The Role of E-cadherin in mouse Embryonic Stem cell pluripotency

Joe Segal and Chris Ward. Stem Cell Research Group, The University of Manchester.

Email: Christopher.ward@manchester.ac.uk; Tel: 0161 275 5182

Background

Our group investigates the role of adhesion molecules in embryonic stem (ES) cell pluripotency, differentiation and tumorigenesis. We have found that the cell adhesion protein E-cadherin plays an important role in regulating pluripotent signalling pathways in mES cells (Soncin *et al* 2009). In addition, E-cadherin repression is associated with ES cell differentiation, which exhibits similarities to tumour cell metastasis (Eastham *et al* 2007).

The principle function of E-cadherin is to maintain epithelial integrity, however, how this process relates to regulation of ES cell pluripotency and molecular processes that define ES cell behaviour and control 'stemness' are not clear. We have shown that expression of E-cadherin is a critical factor in regulating unique signalling pathways in mES cells to maintain pluripotency. For example, while mES cells maintain self renewal through the default LIF/BMP signalling pathway, loss of E-cadherin leads to a switch to the Activin/Nodal and FGF2 signalling pathways which acts independently of LIF to sustain expression of pluripotent transcription factors Oct4, Sox-2 and Nanog (Soncin *et al* 2009). How E-cadherin mediates this hierarchical switch and maintains ES cell pluripotency remains unclear.

We have compared the global transcript expression between E-cadherin null (Ecad-/-) and wild-type (wt)ES cells cultured in the presence of LIF. Loss of E-cadherin expression resulted in significant changes to the gene expression profile, with alterations to >2000 transcripts (Soncin *et al* 2011). Intriguingly, abrogation of E-cadherin affects not only cell adhesion and motility but genes involved in a wide range of biological processes, including primary metabolism, differentiation and apoptosis (Figure 1). Hierarchical clustering of microarray data indicates that the Ecad -/- transcriptome is more phenotypically similar to mouse EpiS cells isolated from post-implantation embryos than ES cells of the inner cell mass, of which the former maintain pluripotency via Activin/Nodal signalling.

To further specify changes in gene expression associated with E-cadherin loss, we have combined the Ecad-/- ES cell microarray expression profile with microarray data obtained from two independently derived ES cell lines which naturally down-regulate E-cadherin expression (ENPS cells). ENPS cells demonstrate significant alterations in their transcript profile and are more phenotypically similar to Ecad-/- cells than wtES cells. Initial analysis of the combined microarray data has identified ~200 upregulated and ~400 downregulated genes

common to all three cell lines compared to wtES cells. Gene changes include transcripts associated with various biological processes including epigenetic regulation, apoptosis and transcription factor activity. Several down regulated genes identified in this analysis have defined roles in regulating ES cell pluripotency, including members of the nuclear receptor protein family and components of the pluripotency transcription factor network. These results have been confirmed RT-PCR.

