STRESS GENE EXPRESSION IN HUMAN AND FISH CARCINOMA CELLS ON DIFFERENT EXTRACELLULAR MATRICES (ECM)

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Whereas virtually all cells in culture express the classical heat shock genes at high levels in response to a variety of proteotoxic stressors, dramatic heterogeneity in the responses of cells *in vivo* has been observed. This phenomenon is usually interpreted as loss of differentiation of cultured cells, but the mechanism is unclear. The ECM is known to play an active role in development and maintenance of cellular differentiation. To date, no detailed studies exploring the effects of ECM on the expression of genes involved in proteotoxic stress responses have been published. Here I report on the initiation of a systematic test of the hypothesis that ECM affects the level of stress gene expression using two carcinoma cell lines, human colon carcinoma cells (HT-29 line) and hepatocellular carcinoma cells (PLHC-1 line) from the desert topminnow *Poeciliopsis lucida* (clearfin livebearer). These lines were chosen because they are relatively easy to culture and because both retain many of the morphological and metabolic characteristics of their differentiated counterparts *in vivo*. By carrying out a comparative study of both human and fish cells, it will be possible to determine whether each step in a putative signaling mechanism that may couple ECM to gene expression is unique to one or the other or shared by both cell lines, and thus likely to be highly conserved evolutionarily and broadly distributed among vertebrates.

A stably transfected line of HT-29 cells expressing enhanced Green Fluorescent Protein (GFP) under the control of a human hsp70 promoter (HT29phspGFP cells) was available from a previous study in my laboratory whereas the creation of such a stable transformant of PLHC-1 cells is an ongoing project. Therefore, most of the results of my initial experiments pertain to the effects of plating HT29phspGFP cells on a variety of different matrices and measuring levels of eGFP expression. Nontransfected PLHC-1 cells were plated on these same matrices as well to obtain some preliminary data on their growth and morphological properties.

HT29phspGFP cells were plated in two wells of each six-well tray. Each tray contained a different ECM including rat tail collagen I, human fibronectin, mouse laminin, mouse collagen IV, and poly-D-lysine (Becton Dickinson [Two Oak Park, Bedford, MA 01730] Biocoat cellware, Variety Pack II). Their cell culture characteristics were compared to cells plated on standard tissue culture plastic and Falcon [Two Oak Park, Bedford, MA 01730] Primaria plates, a modified plastic surface that favors attachment of certain primary cells. The initial attachment rates were estimated by microscopic observation. On collagen I and IV, ~95% of the inoculated cells attached by sixty minutes with ~1% spread; on poly-D-lysine, ~80-90% were attached and rounded; on laminin, ~85% were attached and rounded; on standard plastic and Primaria, ~50-60% were attached but remained rounded; and on human fibronectin, ~25% were attached and rounded. Cell spreading was taken as an indication of more extensive adhesion to substratum. One day later, the cells on collagen I and IV were well-spread and organized into straps and clusters; the cells on human fibronectin were not as well spread out as on the collagens; the cells on Primaria and standard plastic followed in order of spreading with sharp, refractile edges on the cell clusters and a raised or columnar appearance; the cells on poly-D-lysine formed short straps of cells with a columnar

appearance; and cells on laminin were spread least well with raised clusters of cells having refractile edges. Five days after plating, cells on collagens I and IV were confluent; cells on human fibronectin were next in percent confluency followed by standard plastic and Primaria, which were equivalent. Cells on poly-D-lysine continued to grow in clusters and straps with columnar morphologies one cell layer thick. The cells on laminin were the most unusual. They formed raised mounds with little further spreading on the plate surface and took on a 'polyp-like' appearance.

To test the stress response of HT29phspGFP cells on different ECMs, the cultures were treated with 5 mM Zn(II) for three hours, after which the treatment medium was replaced by normal culture medium and the cells were incubated for an additional 16 hours. Accumulation of eGFP was monitored by fluorescence microscopy and by measuring cell extracts of duplicate wells by fluorimetry. For the duplicate wells of each ECM, treated cultures were compared to matched control cultures. The polyp-like assemblies on laminin had the largest amounts of eGFP by both microscopic observation and by fluorimetry (5 fold increase in fluorescence over matched control culture, comparing means of values of duplicate lysates). Interestingly, the cell assemblies in treated cultures were spread out on the plate more than controls, suggesting a change in association with the ECM. The ability of different ECMs to support induction of eGFP was different and decreased in the following order, as estimated by microscopic observation of the numbers of fluorescent cells: laminin ~ poly-D lysine > collagen IV ~ human fibronectin > collagen I ~ Primaria. Zn treatment also affected cell attachment: Laminin ~ poly-D lysine ~ Primaria (>95% cells still attached)> collagen IV ~ fibronectin (mix of rounded, detached cells and attached cells) > collagen I (most affected). Until protein assays can be done on the extracts, only the relative fluorescence values for laminin (5 fold increase) and Primaria (2 fold increase) cultures can be compared reliably. For these two cultures, the qualitative evaluation by microscopy agreed with the fluorometric measurements, indicating that the kind of ECM affects the activation of the *hsp70b* promoter in response to Zn stress. The response of cells on laminin is potentially very interesting since it is the dominant matrix component during certain stages of embryonic development. Primaria will provide an excellent example of a matrix that supports relatively less expression.

Microscopic observation of initial attachment of PLHC-1 cells on these same matrices yielded the following order of attachment: poly-D lysine ~ collagen I > collagen IV > Primaria > human fibronectin >> laminin. By 3 hours of incubation at 30C, collagen I and poly-D lysine both had greater than 70% of the cells attached and approximately half of these had begun to spread on the ECM, clearly the best early attachment on the matrices tested. By 3 days of incubation, the cultures on collagen IV and Primaria appeared to be confluent and cultures on collagen I and poly-D lysine appeared to be close to confluency. Cells growing on laminin and human fibronectin initially formed broad straps of cells that left open areas of plate between them, and these areas were slow to fill in. Overall, it is clear the PLHC-1 cells, like the HT29phspGFP cells, have different morphological and growth properties on different ECM. Once a PLHC-1 cell line stably transformed with the phsp70-eGFP construct is produced, a similar analysis of the level of *hsp70* gene expression will be done. (Supported by an MDIBL New Investigator Award)