Integrin αvβ5 heterodimer is a specific marker of human pancreatic beta cells

Jacqueline V. Schiesser1,2, Thomas Loudovaris3, Helen E. Thomas3,4, Andrew G. Elefanty1,2,5,6 & Edouard G. Stanley1,2,5,6*

The identification of cell surface markers specific to pancreatic beta cells is important for both the study of islet biology and for investigating the pathophysiology of diseases in which this cell type is lost or damaged. Following analysis of publicly available RNAseq data, we identified specific integrin subunits, integrin αv and integrin β5, that were expressed in beta cells. This finding was further elaborated using immunofluorescence analysis of histological sections derived from donor human pancreas. Despite the broad expression of specific integrin subunits, we found that expression of integrin αvβ5 heterodimers was restricted to beta cells and that this complex persisted in islet remnants of some type 1 diabetic individuals from which insulin expression had been lost. This study identifies αvβ5 heterodimers as a novel cell surface marker of human pancreatic beta cells, a finding that will aid in the identification and characterisation of this important cell type.

Abbreviation
T1D  Type 1 diabetes

There is a dearth of cell surface markers available that specifically identify pancreatic beta cells—instead, enrichment strategies based on intracellular zinc granules (for example TSQ1 or Newport Green2 or negative selection are currently used. Given that integrins are often used to distinguish and isolate specific cell types from a variety of sources3,4, we sought to determine whether this group of proteins could identify specific sub-populations within human islets.

Integrins are a large family of cell adhesion receptors that bind extracellular matrix (ECM) proteins and cell surface ligands, regulating a wide variety of processes such as tissue morphogenesis, cell adhesion and cytoskeletal organisation. The receptors themselves are heterodimeric in structure, consisting of non-covalently bound α and β subunits. Each α subunit preferentially associates with specific β subunits, a characteristic that confers an added level of cell type specificity. The αv-integrin subfamily comprises of five members (αvβ1, αvβ3, αvβ5, αvβ6, αvβ8), all of which recognise ECM and other peptides via a Arg-Gly-Asp (RGD) motif5. In the pancreas, interaction between the ECM and islet cells via adhesion-mediated integrin signaling has been shown to play a crucial role in maintenance of islet architecture and beta-cell function6.

In adults, the αv subunit was found to be expressed in the majority of the islet cells in intact tissue, but this expression was substantially decreased in isolated human islets7. Studies performed using mid-gestation (18–20 week) fetal pancreas found that the integrin heterodimers αvβ3 and αvβ5 were expressed in ductal cells and migrating endocrine cells. It was also observed that this expression decreased as the endocrine cells formed larger, more organised islet structures8. Further work demonstrated adult pancreatic cell preparations expressed the αv integrin subunit as well as both the β1 and β5 but not the β3 subunit5. This study suggested that αvβ1 was required for beta cell migration in the fetal pancreas and that the αvβ5 heterodimer had a significant role in adhesion to vitronectin5—an ECM component that has been previously identified in the developing and adult pancreas5.

In the present study, we show that the integrin heterodimer αvβ5 is specifically formed in beta cells of the adult human pancreas, in both control and type 1 diabetic subjects. Interestingly, analysis of pancreatic sections

1Murdoch Children’s Research Institute, The Royal Children’s Hospital, Flemington Road, Parkville, VIC 3052, Australia. 2Department of Paediatrics, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, VIC 3052, Australia. 3St. Vincent’s Institute of Medical Research, Fitzroy, VIC 3065, Australia. 4Department of Medicine, St. Vincent’s Hospital, University of Melbourne, Fitzroy, VIC 3065, Australia. 5Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC 3800, Australia. 6These authors contributed equally: Andrew G. Elefanty and Edouard G. Stanley. *email: ed.stanley@mcri.edu.au
representing individuals with type 1 diabetes showed persistence of heterodimer expression beyond that of insulin, potentially enabling identification of beta cell remnants.

**Methods**

**Ethical approval and tissue donor details.** Use of tissue donor material was approved by the St Vincent’s Hospital Human Research Ethics Committee (approval no. SVH HREC-A 011/04). All experiments were performed in accordance with relevant guidelines and regulations. Details of individual donors are provided in Tables 1 and 2.

