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

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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. Sharks cells were successfully passage twice using standard mammalian tissue culture methods. Further, shark cells and tissues were found to endogenously express high levels of shark telomerase, which suggests that trying to immortalize the cells with human telomerase is not the right approach. Accordingly, future experiments will focus on optimizing the cell culture conditions and immortalizing the cells with SV40 large T antigen and the E6/E7 oncoproteins.

The culture of skate cells provided different challenges. Cultures would succumb to bacterial infections after several days. Some success was found as one culture was maintained for 60 days without contamination. Skate cells were not successfully passaged. Like the shark, skate cells and tissues were also found to endogenously express high levels of skate telomerase, which suggests that trying to immortalize the cells with human telomerase is not the right approach. Accordingly, future experiments will focus on optimizing the cell culture conditions and immortalizing the cells with SV40 large T antigen and the E6/E7 oncoproteins.

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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