Mathematical modelling

What we hope to obtain through mathematical modelling is initial proof-of-concept of our current data showing the role of E-cadherin in regulating ES cell signalling pathway hierarchy (Figure 3). The contents of these pathways are well studied and should allow mathematical modelling and generation of hypothesises that can be tested in our lab. In the longer term we would wish to determine the exact pathways that E-cadherin regulates in ES cells to allow a better understanding of the role of this protein in ES cell identity. Since loss of E-cadherin is also associated with tumour cell spread, these analyses may provide novel agonist pathways for stimulating E-cadherin expression in tumour cells, thereby decreasing cell proliferation and motility. We would expect to submit a grant application with our partners to BBSRC/EPSRC/MRC after the proof-of-concept stage of this project.
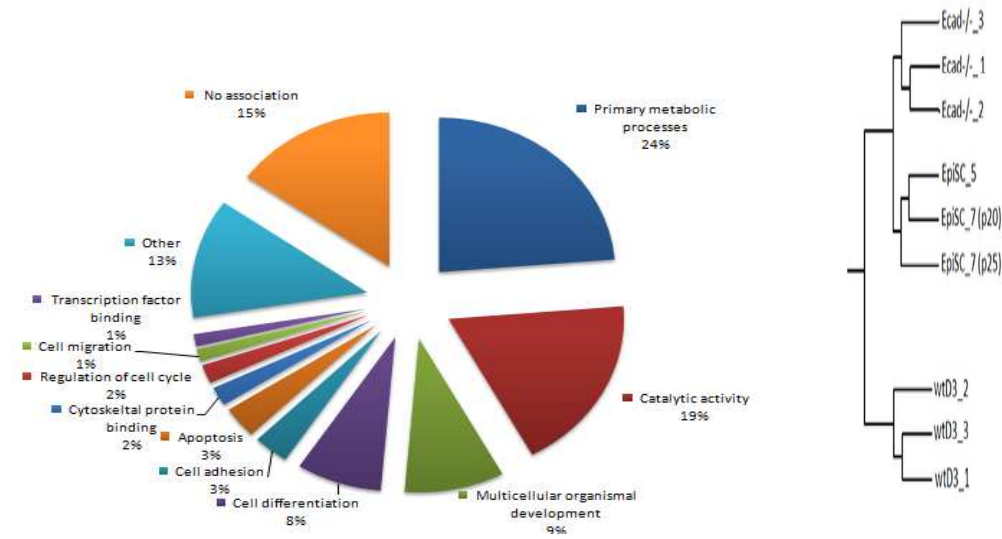

Figure 1: Ecad -/- array Gene ontology and gene expression profile hierarchical clustering: A. Pie chart showing gene ontology terms most represented in microarray analysis comparing Ecad -/- and wtD3 ES cells. Terms relating to a range of Biological processes are influenced by E-cadherin loss. B. .Hierarchical clustering of microarray data shows that the Ecad -/- transcriptome is more phenotypically similar to EpiS cells isolated from post-implantation embryos then wtD3 cells isolated from the ICM. Data for Ecad-/-, wtD3 and EpiS cells were taken from Soncin *et al* (2011) and Brons *et al* (2007). Image adapted from Soncin *et al*(2011).
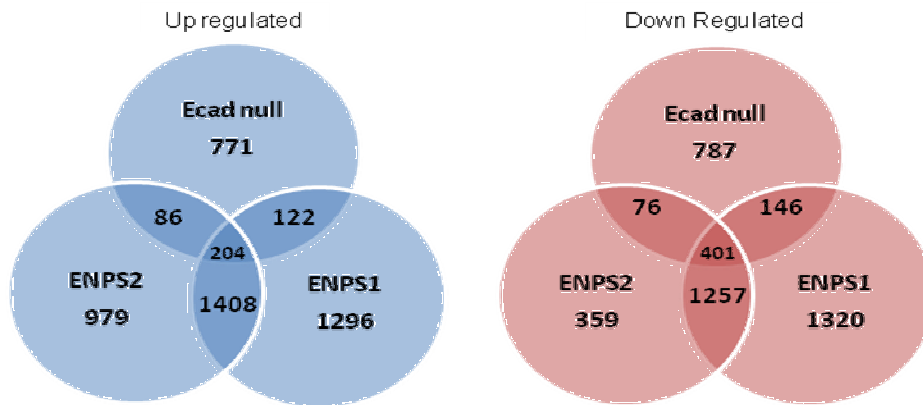
Figure 2: Venn diagram comparing significant changes in gene expression common to three E-cadherin null cell lines: Microarray data of transcript alterations comparing Ecad-/- (gene knockout) and wtD3 was compared to microarray data for ENPS1 and ENPS2 vs wtD3 ES cells to identify up and down regulated genes shared between all three. Comparison shows 204 gene transcripts up regulated in Ecad -/-, ENPS1/2 and 401 gene transcripts downregulated in Ecad -/-, ENPS1/2. Significant changes in gene expression: 1.50 FC (P value <0.05). Data for Ecad-/- and ENPS1/2 microarray taken from Soncin *et al* (2011) and Hawkins et al (unpublished).
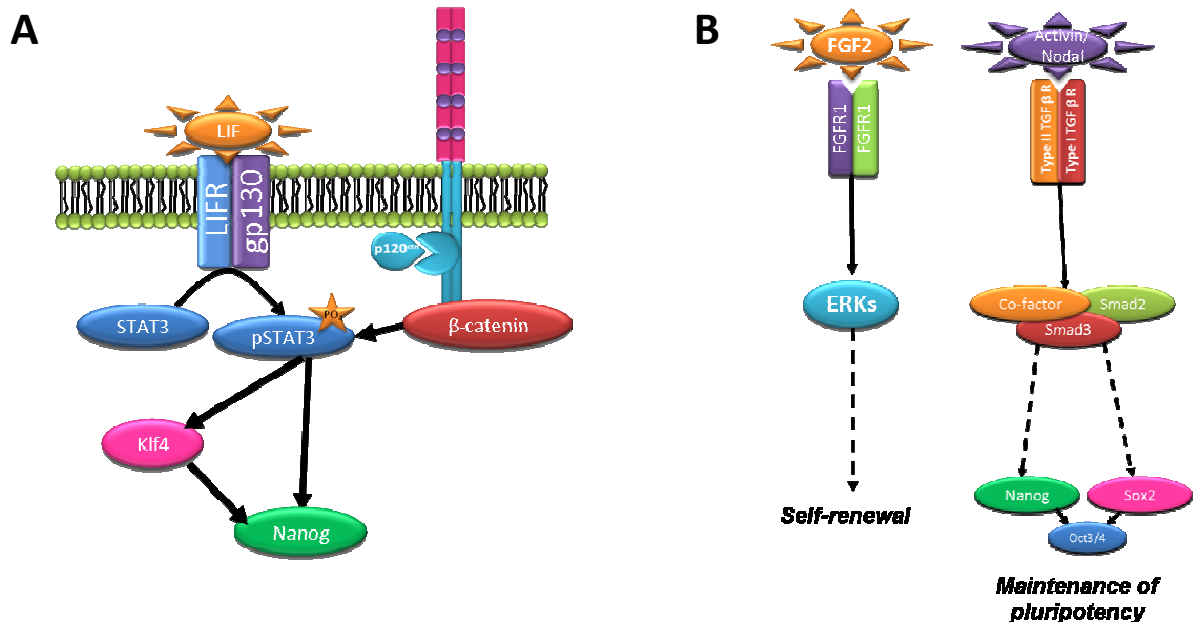

