Polypeptide Growth Factors

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MDBK Cells

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by

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Summary

The purpose of my thesis is to investigate the growth factors produced by the bovine kidney cell line, MDBK, and to determine which of the growth factors may be involved in autocrine stimulation. The MDBK cell line, cultured under serum-free conditions, was found to produce growth factors which stimulated protein synthesis in the rat L-6 myoblast cell line. As a result of characterizing these growth factors it was determined that the MDBK cell line produces, (1) an IGF binding protein, (2) IGF-2 related growth factors, (3) PDGF, and (4) two growth inhibitors which are related to TGF-β.

The IGF binding protein produced by MDBK cells was detected by the ability of fractions collected after acid gel filtration chromatography of conditioned medium to bind ^{125}I-IGF-1 and interfere in an IGF-1 radioimmunoassay. Two samples of partially purified growth factor from MDBK cell conditioned medium, exhibited IGF-2 receptor competing activity after removal of IGF binding protein by acid gel filtration chromatography. The IGF-2-related growth factors stimulated both protein and DNA synthesis in L-6 myoblasts and eluted from a HPLC gel filtration column with approximate molecular weights of 9,000 and 12,000.

The MDBK cell-derived PDGF was purified approximately 2,300-fold from serum-free conditioned medium by cation exchange chromatography and reverse phase HPLC. Growth factor activity was monitored by the stimulation of protein synthesis in L-6 myoblasts. The purified growth factor (referred to as bovine PDGF or bPDGF) appeared as a major and a minor band of approximately 35,000 molecular weight after SDS-polyacrylamide gel electrophoresis under non-reducing conditions. When the same technique was applied to bPDGF under reducing conditions a series of bands with molecular weights ranging from 14,000 to 30,000 appeared. Reduction of bPDGF was associated with the loss of biological activity.

Bovine PDGF, hPDGF-AB and hPDGF-BB all stimulated protein synthesis in the rat L-6 myoblast cell line to a similar maximum level, whereas the hPDGF-AA isoform had very little effect on protein synthesis in L-6 cells. Similar results were obtained when DNA synthesis in L-6 cells was investigated. In an ELISA assay specific for the A chain of PDGF, bPDGF competed with a similar potency to hPDGF-AA standard, whereas only a slight competition was observed between bPDGF and hPDGF-BB standard in a B chain specific ELISA assay. From the above results, and those from radioreceptor and antibody

neutralization assays specific for the different isoforms of PDGF (E.W. Raines, *pers. comm.*, 1989), bPDGF appears to be the bovine equivalent of hPDGF-AB, where the antigenicity of the bovine B chain differs significantly from that of the human form of the PDGF B chain.

Two growth inhibitors (referred to as GI-1 and GI-2) were partially purified from MDBK cell conditioned medium by cation exchange chromatography and reverse phase HPLC. Detection of growth inhibitor activity was by inhibition of DNA synthesis in L-6 myoblasts. Both growth inhibitors were determined to have a molecular weight of approximately 25,000 as determined by gel filtration HPLC. An anti-TGF-β antibody neutralized 70-75% of the inhibitory activity of GI-1 and GI-2 on L-6 myoblasts, indicating that both growth inhibitors are related to TGF-β.

Various growth factors were examined for their effects on protein synthesis, protein degradation and DNA synthesis in MDBK cells. The insulin-like growth factors (represented by IGF-1, des-(1-3)-IGF-1 (a more potent truncated derivative of IGF-1) and IGF-2) and insulin decreased protein degradation, and stimulated protein and DNA synthesis, whereas bPDGF, hPDGF-BB and hPDGF-AA had no significant effect in any of these assays on MDBK cells. The two MDBK cell derived growth inhibitors (GI-1 and GI-2) and TGF-β1, were found to inhibit DNA synthesis and to slightly decrease protein degradation in MDBK cells.

It is concluded from these studies that PDGF-AB produced by MDBK cells is not involved in an autocrine stimulation of this cell line. However, endogenously produced IGF-2 may be involved in autocrine stimulation, although it is considered unlikely due to the production by MDBK cells of an IGF binding protein that preferentially binds IGF-2. Hence, the relative serum independence of the MDBK cell line appears to be due to factors other than the production and secretion of autocrine growth factors.