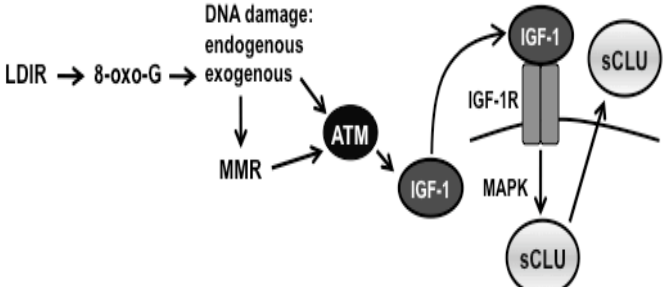
**DNA damage induced clusterin expression - A sensitive measure of genomic instability.** Eva Goetz[1], Long Shan Li[1], Julio Morales[1], Yonglong Zou[1], Robert Bachoo[2] and **David A. Boothman**[1] .[1]Dept.of Oncology, [2]Dept of Neuro Oncology, Program in Cell Stress and Cancer Nanomedicine, University of Texas Southwestern Medical Center at Dallas, TX 75390, USA. David.Boothman@UTSouthwestern.edu

Secretory clusterin (sCLU) is a glycoprotein secreted from cells following exposure to genotoxic stress, and sCLU expression is elevated in many different disease states. sCLU is a pro-survival protein that acts as a molecular chaperone to remove cell debris caused by trauma to cells and tissues *in vivo*. sCLU expression is extremely sensitive to oxidative stress and DNA damage and can be induced by low dose ionizing radiation (LDIR), as low as 2 cGy. We previously demonstrated that sCLU was induced after LDIR by activation of the Insulin-like Growth Factor 1 Receptor (IGF-1R), and downstream stimulation of Src/MAPK/Erk-1/2 to promote binding of the Egr-1 transcription factor to the CLU promoter.

**Proposed MMR-dependent stimulation of ATM-IGF-1-sCLU expression after low doses of ionizing radiation (LDIR).**




Since sCLU is induced at by LDIR, and low doses of IR induce 8-oxoguanine (8-oxo-G) DNA lesions are detected by DNA mismatch repair (MMR), we examined sCLU induction at low doses in genetically matched HCT116 (MMR-deficient) *versus* HCT116:3-6 (MMR-competent) cells, corrected for MMR by chromosome 3 transfer (HCT116:3-6). sCLU was induced in HCT116:3-6 cells and knockdown of sCLU using stable lentiviral shRNA-sCLU expression, sensitized cells to 0.1 Gy priming doses, as well as high dose IR challenges. These data suggest that MMR-detected 8-oxoguanine lesions can trigger and augment ATM-IGF-1-sCLU expression. We are currently examining the role(s) of this pathway in survival and mutagenesis 'adaptive survival' responses.

Since sCLU can be induced by low doses of IR exposure ($\leq$2 cGy), we hypothesized that cells with increased endogenous DNA damage or genomic instability may initiate signals in a manner very similar to those exposed to exogenous insults. We tested this by studying the IGF/IGF-1R/sCLU pro-survival pathway in genomically unstable cells. Murine embryonic fibroblasts (MEFs), deficient in H2AX, MDC1, NBS1, or the RNA component of telomerase (mTR), have elevated IGF-1 and sCLU expression compared to wild-type MEFs. Additionally, HCT116 cells had higher basal sCLU expression compared to HCT11:3-6 cells. Additionally, AG1024 treatment decreased basal sCLU levels in all the genomically unstable cells. To determine the initiating signal, cells were treated with an ATM/ATR inhibitor, and this reduced basal sCLU expression. ATM deficient fibroblasts failed to induce sCLU after IR and UV exposure. Hence, we propose that activated ATM (by any mechanism) up-regulates the IGF-1/IGF-1R/sCLU pro-survival pathway. Together these results strongly suggest that sCLU expression can be used as a very sensitive marker of low dose IR exposure and genomic instability, and is an important biomarker monitor DNA damage in radiation workers.

***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"***