**Islet isolation.** Healthy human pancreata were obtained with informed consent from next of kin, from heart-beating, brain-dead donors, with research approval from the Human Research Ethics Committee at St Vincent’s Hospital, Melbourne. Human islets were purified by intraductal perfusion and digestion of the pancreases with collagenase AF-1 (SERVA/Nordmark, Germany) followed by purification using Ficoll density gradients. Purified islets were cultured in Miami Media 1A (Mediatech/Corning 98-021, USA) supplemented with 2.5% human serum albumin (Australian Red Cross, Melbourne, VIC, Australia), in a 37 °C, 5% CO2 incubator.

**Immunofluorescence staining.** Paraffin sections of donor human pancreas were obtained from the Tom Mandel Islet Isolation Program (St Vincent’s Hospital, Victoria). Paraffin was removed using xylene, samples were rehydrated, and antigen retrieved using 10 mM citrate buffer. Samples were blocked for 1 h at room temperature in staining buffer (10% foetal calf serum (FCS) (Sigma-Aldrich; 12003C) in PBS) and 0.1% Triton-X (Sigma-Aldrich; T9284), stained overnight with primary antibodies at 4 °C, stained for 1 h at room temperature with secondary antibodies, and stained with DAPI (Sigma-Aldrich; D9542) for 5 min. Antibody details are provided in Table 3. Samples were mounted using Fluoromount-G (Southern Biotech; 0100-01) and imaged using a LSM780 inverted confocal microscope (Zeiss). Image analysis was performed using ImageJ (version 1.0).

**Flow cytometry and sorting.** Isolated human islets obtained from the Tom Mandel Islet Isolation Program were digested by resuspension in Accutase (Sigma-Aldrich; A6964) solution for 15 min at 37 °C. Following trituration, cells were washed in PBS and stained with primary antibody in FACS buffer (2% FCS in PBS) for 30 min on ice. Cells were then washed twice with FACS buffer and stained with the appropriate secondary antibody for 30 min on ice. Antibody details are provided in Table 3. Cells were then washed twice with FACS buffer and then resuspended in 1 μg/ml propidium iodide (Sigma-Aldrich; P4864) to exclude dead cells, prior to cell sorting. Flow sorting was performed on an BD Influx (BD Biosciences) or BD FACSAria Fusion (BD Biosciences). Isolated cells were resuspended in RLY buffer (Bioline) as a prelude to preparation of RNA.

**Bulk RNAseq analysis.** RNA extraction of FACS purified populations was performed using an Isolate II RNA microkit as directed by the manufacturer (Bioline, BIO-65042). RNA samples were processed, quality control performed, and sequenced by the Victorian Clinical Genetics Service, Melbourne (VCGS). Sequencing

| Table 1. Information for donor material used in immunofluorescence studies. |
|---|
| Control-1 | Control-2 | Control-3 | T1D-1 | T1D-2 | T1D-3 |
| Unique Identifier | SVI-007-19 | SVI-009-19 | SVI-025-18 | SVI-012-19 | SVI-004-18 | SVI-007-19 |
| Donor age (years) | 37 | 52 | 25 | 59 | 46 | 44 |
| Donor sex (M/F) | M | M | F | F | M | F |
| Donor BMI | 27.4 | 26.5 | 41.4 | 28.7 | 29.0 | 24.0 |
| Cause of death | Hypoxic brain injury | Spontaneous intracranial haemorrhage | Cerebral hypoxia/ischemia | Cerebral hypoxia/ischemia | Hypoxic brain injury | Cerebral infarction |
| Donor history of diabetes | No | No | No | Yes, T1D | Yes, T1D | Yes, T1D |
| Donor HbA1c | 9 | 7.5 | 11.4 |
| Diabetes duration (years) | 13 | 24 | 11 |

