The role of connexins in β-cell communication

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1.Introduction

- There are currently over 2 million people with diabetes in the UK and up to another 750,000 people with diabetes who have the condition, but are unaware of it (data from *www.diabetes.org.uk*)
- Diabetes mellitus is a disorder that affects body's ability to efficiently utilise blood glucose. Uncontrolled hyperglycaemia is a direct cause of many of the problems associated with diabetes. The diagram below represents localisation of pancreatic islets.

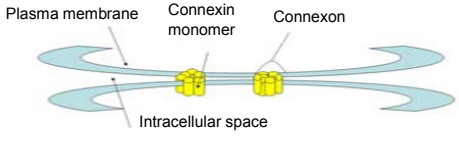
a. Location of the pancreas in humans

b. Gross anatomy of the normal pancreas.

c. Micro-structure of the pancreatic islet.

- Pancreatic islets are required for efficient insulin secretion, with the loss of islet architecture and communication between cells dramatically reducing insulin output.
- Regular insulin injections are used as a treatment for patients with type I diabetes. It is, however very difficult to maintain optimal blood glucose.
- New approach focuses on islet transplantation. However, since the pool of donors is low an alternative source of transplantable material is needed. However, it is insufficient to merely generate large amounts of insulin-secreting cells. An islet secretes more efficiently than the same number of cells in isolation and we need to understand how cells communicate to maximise function in the *in vitro* development of replacement therapy.

The role of connexins in formation of pancreatic gap-junctions…



The diagram represents gap-junctions, that allow the direct movement of small molecules and ions between adjacent cells to synchronize activity.

2. Aims

- The aim was to assess the localisation of gap junction-forming connexins Cx43 and Cx36, in MIN6 β-cells cultured as sparsely coupled monolayers or high contact pseudo-islet structures.
- Localise and assess expression of two cell adhesion proteins involved in targeting the connexins to the plasma membrane : E-cadherin and β-catenin.
- Consequently, to assess how important is cell adhesion in altering the expression and localisation of gap junctions.

3. Methods

- MAINTANANCE AND PREPARATION OF MIN6 CELLS

MIN6 β-cells passage (54-57) were maintained at 37˚C (95% O_2 5% CO_2). Both pseudo-islets and monolayers were cultured under identical conditions. Monolayers were trypsynised when confluent, whereas pseudo-islets were grown in pre-coated 1% gelatine flasks.

- WESTERN BLOT

Expression of connexin 36 and 43 protein was compared between pseudo-islets and monolayers via standard Western blot analysis.

- IMMUNOCYTOCHEMISTRY

The samples were mounted on APES coated cover slips, after the triple stain for insulin (Guinea pig anti insulin C3 1:2500), cytoskeleton (TRITC-conjugate Phalloidin 1:100) and nucleus (DAPI 1μM) was performed.

The effect of altering adhesion between cells was assessed by immuno-neutralising the key cell surface adhesion protein E-cadherin. The trafficking and insertion of Cx-36 was monitored via fluorescence microscopy.

4. Results – *In vitro* culture of MIN6 pseudo-islets and monolayers

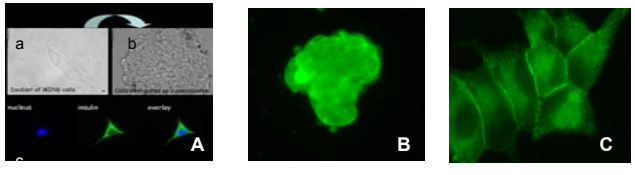


Figure 1: *In vitro* culture of MIN6 cells. (A) Normal morphology of MIN6 cells cultured either as a monolayer (a) or as a pseudoislet grown on 1% gelatine coated plastic (b; phasex40magnification). Immunocytochemical identification of insulin immuno-reactivity is shown in the lower panels of 4A (insulin, green; nucleus, blue). A single pseudoislet (B) and monolayer cell cluster (C) are visualised via fluorescent microscopy.

Expression and localisation of Cx-36 and Cx-43

A. Western blot for Cx-36 **B. Western blot for Cx-43**

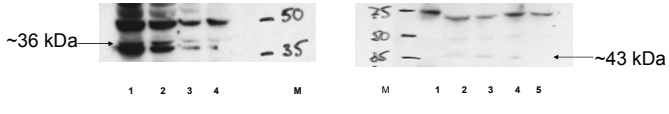
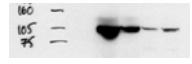


Figure 2: Expression and localisation of Cx-36 and Cx-43 Western blot analysis confirms expression of both connexin isoforms in MIN6 cells. However, in both panels (A) and (B) there are multiple bands of non-specific binding, suggesting that the conditions used for determining protein expression needs to be optimised. We were unable to localise connexin expression using the same poly-clonal antibody in immunocytochemistry studies.

Expression and localisation of E-cadherin.

A. Western blot for E-cad



B. Immunocytochemistry

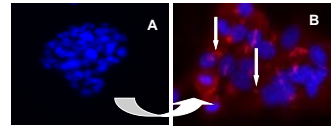


Figure 3: Expression and localisation of E-cadherin. A band of the expected size for E-cad expression (120kDa) was determine by Western blotting (A). Localisation of E-cad (red) to the cell membrane, was visualised by immuno-cytochemistry

Localisation of key adhesion molecule : β-catenin

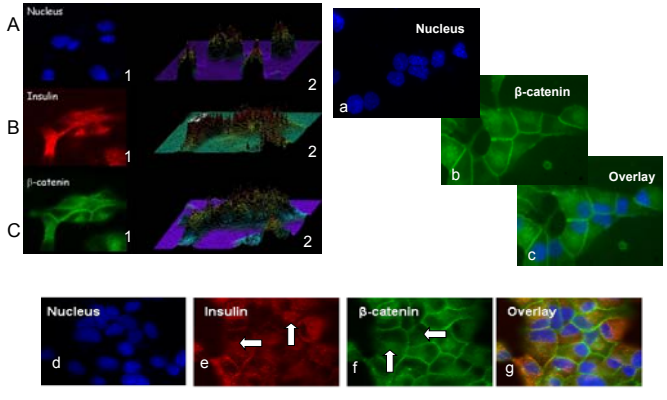


Figure 4: Localisation of β-catenin in MIN6 monolayers. Immuno-cytochemistry (ABC-1) visualises nucleus (blue), insulin (red) and β-catenin (green) stain respectively. (ABC-2) represents corresponding intensity profiles. (a-g) Triple stain on two sets of monolayer sections. Insulin granules are aggregated at the cell periphery with β-catenin (overlay image (g)).

5. Conclusion

- Intra-islet communication is crucial for efficient insulin secretion and gap junction-forming connexins play a central role in synchronising activity.
- This study highlights the potential importance of Cx-36 and Cx-43, and highlights the need for further optimisation in the conditions necessary to resolve clear expression of these proteins.
- Knowing how and where connexins are inserted into the membrane to facilitate information transfer between coupled cells is essential in deciphering why adhesion between cells augments insulin output.

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