C. Final Progress Report:

We completed all of the Aims of our prior grant, DE-FG-022178-16, that ended in March, 2006. The data that resulted led to publications on the regulatory processes and functions of sCLU in cells after low dose exposures of IR (see below). Our accomplishments include:

1. In Aim 1, we discovered a low dose IR-inducible, delayed IGF-1/IGF-1R/Src/Raf/MAPK/Egr-1 signaling pathway that activated the CLU promoter. In Aim 2, we discovered that sCLU was a pro-survival factor in MCF-7 cells, wherein expression knockdown using siRNA specific for sCLU enhanced lethality after clinically relevant doses of IR.

2. In Aim 1, we generated MCF-7 cells stably containing the hCLU promoter-firefly luciferase reporter, and then developed technology to visualize CLU promoter activity in cells in culture. We then developed an IR-inducible transgenic mouse containing the human CLU promoter controlling the firefly luciferase reporter for in vivo bioluminescent, noninvasive imaging of CLU promoter activity before and after IR. Using these mice, we showed CLU promoter activity induced after IR (by ≥10 cGy) in specific organs (bone marrow, colon, thymus, skin, spleen) with temporal and dose-response induction characteristics identical to IR-induced endogenous sCLU protein levels. (below, Refs. b,c). The human and mouse CLU promoters are highly conserved, particularly in functional domains.

3. In Aim 1, we discovered that p53 suppressed sCLU expression (Refs d). We subcloned a longer region (−4250 to +1) of the human
CLU promoter and fused this in-frame with firefly luciferase to elucidate transcription factors that regulate CLU promoter activity and to elucidate the mechanism(s) by which p53 suppresses CLU promoter activity (Refs. a). This construct enables transient transfection of cells for analyses in any cell of choice. We also developed a lentivirus system for sCLU expression in CLU- cells, and for expression of small hairpin RNA specific to CLU (shRNA-sCLU) for stable sCLU-specific knockdown; nuclear clusterin levels are not affected (Ref. a).

4. In Aim 2, we developed LNCaP cells stably over-expressing sCLU and demonstrated that these cells were resistant to IR compared to CLU-deficient vector alone LNCaP cells. A similar LNCaP system was previously reported by Gleave et al., (169, 280). These cells will be used in our new Aims 2 and 3 to investigate the role(s) of sCLU in bystander and adaptive responses after low doses of IR.

5. In Aim 3, we discovered that TGF-β1 treatment of human cancer cells induced sCLU in a signal transduction process that depended on Smad 4 and Smad 3 activation. We defined Smad Binding Elements (SBEs) in the CLU promoter and characterized Smads 3 and 4 binding. (Ref. e). We are now in a position to test the hypothesis that sCLU suppresses TGF-β1 signaling in Aim 2.

6. In Aim 3, we showed that unlike IR, TGF-β1 exposure of cells induced sCLU in cells with functional p53, and we provided evidence that induction by TGF-β1 was the result of activated Smad-dependent, increased Hdm-2 expression, which down-regulates p53 allowing induced CLU promoter activity (Ref. f). We now provide direct, preliminary evidence that sCLU can suppress TGF-β1 induction of CLU promoter activity (below). These data support a role for sCLU as a negative feedback factor to suppress TGF-β1 responses. Potential bystander effects of sCLU will be examined in Aim 2.

7. In Aims 1 and 2, we defined the mechanism by which the nuclear clusterin (nCLU) pro-death protein was synthesized and activated (Ref. g). We recently discovered that nCLU regulation is controlled by nuclear export sequences (NESs), as well as CRM1-mediated processes (Refs. h,i).

Articles Published: Peer-reviewed (9 papers published, in press, or submitted):


h. Leskov, K., Araki, S., and Boothman, DA. CRM 1-mediated nuclear export of nuclear clusterin (nCLU), a pro-death protein activated by ionizing radiation. JBC, Submitted, 2006.


j. Related Peer- and non-peer-reviewed articles published as a result of this grant:


