

P014 Cellular responses to the HepII domain of fibronectin require N-sulphation of cell-surface heparan sulphate proteoglycan Yashithra Mahalingam¹ John T Gallagher² and John R Couchman¹

¹*Division of Biomedical Sciences, Imperial College London*

²*Cancer Research UK Department of Medical Oncology, Paterson Institute for Cancer Research, University of Manchester.*

In addition to integrins, cell adhesion and cytoskeletal reorganisation in response to fibronectin involves signalling from the cell surface proteoglycan syndecan-4. This proteoglycan interacts with high affinity to the major heparin binding site (HepII domain) of fibronectin via its heparan sulphate chains. *In vitro* experiments suggest that highly sulphated regions (S domains) of heparan sulphate bind to the HepII domain and that their length and pattern of sulphation are critical for optimum binding. However, it is not known whether this extends to cell surface heparan sulphate and cell adhesion. Cell attachment to the HepII domain in the presence of competitive sulphated and desulphated heparin and heparan sulphate oligosaccharides showed that optimal interaction required 14 or more sugar residues and only N-sulphated glucosamine; sulphation at other positions was dispensable. However, focal adhesion formation in response to fibronectin's HepII domain needed, in addition, glucosamine 6-O-sulphate. The use of wild-type and mutants defective in elements of heparan sulphate synthesis concurred with these requirements. Therefore, cell surface responses to an extracellular matrix heparin-binding domain are specific and show increasing complexity where transmembrane signalling events are involved.