Low dose IR stimulation of TGF-ß1 *in vivo* and downstream activation of the IGF-1-sCLU pro-survival expression axis: A bystander and adaptive survival mechanism *in vivo*.

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Over the past few years our laboratory has elucidated the regulatory mechanisms governing the insulin-like growth factor-1 (IGF-1) - secretory clusterin (sCLU) expression axis, which can be induced by >2 cGy *in vivo* and *in vitro* with the same dose-response kinetics. IGF-1-sCLU expression can be induced by (A) TGF-ß1 and (B) IR, but is suppressed by the p53 and Klotho tumor suppressors. Interestingly, there is a substantial difference between the regulatory mechanisms of IGF-1-sCLU induction after TGF-ß1 and IR exposures, where p53 suppresses IR responses but does not suppress after TGF-ß1 exposures. This work focused on the mechanisms by which TGF-ß1 can over-come p53 suppression and describes the discovery that Klotho, an agent important in aging, can suppress TGF-ß1-induction of IGF-1-sCLU expression.

We show here that exposing CLU promoter-luc reporter animals with low doses of IR induces IGF-1-sCLU in all IR-sensitive tissues, including large and small intestines, bone marrow, thymus, and spleen, but not other non-sensitive tissues to doses 0.02-2 Gy. Mechanistically, we demonstrate that TGF-ß1 is activated by low doses of IR in these, but not in the non-responsive, tissues. We show that TGF-ß1 is able to circumvent p53 suppression of IGF-1-sCLU by inducing Mdm2, which in turn degrades p53 at delayed times (24-72 or longer h). Furthermore, we can augment the IGF-1-sCLU responses by knocking down Klotho. Furthermore, Klotho can suppress the responses *in vitro* by suppressing both IGF-1 and TGF-ß1 downstream signaling events.

These results have important implications on risk assessment. First, we have developed ELISA assays for IGF-1 and sCLU as well as Klotho. Second, along with being able to analyze the robust IGF-1-sCLU expression in animals from blood or tissue and being able to predict exposure doses in these patients, IGF-1-sCLU offers both a bystander as well as adaptive response mechanism for IR protection of tissue-especially clearing cell debris by sCLU. For cancer cells, we show that IGF-1-sCLU expression can augment migration and metastases. For normal tissue/cells, IGF-1-sCLU can offer radioprotection. Thus, this expression pathway is good for normal tissue, however, if the remediation worker has an initiated or cancer cell, this low dose IR stress response may augment cancer progression. We welcome collaborations for analyzing TGF-ß1/IGF-1/sCLU expression as well as Mdm2, p53 and Klotho in blood and animal tissues, where applicable, before and after low doses of IR to start to understand risk assessment analyses of these important functional factors.

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