| Table 2. Information for donor material used in flow sorting studies. |
|---|
| Sort-1 | Sort-2 |
| Unique Identifier | SVI-004-20 | SVI-025*19 |
| Donor age (years) | 44 | 48 |
| Donor sex (M/F) | F | M |
| Donor BMI | 30.4 | 25.4 |
| Cause of death | Traumatic brain injury | Suicide/hanging |
| Donor history of diabetes | No | No |
publicly available RNAseq data showed that of the potential heterodimerisation partners, islets expressed beta
strated that the integrin subunit αv was expressed in the endocrine cells of the pancreas. Subsequent analysis of
associated with pancreatic endocrine development and functionality of adult beta cells. This analysis demon-
cytometry. We paid particular attention to members of the integrin family, some of which have been previously
identified as potential cell surface markers that could be used to quantify islet composition using live cell techniques, such as flow
the identification of clusters corresponding to endocrine (alpha, beta, delta, gamma), exocrine (acinar, ductal) and
clusters based on the expression of a key set of signature genes (Supplementary Fig. 3a,b). This process enabled
projection (UMAP) analysis placed cells within distinct clusters (Fig. 1a). We assigned nominal identities to these
samples was performed using an NovaSeq 6000 (Illumina) instrument. Between 20 and 30 million 150 bp
paired-end reads were obtained per sample. Individual fastq files were aligned to the reference genome (GrCh38
assembly) with the Spliced Transcript Alignment to a Reference (STAR) software (version 2.7.3) using default	parameters. Nonuniquely mapping reads and read pairs with unpaired alignments were excluded. Read counts
for each gene were determined using featureCounts as part of the Rsubread library. RNAseq analysis
was performed on the raw count table using the limma and edgeR packages within R. Briefly, the counts
per million (CPM) value was calculated using the cpm() function in edgeR, and genes expressed at low levels
(defined as a CPM value below 0.5 in any sample) were filtered out. The filtered matrix was then used to create a
differential expression list object in edgeR, and the object TMM normalized using the calcNormFactors() function. Highly variable
genes were identified by estimating the variance of each gene across samples, then sorting genes according
to variance value. Unsupervised hierarchical clustering of samples was then performed using the top 500 variable
genes. Heatmaps were created for the top 500 variable genes using the heatmap2() function in the gplots
package (Supplementary Figs. 1, 2).

Single cell RNAseq analysis. Processed RNA sequencing data was downloaded from GEO (GSE114412).
The count matrix was filtered to remove mitochondrial-encoded genes, genes with less than 1000 UMIFM
counts and cells with greater than 25% mitochondrial-DNA content. Variation in the total counts of individual
cells was then removed by normalizing the sum of counts for each individual cell to 10,000. The normalized
counts were then used for dimensionality reduction and clustering for each dataset, which was performed using the Seurat package within R. Briefly, highly variable genes were identified using the FindVariableFeatures() function. Principle components were then computed and clustering was performed using Louvain
community detection in the space of the first 30 principle components. UMAP projections were then computed
using the first 30 principle components. Differentially expressed genes within clusters were then computed using the FindAllMarkers() function within Seurat and cluster identity assigned using the genes identified in Supplementary Fig. 1.

Results
In order to search for cell surface markers associated with specific cell types within human islets, we interrogated a previously published single cell transcriptional profiling (scRNAseq) data set, representing islets isolated from 4 independent non-diabetic donors. Visualisation of this data set using uniform manifold approximation and projection (UMAP) analysis placed cells within distinct clusters (Fig. 1a). We assigned nominal identities to these clusters based on the expression of a key set of signature genes (Supplementary Fig. 3a,b). This process enabled the identification of clusters corresponding to endocrine (alpha, beta, delta, gamma), exocrine (acinar, ductal) and immune (macrophage, mast cell, T-Cell, B-Cell) cells, as well as other cell types (endothelial, stellate, pericyte).

