DERIVATION OF CONTINUOUS MARINE CELL LINES FOR MODEL SYSTEMS IN TOXICOLOGY AND CELL BIOLOGY

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In recent years, methods have been developed for establishing cell lines from freshwater and marine fish (Collodi, P. et al., Cell Biol. and Tox. 8:43-61, 1992; Bradford, C. et al., Mol. Marine Biol. and Biotech. 6:270-288, 1997; Buck, C. et al., Marine Biotech. 3:193-202, 2001). These lines have provided models for both molecular developmental biology and toxicology (Collodi, P. et al., Xenobiotica, 24:487-493, 1994; Singh, N.N. et al. Marine Biotech, 3:27-35, 2001). Many of these cultures retain normal karyotype and at least partial differentiated function. The cultures do not show growth crisis, unlike mammalian cells cultured in conventional media. The goal of this project is to derive continuous cell lines for model systems used at MDIBL. One of these is the dogfish shark (*Squalus acanthias*) rectal gland (Frizzell, R.A., Physiol Rev. 79(Suppl):S1-2, 1999; Aller, S.G. et al., Am. J. Physiol. 276:C442-9, 1999; Waldegger, S. et al., Pflugers Arch. 437:298-304, 1999). Other models include skate (*Raja erinacea*) hepatocytes (Rebbeor, J.F. et al., Am. J. Physiol. Gastrointest. Liver Physiol. 279:G417-25, 2000; Ballatori, N. et al., Am. J. Physiol. Gastrointest. Liver Physiol. 278:G57-63.2000) and killifish (*Fundulus heteroclitus*) tissues (Hossler, F.E. et al., J. Morphol. 185:377-86,1985; Mickle, M. and Cutting, G. R., Med Clin North Am. 84:597-607, 2000; Moyer, B.D. et al., J. Clin. Invest.104:1353-1358, 2000).

The methods used to establish fish cell cultures rely on the replacement, reduction or supplementation of the usual bovine serum supplement with a variety of purified peptide growth factors, nutritional supplements and other proteins specific for the cell type (Helmrich, A. and Barnes, D. Meth. Cell Biol. 59:29-38, 1999). For instance the peptides epidermal and fibroblast growth factor in combination with extracts of trout embryo, shark egg yolk and serum from trout or eel have proved effective in specific cases. Attention is also given to optimization of the basal nutrient medium for fish cell culture. Conveniently, cultures often can be weaned from some of the more exotic supplements, once dependably proliferating cells have been derived. In some cases it is necessary to alter the substratum or passaging procedure or provide conditioned medium from other established fish lines to allow derivation of functional cultures.

Primary cultures of dogfish shark rectal gland were examined for growth responses to a wide range of peptide hormones and other potential effectors of proliferation in both primary and secondary cultures. Passaging protocols were compared, using a variety of hydrolases (eg., trypsin, collagenase, hyaluronidase) and procedures, and it was found that a simple increase in temperature of about 5 degrees C

for the period of the enzyme treatment improved survival of passaged cells. This may be related to a protective alteration of the membrane through lipid phase changes for the period of enzyme treatment, and is not simply the result of increased enzyme activity at the higher temperature. Extended incubation of primary cultures revealed that a low percentage of proliferating fibroblastic cells are present in the cultures. A range of substratum treatments was assessed for improvements in cell adhesion, and none were found better than plating the cells on a collagen II gel, identical to that used in the primary cultures.

A combination of dibutryl c-AMP (1 mM) and forskolin (10 microM) increased cell survival and markedly altered the morphology of the cells, leading to a more compact appearance. Cyclic AMP is recognized to be involved in signaling for transport phenomena in these cells, and may also have a role in cell survival and proliferation. A similar phenomenon is seen with some mammalian kidney cell cultures, in which cAMP influences both cell proliferation and expression of differentiated physiological function. Because of the naturally slow growth rate of the cells, results have been limited, and considerably more insight will be required to establish multipassage proliferating cultures of rectal gland cells. Cells from skate liver also were initiated as primary cultures and have been maintained with evidence of metabolic activity over more than six months. The cultures have been best maintained as multicellular aggregates rather than populations of individual, autonomous cells.

Attempts were made to develop cell lines from a transgenic medaka strain carrying a bacteriophage lambda cII transgene target (Winn R. et al., Proc. Natl. Acad. Sci. U S A 97:12655-60, 2000). This system provides a means for quantitative assessment of some classes of mutations and assay of genotoxic activity of purified environmental toxins and mixtures of known or unknown toxins. Actively proliferating cultures of liver and gill cells from the transgenic medaka were obtained using variations of media developed for zebrafish. Similar results were also obtained with cultures from *Fundulus* spleen and ovary. The transgenic medaka liver cells were successfully passaged and behaved much like zebrafish liver cells in culture. Unfortunately, these cultures were lost to contamination, but it is likely that the cultures can be reproducibly initiated.

A permanent, reproducible source of experimental material and the ability to manipulate both the environment of the system and the genetics of the cells in vitro will provide additional tools for exploitation of the unique properties of the shark rectal gland). This and the other culture systems also will be useful for heavy metal or other toxicity studies (Forrest, J.N. et al., J Exp Zool. 279:530-6, 1997) and will facilitate examination of xenobiotic metabolism and transport (Miller, D.S. et al., Am. J. Physiol.. 275:R697-705, 1998). The culture of cells from the transgenic medaka will allow comparison of mutagenic activity for genotoxins in vivo and in vitro, as well as providing a system for identifying modulators of mutagenesis and mechanisms of action. The ability to transfect and select fish cells in vitro also allows the use of these cultures in studies of molecular biology and mechanisms of action of physiological and hormonal mediators of cellular behavior. Supported in part by a New Investigator Award to D.B

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