CHARACTERIZATION THROUGH PROTEOMICS OF THE GILL DERIVED RAINBOW TROUT CELL LINE, RTGILL-W1 GROWN UNDER VARYING OSMOTIC CONDITIONS

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Quantitative and qualitative measurements of protein changes in response to biological perturbations such as disease, hormonal or drug treatments and response to toxicants can be readily evaluated using proteomic analysis. Observation of changes at the protein level, unlike genomics, allows for functional analysis of cells and organisms, and the determination of mechanisms of action at the cellular level. However, the complexity and variability of organismal responses make it difficult to evaluate whole organism proteomes. In vitro assays have been instrumental in environmental toxicology and fish cell lines have been crucial in screening and elucidating mechanisms of action of various aquatic pollutants. Thus a proteomics approach to evaluate changes in fish cell lines in response to environmental contaminants has been initiated.

One of the first sites of action of aquatic pollutants in fish is at the gill surfaces and evaluation of chemical effects using gill cells in culture have been difficult because of the confounding effects of culture media and serum additives that may bind toxicants. In the present study, the RTgill-W1 cell line (Bols et al. 1994), was tested for growth under varying water concentrations and changes in their proteome under varying osmotic conditions were evaluated using 2D gel electrophoresis. RTgill-W1, which is an epithelioid cell line derived from gill explants of rainbow trout and is available from the American Type Tissue Collection (CRL2523), were maintained for over 6 months in tissue culture

inserts with water on the apical surface and culture media in the basolateral surfaces. Moreover, cells were adapted to grow under hypoosmotic and hyperosmotic conditions and were passaged several times. Hypoosmotic conditions included growth in 25% media: 75% water, 50% media:50% water, while hyperosmotic conditions included growth in 75% media:25% seawater, 50% media:50% sea water. Although growth rates were significantly reduced under the varying osmotic conditions, cells could be maintained for prolonged time periods especially in the 50:50 mixtures. Analysis of 2D gels of control, hypoosmotic (50% water), and hyperosmotic (50% seawater) cells showed slight differences in protein profiles which is currently under analysis (Fig.1).

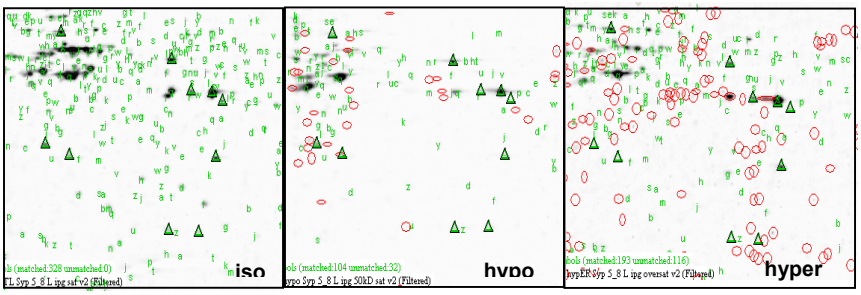

Fig.1 Protein profile of RTgill-W1 grown in varying osmotic states separated by IEF 5-8 in the first dimension and by 12.5% SDS-PAGE in the second dimension. Proteins were visualized by Sypro Ruby staining. Circles indicate protein spots not present in control isosmotic cells. Triangles indicate landmarking protein spots.

Morphologically, RTgill-W1 in hyper- or hypo-osmotic media were more epithelial than in the iso-osmotic control media (Fig.2). Granulated cells were observed in the hyper- and hypo-osmotically grown cells, but this was more predominant in the hyperosmotic medium. Tests are currently being performed to assess whether the granular cells are chloride-like cells. The above findings make RTgill-W1 a readily available and useful model for physiological and toxicological studies of fish gills. Aquatic contaminants could be directly added to the cells and comparisons in protein profiles could be made and changing protein patterns could then be further analysed to elucidate mechanisms of toxicant action.

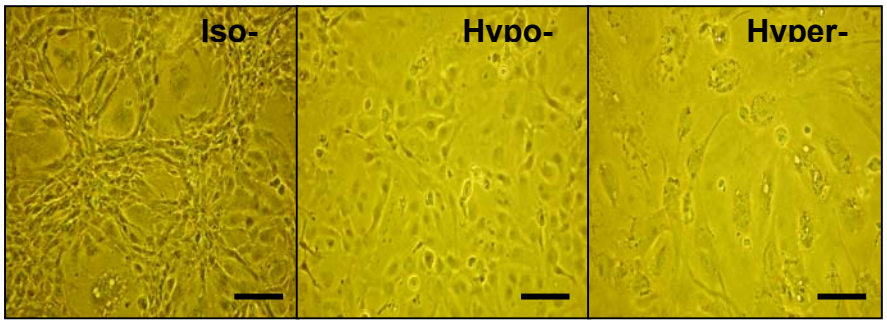

Fig. 2. Morphology of RTgill-W1 grown in varying osmotic states. Phase contrast micrographs taken at 100x magnification. Bar=100µm.

References

Bols, N. C., A. Barlian, M. Chirino-Trejo, S. J. Caldwell, P. Goegan and L. E. J. Lee (1994) Development of a cell line from primary cultures of rainbow trout, *Oncorhynchus mykiss* (Walbaum), gills. J. Fish Diseases 17: 601-611

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