We examined the gene expression profile associated with each cell cluster with a view to identifying potential cell surface markers that could be used to quantify islet composition using live cell techniques, such as flow cytometry. We paid particular attention to members of the integrin family, some of which have been previously associated with pancreatic endocrine development and functionality of adult beta cells. This analysis demonstrated that the integrin subunit αv was expressed in the endocrine cells of the pancreas. Subsequent analysis of publicly available RNAseq data showed that of the potential heterodimerisation partners, islets expressed beta

| Antibody | Source | Catalogue number | Dilution | RRID |
|----------|--------|------------------|----------|-------|
| Guinea-pig anti-Insulin | Dako | A0564 | 1:1000 | AB_10013624 |
| Rabbit anti-integrin alpha v | Abcam | ab179475 | 1:100 | AB_2716738 |
| Mouse anti-integrin alpha v + beta 5 | Abcam | ab177004 | 1:100 | AB_448231 |
| Mouse anti-glucagon | Sigma-Aldrich | G2654 | 1:500 | AB_259852 |
| Rabbit anti-integrin alpha v + beta 1 | Biozzi | orbi5155 | 1:100 | AB_10748950 |
| Sheep anti-hIntegrin b5 -biotin | R&D systems | BAf3824 | 1:50 | AB_2129279 |
| Mouse Biotin anti-human CD51 (ITGAV) | Biolegend | 327906 | 1:100 | AB_940568 |
| Rat anti-integrin alpha v + beta 6 | Abcam | ab97588 | 1:100 | AB_10715984 |
| Goat anti-guinea pig IgG (H + L), Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | ThermoFisher | A-11073 | 1:1000 | AB_2534117 |
| Goat anti-mouse IgG (H + L), Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 | ThermoFisher | A-21235 | 1:1000 | AB_2535804 |
| Goat anti-rabbit IgG (H + L), Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 | ThermoFisher | A-11011 | 1:1000 | AB_143157 |
| Goat anti-rat IgG (H + L), Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 | ThermoFisher | A-21247 | 1:1000 | AB_141778 |
| Goat anti-mouse IgG (H + L), Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 | ThermoFisher | A-11032 | 1:1000 | AB_2534091 |
| Alexa Fluor 647 Streptavidin | Biolegend | 405237 | 1:1000 | |
chains 1, 3, 5 and 6 (Supplementary Fig. 3c,d). As expression of β1 has already been examined in the adult islet, we focussed on the next most highly expressed beta subunit, β5. In order to examine how the expression pattern of αv and β5 proteins related to the pattern suggested by RNAseq analysis, we used FACS to isolate specific islet subpopulations. Flow cytometry analysis showed that the vast majority of islet cells expressed some level of both the αv and β5 subunits. Islets also contained a smaller population that exclusively expressed the αv subunit (Fig. 1b). Using our analysis of single cell sequencing data as a guide, we purified populations expressing both subunits from isolated human islets and subjected these to bulk
**Figure 2.** Expression of the integrin heterodimer αvβ5 and its component subunits in human pancreatic islets. (a, b) Immunofluorescence analysis of integrin subunits αv (a) and of β5 (b) expression in pancreatic sections representing control and T1D tissue donors co-stained with antibodies recognising INSULIN (green) and GLUCAGON (red). (c) Immunofluorescence analysis of αvβ5 heterodimer formation (grey) in pancreatic sections representing control and T1D tissue donors, co-stained with antibodies recognising INSULIN (green) and GLUCAGON (red). Scale bars for all images are 25 μm.
Mapping the 20 most highly expressed genes in each isolated cell fraction to the gene expression profiles associated with specific cell clusters identified by scRNAseq analysis enabled us to estimate the cellular composition of the isolated cell populations (Fig. 1c–e). These associations can be visualised by means of a heat map (Fig. 1c and Supplementary Fig. 4). This analysis suggested that the αvβ5hi population was enriched for endocrine cell types including alpha, delta and gamma cells (Fig. 1c–e) whilst the αvβ5lo population was enriched for pericytes and stellate cells (Supplementary Fig. 4b). Interestingly, the αvβ5neg reference population appeared to be enriched for myeloid cells, most likely mast cells (Supplementary Fig. 4a).

