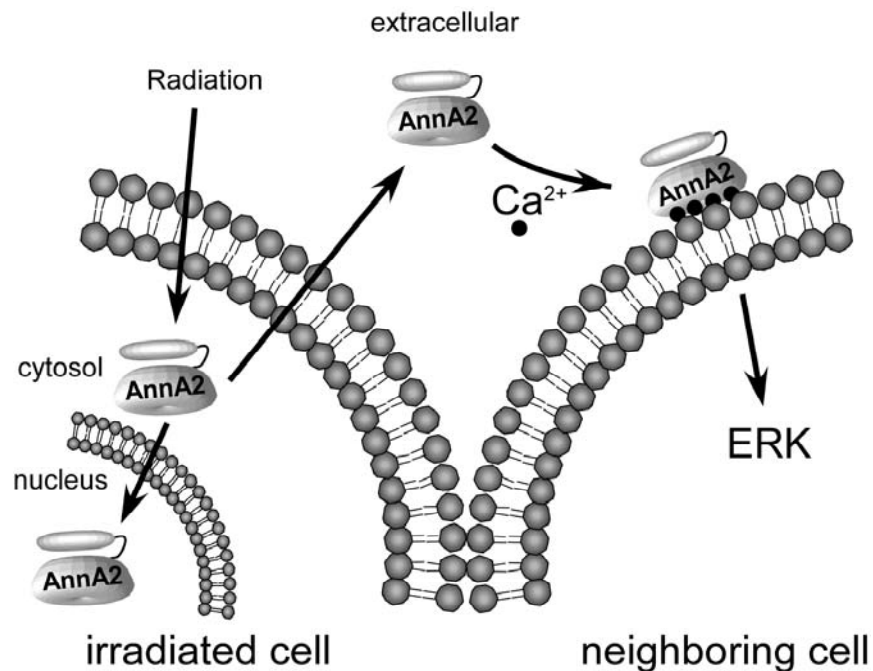


Annexin A2 Regulates A Low Dose-Specific Stress Response To Radiation. Thomas J. Weber (PI), Greg J. Newton, Ryan D. Quesenberry, Janani I. Shutthanandan, Nikki Bollinger, Heather E. Engelmann and Lee K. Opresko. Cell Biology and Biochemistry Group, Pacific Northwest National Laboratory, Richland, WA 99354.

Previous studies have demonstrated that JB6 cells release cell transforming paracrine factors following exposure to a low dose of radiation (10 cGy). Investigation of secreted proteins by SDS-Page and silver stain led to the identification of a 36 kDa band whose levels were increased in medium from irradiated cells, relative to sham controls. The 36 kDa band was identified as annexin A2 by mass spectrometry. Western blot analysis confirmed a dose-dependent increase in annexin A2 levels associated with medium from irradiated (10-100 cGy) cells, relative to sham controls. Annexin A2 is known to bind to the extracellular plasma membrane in a calcium-dependent fashion. To determine whether secreted annexin A2 binds to bystander cells, sham exposed or 10 cGy irradiated JB6 cells (seeded in top inserts) were co-cultured with LNCaP cells (bystander cells seeded in bottom chamber) for 24 hr, followed by Western blot analysis of LNCaP-associated annexin A2. LNCaP-associated annexin A2 levels were increased in co-culture with 10 cGy irradiated JB6 cells, relative to co-culture with sham exposed controls. Treatment of JB6 cells with recombinant annexin A2 resulted in the activation of extracellular signal regulated kinases (ERKs), suggesting that extracellular annexin A2 is capable of activating transformation relevant signal transduction pathways in bystander cells. To further investigate the role of annexin A2 in the cellular response to radiation, we have developed a JB6 annexin A2 knockdown model. Cells expressing annexin A2 shRNA exhibit dramatic reduction of annexin A2 mRNA and protein (95%). To determine whether annexin A2 knockdown modulates the radiation response, we employed a cell proliferation assay as a sensitive measure of cell damage. Radiation reduced cell number in a dose-dependent manner with two distinct features in the dose-response curve. At low dose exposures, there was a small reduction of cell number (approximately 15%), relative to sham control, that was comparable at doses between 10 and 100 cGy. A linear reduction of cell number was observed at higher dose exposures (100 – 500 cGy). We subsequently compared cell number in vector control and annexin A2 shRNA cells 72 hr following X-Ray radiation exposure. Reduced cell number was observed in vector control cells exposed to 10-500 cGy X-Ray radiation as previously observed. In contrast, a significant increase in cell number was observed for annexin A2 shRNA cells exposed to low (5 - 25 cGy), but not high (50 – 500 cGy) doses of X-Ray radiation. Nuclear localization of annexin A2 is associated with inhibition of cell proliferation, therefore, the differential response of annexin A2 knockdown cells could be accounted for by loss of this negative regulatory signal. Western blot analysis demonstrated increased annexin A2 levels associated with nuclear extracts prepared from JB6 cells exposed to 10-250 cGy X-Ray radiation, relative to sham controls. Nuclear annexin A2 levels peaked at 40 min following radiation exposure. Parallel measurements of P-JNK in nuclear extracts demonstrated peak nuclear P-JNK levels at 40 min in cells exposed to a high radiation dose (250 cGy), while P-JNK was not detected in cells exposed to a low dose (10 cGy). To confirm this observation, we examined nuclear annexin A2 levels *in situ*. Initial observations were consistent with increased nuclear translocation of annexin A2 in response to 10 cGy X-Ray radiation and were associated with the appearance of both diffuse and focal nuclear staining patterns. However, the

intensity of the foci was relatively weak and difficult to resolve over the high background associated with extranuclear annexin A2. To improve signal-to-noise we investigated an annexin A2 nuclear trapping strategy. Annexin A2 is exported from the nucleus by the CRM1 export pathway. Leptomycin B (LB) inhibits CRM1, resulting in the retention of annexin A2 in the nucleus. Evidence for CRM1-dependent regulation was observed in JB6 cells where LB-treatment resulted in the nuclear retention of native annexin A2 and an annexin A2-YFP chimera. Under these conditions, immunofluorescent detection of nuclear foci was more robust and could be co-localized with nucleophosmin, a marker of nucleoli, in a manner consistent with nucleoli identification by DAPI staining. Using this trapping strategy, a significant increase in nuclear annexin A2 levels for both the native and annexin A2-YFP chimera could be detected in JB6 cells exposed to 10 cGy X-Ray radiation, relative to sham controls, that would have otherwise been dismissed. Collectively, these observations suggest that low dose radiation induces the mobilization of annexin A2 to extracellular and nuclear compartments. A functional role for annexin A2 in the stress response to radiation is supported by the differential response of annexin A2 shRNA cells to low dose radiation exposures. Further, our data indicate that competing nuclear export activities should be considered when defining nuclear translocation events triggered by subtle stresses. Because annexin A2 is implicated in human disease, activation of this pathway by low doses of radiation warrants further investigation.



Radiation-induced mobilization of annexin A2 (AnnA2) to extracellular and nuclear compartments.