Induction of secretory clusterin via ATM and IGF-1 signaling during replicative senescence: Similarities to responses to low-dose ionizing radiation. Masatoshi Suzuki, Eva Goetz, Bhavani Shankar, Longshan Li, Julio Morales, Yonglong Zou, and David A. Boothman, Department of Oncology, Program in Cell Stress and Cancer Nanomedicine University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390.
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Secretory clusterin (sCLU) is induced in response to a broad range of ionizing radiation (IR) doses (from 2 cGy) and is transcriptionally induced in a dose- and time-dependent manner. Since multiple and long-term exposure to low-dose IR effectively induces stress-induced premature senescence (SIPS) and sCLU is also induced in replicative senescent cells, we hypothesized that sCLU expression was related to senescence induction by low-dose stimulation. Replicative senescent cells have a relatively low level of DNA double-strand breaks (DSBs), and approximately 5 cGy of X-rays generates a similar amount of DSBs. We propose that replicative senescent cells represent a good model of multiple low-dose IR exposures. Activation of the IGF-1/IGF-1R/MAPK/Egr-1 pathway induces sCLU after high-dose IR, therefore, it is possible that the IGF-1-sCLU pathway is activated with replicative senescence. Furthermore, the *Ataxia telangiectasia* mutated (ATM) protein plays an important role as a sensory protein for DSBs, and it is well known that ATM is activated immediately after DSBs are introduced. With this understanding, we hypothesized that ATM regulates IGF-1-sCLU pathway during replicative senescence.

We found that sCLU is gradually and transcriptionally induced during replicative senescence in IMR-90, normal human lung fibroblasts. Upon induction, sCLU was gradually secreted outside the cell and its level peaked in senescent cells. sCLU was induced by ionizing radiation before cells displayed senescent phenotypes. However, sCLU was already expressed in senescent cells and exposure to IR did not cause further increase in sCLU expression. These results suggest that sCLU is expressed through the same pathway in replicative senescent. as in young cells after IR-treatment. Interestingly, induction of the IGF-1 receptor (IGF-1R), as well as its phosphorylation form (pIGF-1R) was noted during replicative senescence. Consistent with the activation of pIGF-1R, phosphorylation of Src and ERK1 were noted and both phosphorylated levels] increased from middle-aged to senescent cells. We also observed the secretion of IGF-1 in senescent cells. These data strongly suggest that the IGF-1 signaling pathway was activated in senescent cells. Treatment with AG1024, which is an inhibitor of IGF-1R kinase activity, greatly reduced sCLU expression in senescent cells, but did not affect senescence-induced IGF-1 levels. Interestingly, in primary AT cells that lacked functional ATM, sCLU induction during replicative senescence was not observed. Interestingly, IGF-1 receptor levels were dramatically lower in AT cells compared to isogenic wild-type (reconstituted with ATM) cells. In contrast, wild-type cells expressed much greater levels of IGF-1R. These data suggest that the mechanism of sCLU induction consists of two additive steps, one is the ATM-dependent IGF-1 receptor expression, and the

other is IGF-1-dependent signal transduction during replicative senescence. ***This work was supported by DOE grant # DE-FG02-06ER64186, entitled "IGF-1/IGF-1R-MAPK-secretory clusterin (sCLU) pathway: Mediator of a low-dose IR-Inducible bystander effect"***