# ESTABLISHING CELL LINES IN FOUR MARINE ANIMALS: BOWHEAD WHALE, (*BAELAENA MYSTICETUS*), BELUGA WHALE (*DELPHINAPTERUS LEUCAS*), LITTLE SKATE (*RAJA ERINACEA*), AND SPINY DOGFISH (*SQUALUS ACANTHIAS*)

John Pierce Wise, Sr., Peter G. Antonucci, and B. H. Bryant
Laboratory of Environmental and Genetic Toxicology
Division of Environmental Health Sciences,
Department of Epidemiology and Public Health
Yale University School of Medicine
New Haven, CT 06520

The long-term objective of this research is to develop models of metal-induced carcinogenesis in cells from marine animals for comparison with human models. This is important because several metals are established carcinogens and both human and marine animals are heavily exposed to metals. Currently, there are no models and little or no data concerning the genotoxic effects of metals in marine animals. Thus, the short-term objective of this research is to focus on one metal of significant public health concern (hexavalent chromium (Cr(VI)), and establish the necessary cell lines to develop this model.

Skate hepatocytes were obtained from Dr. James Boyer. Shark rectal gland epithelial cells were obtained from Dr. John Forrest. Beluga skin fibroblasts were obtained from the Mystic Aquarium in Mystic, Connecticut and bowhead kidney cells were obtained from Thomas Goodwin at NASA in Houston., Texas. The approach was to immortalize the cells with hTERT the catalytic subunit of telomerase, E6/E7 the oncoproteins produced by human papilloma virus and large t-antigen the oncoprotein produced by Simian virus 40. Each immortalizing vector contained the immortalizing gene and a drug resistance gene. Thus, cells expressing the immortalized gene were selected based on their resistance to geneticin or neomycin. Once immortalized the cell lines were to be derived from single cells and analyzed for genotoxicity after Cr(VI) exposure.

The project presented many technical challenges. The culture and reagents of the shark and skate cells require additional salts and urea to maintain a high osmolality. Because of the need and relevance of these cell lines for the laboratory as a whole, the decision was to focus on these cells first. MDIBL is quite skilled in the culture of initial primary cells from these two species, so infections with immortalizing agents were able to proceed rapidly. Selection of cells with the immortalizing gene was done by drug selection. Skate cells did not develop drug resistance suggesting that they did not express the immortalizing agents, however the shark cells did acquire drug resistance indicating that they did. Thus the infection technique used for mammalian cells was also successful for marine animal cells.

Further investigations revealed that the difficulty with the skate cells was because they were fully differentiated and not dividing. The immortalization techniques require that the cells be dividing. Thus, more work is needed on the primary culture of the skate cells to prevent them from fully differentiating before immortalization. Difficulties were also encountered with the shark cells. First, it was discovered that the cells were highly resistant to trypsinization and could not be removed from plasticware. After much work a successful trypsinization protocol

was defined. Then it was discovered that the cells were not able to replate, making passaging impossible. There was preliminary success in determining media requirements that allow replating, but more work is needed to confirm its success.

Important steps were accomplished with the project regarding the culture of shark and skate cells. The next steps are: 1) define new culture media for the skate; 2) optimize the protocol for passaging the shark cells; 3) infect the shark, skate and whale cells; 4) begin defining culture variables for the killifish; and 5) evaluate the genotoxicity of Cr(VI) in each. The last step will be to compare any observed genotoxicity with data concerning the genotoxicity of Cr(VI) in immortalized human cells that has already been determined by the Principle Investigator.

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