Differential Cancer Biomarker Secretion of Cultured Human Cells

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Abstract

Diagnostic biomarkers are a key element in cancer research. Research has focused primarily on intracellular biomarkers, such as HER2 or BRAC1, that identify specific tumors, or provide genetic or phenotypic information that can clarify the process of oncogenesis. Recently more attention is being placed on identifying soluble extracellular circulating biomarkers, which can provide information on the body response to cancer, as well as the relationship between a tumor cell and its environment. Because cancer is a series of different disease states, the study of individual biomarkers is usually inadequate to study the complex relationship between a tumor and its environment. Using a panel of known tumor biomarkers to characterize tumor cell lines of known lineage under different conditions provides a better understanding of the biology specific to different tumor types. Using a 22-plex Cancer biomarker assay kit from EMD Millipore we characterized the secretion of cancer biomarkers by several human tissue culture cell lines under normal and low serum conditions. In addition to the established cell lines, three mesothelioma cell lines included two cell lines derived from different individuals were characterized. Comparison data from all cell lines will be provided.

Introduction

Many different cell types, including tumor cells secrete cellular messengers than can influence growth via receptor mediated signaling. If the cell in question secretes a compound that will interact with its own receptors this is referred to as autocrine secretion. If the secreting messenger interacts with adjacent cells this phenomenon is referred to a paracrine secretion (Figure 1). Endocrine secretion involves the flow of the messenger signals through capillary vessels and transportation to remotely located cells. In culture only autocrine and paracrine secretion are possible. The assay is based on Luminex® xMAP® technology. Distinct internally color-coded magnetic microspheres each coated with a specific antibody capture and quantitate different analytes. By using multiplexed bead sets several analytes can be quantitated simultaneously.

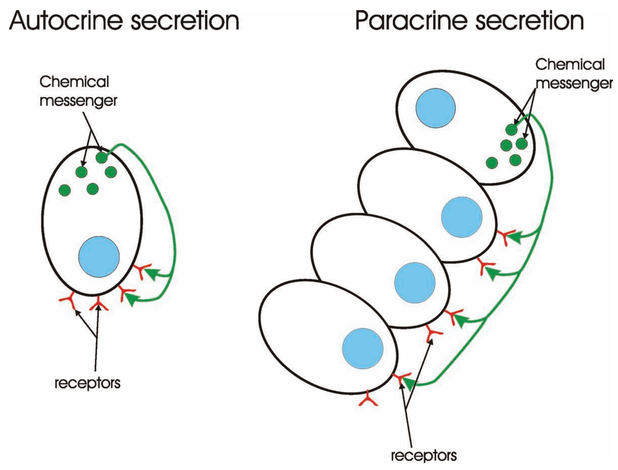

Figure 1 – *Autocrine and paracrine secretion.*

Materials and Methods

The human tissue culture cell lines HepG2, HT1080, and HEK293 were obtained from ATCC. LP9, H-Meso, GA and MI cells were from the Vermont Cancer Center at the University of Vermont. DMEM, F12, MEM, F12/DMEM growth media, glutamine and fetal bovine serum (FBS) were obtained from Invitrogen. Hydrocortisone (Cat # H0135) and ITS (Cat # I1884) were from Sigma-Aldrich. MILLIPLEX® map kits for human circulating cancer biomarker magnetic bead panel were obtained from EMD Millipore. HepG2 and HT1080 cells were maintained in MEM supplemented with 10% FBS and 2 mM glutamine. HEK 293 cells were cultured in DMEM supplemented with 10% FBS. LP9, H-Meso, GA and MI cell lines were maintained in DMEM/F12 supplemented with 10% FBS, 2 mM glutamine, hydrocortisone 100 ng/mL, and ITS. Aliquots (1.5 mL) of conditioned media were obtained from cultures in log phase growth in the presence of 0.25% or 10% FBS and centrifuged at 14,000 RPM in an Eppendorf 5415C microcentrifuge to remove any particulates.

Results

When human cell lines are tested for cancer biomarker secretion, very different patterns of expression are observed. All of the established cell lines tested secreted IL-8 and MIF, both markers for inflammation. HepG2 cells secreted the greatest number of different tumor markers of the cell lines tested. HepG2 cells were observed to secrete large amounts of the liver specific protein AFP when cultured with or without serum.