To complement our scRNAseq and flow cytometry analyses, we performed immunofluorescence staining on human pancreas from both control and T1D donors (Fig. 2a). Similarly, immunofluorescence analysis showed that β5 was expressed in endocrine cells (Fig. 2b), in addition to being present within endothelial and pericyte populations (Supplementary Fig. 6). Immunofluorescence labelling also enabled us to examine the distribution of αvβ5 heterodimer using an antibody that specifically detects this complex. This analysis suggested that expression of the αvβ5 heterodimer was restricted to beta cells (Fig. 2c), as defined by the expression of INSULIN. In order to quantify the co-localisation of the αvβ5 heterodimer with either INSULIN or GLUCAGON in the islets of a control donor, we performed co-localisation analysis using the coloc2 plugin within ImageJ. This enabled us to calculate the Pearson’s correlation coefficient for the proteins in question and to provide a quantitative measure of co-expression. From this analysis, we found that the mean Pearson’s correlation coefficient for INSULIN and αvβ5 heterodimer was 1.00194 (Range 0.9434–1.0646, n = 5) indicating that these two proteins are indeed co-expressed. In contrast, we found that the mean Pearson’s correlation coefficient for GLUCAGON and αvβ5 heterodimer was 0.0001 (Range –0.286 to 0.198, n = 4) affirming that there is no co-expression of these two proteins in the islets of control donors. To enable quantification of the relationship between insulin and αvβ5 expression within islets, we examined four fields of view at 5x magnification from sections representing three control donors and 3 T1D donors that had been stained for insulin, glucagon and αvβ5. We counted the number of islets (defined by clusters containing cells that expressed insulin and glucagon) and scored them for the co-expression of αvβ5. This analysis showed that all islets expressed αvβ5 irrespective of whether they were from a control or T1D donor. Furthermore, we also identified clusters of αvβ5 expressing cells and scored these for the presence of insulin. As expected, for control donors, this analysis showed that there were no examples of αvβ5 expressing cell clusters that did not also express insulin. Conversely, in a subset of sections derived from T1D donors, we found rare instances of αvβ5 cell clusters that lacked insulin expression (Supplementary Fig. 7). In these cases, clusters of insulin negative αvβ5 positive cells also expressed glucagon, consistent with the possibility of trans-differentiation of beta cells within the islets of individuals with type 1 diabetes20 (Donor T1D-2, Fig. 2c, see Supplementary Fig. 3c for additional donor samples). We also examined expression of other αv heterodimers—αvβ1 and αvβ6. However, using validated commercially available antibodies, we were unable to detect specific expression of either heterodimer within the pancreas for either control or type 1 diabetic donors (Supplementary Fig. 8).

Discussion

During a search for novel beta cell surface markers, we identified integrin subunits, αv and β5, whose expression overlapped in beta cell enriched islet sub-fractions. Using immunofluorescence analysis, we found that αvβ5 heterodimers were specifically formed in beta cells. Surprisingly, specificity in heterodimer formation was not simply a function of the overlap in expression of its component parts, with both the αv and β5 subunits showing much broader expression across all endocrine cells within the islet. This observation raises the possibility of cell intrinsic factors capable of regulating heterodimerisation.

Although antibodies directed against the integrin subunits αv and β5 could be used to enrich for endocrine cells from disaggregated islets, commercially available antibodies recognising the αvβ5 heterodimer were unsuitable for isolation of live cells using FACS. It is unclear if this limitation of the antibody was due to the position of epitope which it recognised (intra- versus extra-cellular) or a consequence of epitope destruction caused by enzymatic disaggregation. Nevertheless, our observation that heterodimer formation is restricted to beta cells suggests that this cell type has a unique relationship with its extracellular environment, potentially impacting its function and biology.

Data availability

Bulk RNA-seq data used in this study has been deposited in the Gene Expression Omnibus (GEO) data base and are available under the accession number GSE167589. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

J.V.S. and E.G.S. contributed to the acquisition and analysis of data for this work. J.V.S., T.L., H.E.T., A.G.E. and E.G.S. contributed to the conception and design of the experiments or to the analysis or interpretation of the data for this work. J.V.S. and E.G.S. wrote the manuscript and all authors made important contributions to editing and revision of the manuscript. All authors have approved the final version of the manuscript. E.G.S. and J.V.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to E.G.S.

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