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Effect of glycosaminoglycans on growth factor-stimulated trophoblast invasion

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Objectives

To determine the effect of glycosaminoglycans and a series of growth factors on the viability and invasion of the extravillous trophoblast cell line SGHPL4.

Methods

Cells were cultured in Hams F10 media supplemented with fetal bovine serum and L-glutamine. For viability studies cells were seeded into 96-well culture plates (10^4 cells/well), maintained in serum free medium for 24h and then incubated with glycosaminoglycans (heparin, heparin sulphate and hyaluronic acid; each 100ng/ml) \pm growth factors (VEGF, FGF and HB-EGF). Cell viability was measured in cells using the MTS assay. Cellular invasion was assessed using the FluoroBlok invasion assay. Cells were serum-starved for 24 h, incubated with the fluorescent dye DilC₁₂(3) (10 μ g/ml) for 1 hour prior to seeding onto an artificial extracellular matrix-coated 8 mm FluoroBlok porous membrane inserts (2.5×10^5 cells per insert). Growth factors \pm GAGs were added to the cell suspension and the inserts were lowered into a 96-well plate containing 10% fetal calf serum. Plates were incubated at 37°C for 24h. Invasion was determined by measurement of fluorescence of invaded cells using a fluorescent plate reader (Ex549/Em565 nm).

Results

Cell numbers were significantly increased following incubation with VEGF, FGF and HB-EGF. Cell number was also increased after incubation with each of the glycosaminoglycans tested. The largest increase was observed following incubation with heparin sulphate. Cell numbers were further increased when the GFs were incubated with HS and heparin, but not with hyaluronic acid. Invasion was increased following incubation with VEGF, HBEGF and HGF. Heparan sulphate and heparin increased invasiveness in a dose-dependent manner. In contrast, hyaluronic acid had no significant effect.

Conclusion

This study demonstrates a role for glycosaminoglycans in key features of trophoblast function.