Figure 3. Signalling pathway hierarchy controlled by E-cadherin in embryonic stem cells. A. LIF pathway is positively regulated by E-cadherin and is the default pathway in wild-type ES cells. B. Upon loss of E-cadherin, ES cells revert to the Activin/Nodal and Fgf2 pathways to maintain pluripotency and proliferation.

References

Eastham A.M., Spencer H., Soncin F., Ritson S., Merry C.L., Stern P.L and Ward. (2007). Epithelial-mesenchymal transition events during human embryonic stem cell differentiation. *Cancer Res.* 67 11254-11262.

Mohamet L., Lea M., Ward C.M. (2011). Abrogation of E-Cadherin-Mediated Cellular Aggregation Allows Proliferation of Pluripotent Mouse Embryonic Stem Cells in Shake Flask Bioreactors. *PloS One.* 5.9. e12921.

Soncin F., Mohamer L., Eckardt D., Ritson S., Eastham A., Bobola N., Russel A., Davies S., Kemler R., Merry C.L.R and Ward C.M. (2009). Abrogation of E-cadherin Mediated Cell-cell Contact in Mouse Embryonic Stem Cells Results in Reversible LIF-independent Self-renewal. *Stem Cells.* 7 2069–2080.

Soncin F., Mohamet L., Ritson S., Hawkins K., Bobola N., Zeef L., Merry C.L.R and Ward C.M. (2011). E-cadherin Acts as a Regulator of Transcripts Associated with a Wide Range of Cellular Processes in Mouse Embryonic Stem Cells. *PLoS one.* 6(7) e21463.

Soncin F and Ward C.M. (2011). The Function of E-cadherin in Stem Cell Pluripotency and Self-Renewal. *Genes.* 2 229-259.