The response to low serum in the established cell lines was quite varied. No cell line was observed to produce a biomarker with low serum that was not secreted in the presence of 10% serum. Poorly differentiated cells such as HEK293 were induced to produce greater amounts of several of the secreted biomarkers, while the differentiated cells generally produced less biomarker under low serum conditions. These differences may be explained by differences in cell growth, which was not examined.

The mesothelioma cell lines surprisingly did not have the same pattern of secreted biomarkers. They, like the mesothelial cell line LP9, also produce IL-6, suggesting that mesothelial derived cell lines secrete IL-6. Interestingly, the GA and MI cell lines also secrete CYFRA21-1, while the H-Meso cell line does not. GA and MI cell lines have only been isolated relatively recently as compared to H-Meso cell line. As with the more undifferentiated established lines (i.e. HEK293) the mesothelioma cell lines respond to low serum by producing more of some the markers.

Assay Process

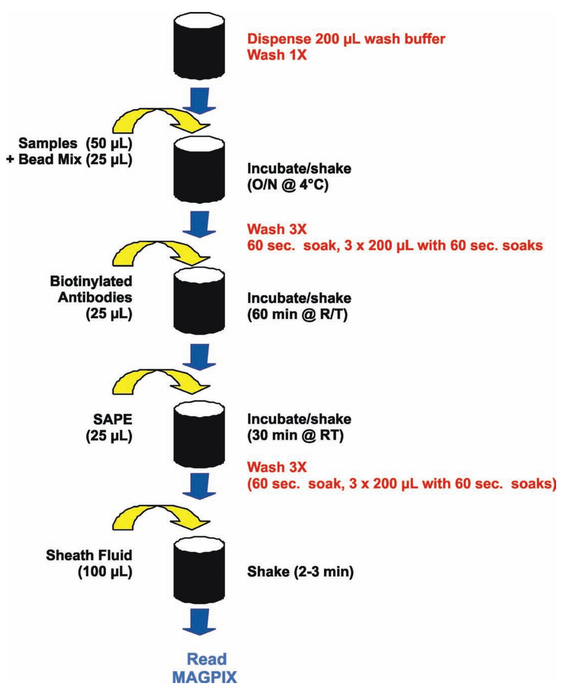

Figure 2 – *EMD Millipore Cancer Biomarker Assay Process. Red text indicates automated processes carried out by the ELx50 Automated Microplate Strip Washer.*

The EMD/MILLIPLEX assay was performed according to the kit instructions (Figure 2). The assay plate was first washed one time using the supplied assay wash buffer to remove any residue. Eight working multiplex standards were generated by serial dilution (1:3) of the reconstituted human cytokine standard, which contained 22 different analytes. After reconstitution, 50 µL each of standards and samples were pipetted into bead containing wells of the assay microplate. In parallel, the bead master mix was prepared by combining 150 µL of each individual bead suspension. 25 µL aliquots of the master mix were added to each well and the reactions were allowed to incubate overnight at 4°C with agitation on a plate shaker. The following day, the plate was washed 3 times as previously described [2]. After washing, 25 µL of detection or secondary antibody reagent was added and allowed to incubate for 60 minutes at RT with agitation. The beads were again washed three times followed by the addition of 25 µL of SAPE reagent. After 30-minute incubation with agitation to allow for reporter tag binding to occur, the plate was again washed as described in the washing instructions. The samples and standards were then resuspended in 100 µL of sheath fluid. Samples were then read on either a Luminex MAGPIX® or a LX100 reader with XPONENT software using the parameters outlined in the assay kit instructions.

