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NUCLEAR APOJ: A LOW DOSE RADIATION INDUCIBLE REGULATOR OF CELL DEATH

Final Report

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Bruce J. Aronow

Email: bruce.aronow@chmcc.org
Children's Hospital Medical Center
3333 Burnet Avenue
Cincinnati, OH 45229

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Overview:

This project entitled "NUCLEAR APOJ: A LOW DOSE RADIATION-INDUCIBLE REGULATOR OF CELL DEATH" was a joint project between Dr. Bruce Aronow, Children's Hospital Medical Center, Cincinnati, Ohio, with Dr. David Boothman, at the Case Western Reserve University, Cleveland, Ohio. The project was devoted to testing the hypothesis that apoJ, also known as clusterin and XIP8 is an effector of programmed cell death in response to a DNA damage.

This project was based on preliminary data that was published by Dr. Boothman (Yang et al. 2000) which indicated a strong induction of apoJ gene expression, increased secretion of the protein, and accumulation of an apparently somewhat different form of the apoJ protein in the nucleus of MCF-7 breast carcinoma cells undergoing response to DNA damage. A clone expressing apoJ protein was isolated that was capable of interacting with Ku80, a component of the double strand break repair complex that is essential for the successful repair of rearranging immunoglobulin and T-cell receptor genes as evidenced by failure to produce mature B and T cells in the absence of Ku70. ApoJ clones isolated and characterized by Dr. Boothman bound strongly to a Ku-70 "bait" protein. Over-expression of these same clones in a cell line was capable of killing the cell. ApoJ is very strongly induced in many instances of programmed cell death and has been proposed repeatedly to play some sort of effector role in the process.

Our principle hypothesis for this study was that the strong induction of the apoJ gene and the particular expression of a nuclear form of the protein was potentially a causal factor in the decision point made by the cell as it attempts to repair double-strand breakage based DNA damage. The hypothesis was that if sufficiently high damage occurred, it would be deleterious to maintain the cell's viability through continued DNA repair. One method to inhibit DNA repair might be by inhibiting proteins such as Ku-70 that are necessary for double-strand break repair. If apoJ does play a critical role in tipping the decision balance over to cell death, we reasoned that deficiency of apoJ would cause increased accumulation of cells with DNA damage and that this might decrease cell death in response to DNA damage and increase tumor occurrence rates.

To test this hypothesis and its potential implications, we exposed wildtype and apoJ deficient animals that we constructed through gene targeting to increasing levels of ionizing radiation from a Cesium source. Data gathered under the support of this grant application initially indicated that apoJ deficient animals were more resistant to radiation, but as we accumulated more and more data points and covered a tighter exposure range, the genotype-based differences became insignificant. However, the possibility existed that because mortality based radiation-resistance could be attributable to mechanism for which nuclear apoJ was not rate determining, we maintained a very large of colony of apoJ knockout and wildtype animals in both the C57/Bl6 and Cv129 strain backgrounds that were exposed to sub-lethal levels of ionizing radiation to monitor for the occurrence of tumors. These animals were allowed to fully recover and age normally in either germ free or normal animal housing. Our results demonstrated no significant differences between wildtype and apoJ knockout animals over a period that extended up to 30

months for individual animals. We recorded similar weight gain, a relatively low mortality rate, and a similar mixture and rate of sarcoma and adenocarcinomas after surviving the initial ionizing radiation exposures. Thus we conclude that apoJ gene function, which was totally eliminated by our gene targeting, did not influence radiation sensitivity or serve as a tumor suppressor in response to DNA damage.

An additional aim that we approached was to identify gene regulatory elements within the apoJ gene responsive to radiation exposure. We have now identified cooperating regulatory elements in both proximal and distal promoter, first intron, and sixth intron that contribute to apoJ gene regulation. The proximal promoter in particular has a cluster of cis-elements that include AP-1, HSE (heat shock), and STAT responsive elements. We have mutated these separately and in combination and surprisingly when analyzed in vivo in transgenic mice there remains a considerable injury inducible capability of the promoter. The realization that cis-elements for apoptosis-related apoJ gene induction are distributed over large genomic regions and our inability to recognize functional control regions has prompted us to design a comparative genomics tool (http://trafac.chmcc.org/). The system is able to visualize clustered occurrences of cis-element motifs that are conserved in the context of conserved sequence blocks. We have begun to populate the database that underlies the application with many more genes, including groups of genes that we demonstrate are coordinately regulated using microarray analyses. Using genes with well defined regulatory regions and promoters as a training set, we are attempting to develop multigene cis element motif-cluster recognition algorithms that may predict cell type specific properties of undefined regulatory regions.

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