Human Tissue Culture Cell Lines

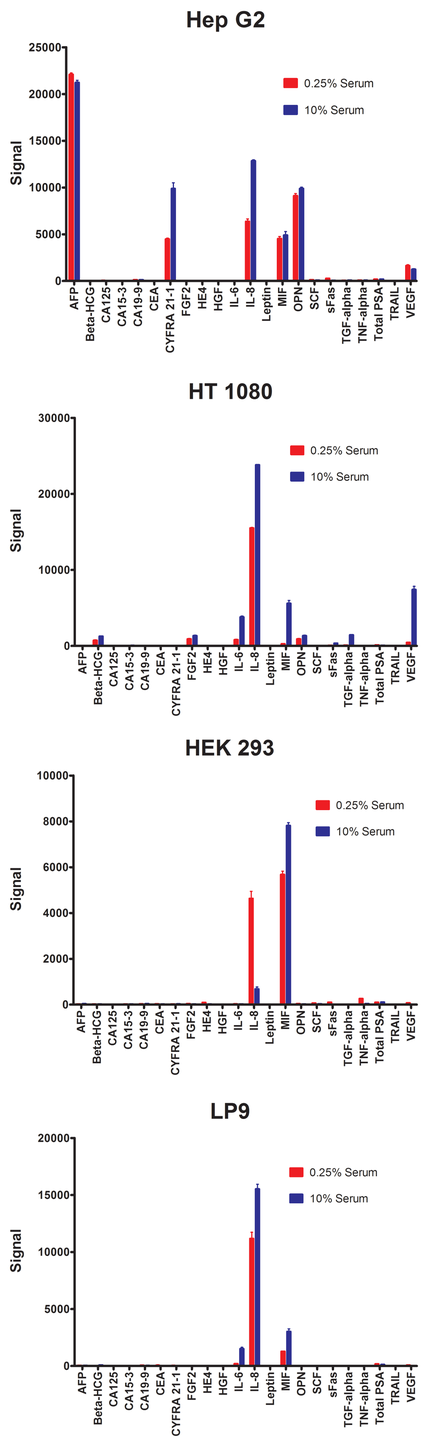

Figure 3 – *Established Tissue Culture Cell Line Biomarker Secretion.*

BioTek Instrumentation

Figure 4 – *ELx50™ Microplate Strip Washer.*

Mesothelioma Cell Lines

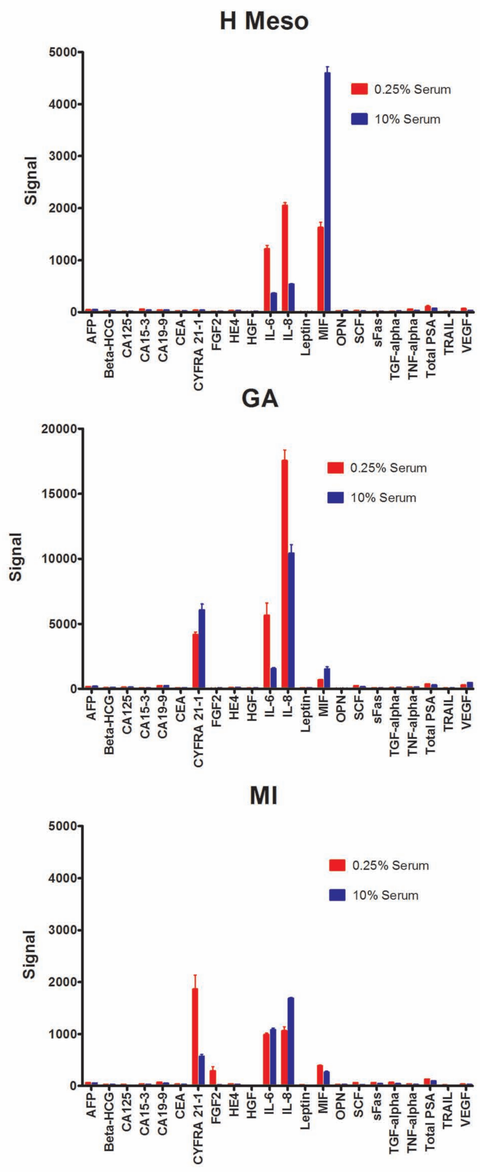

Figure 5 – *Mesothelioma Cell Line Biomarker Secretion.*

Conclusions

- Different human cell types grown in culture express markedly different biomarkers
- Different Primary Human mesothelioma cell lines express different biomarkers
- Inflammatory cytokine IL-8 is consistently expressed in all cell lines tested
- Biomarker secretion in response to exogenous serum supplementation varies by cell type and biomarker
- ELx50 Bead Washer is capable of automating the wash steps of magnetic bead assays

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