[Frontiers in Bioscience S1, 477-491, June 1, 2009]

Integrins and extracellular matrices in pancreatic tissue engineering

Mansa Krishnamurthy[1,2], Rennian Wang[1,3,4]

[1]Childrens' Health Research Institute, University of Western Ontario, London, ON, Canada, [2]Department of Pathology, University of Western Ontario, London, ON, Canada, [3]Physiology and Pharmacology, University of Western Ontario, London, ON, Canada, [4]Medicine, University of Western Ontario, London, ON, Canada

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1. ABSTRACT

The role of integrin receptors in regulating numerous cellular programs have been well studied in the endocrine pancreas. These adhesion receptors and their interactions with the extracellular matrix (ECM) are important determinants of islet cell biology as they influence the development, survival and function of the islets of Langerhans. In this review, we will discuss the profound role of integrin-ECM relationships in controlling pancreatic tissue morphogenesis and the anti-apoptotic properties that these receptors confer through their dynamic and unique signaling pathways. Relationships between the ECM-integrin receptors will also be discussed in light of islet-based therapies including transplantation procedures and pancreatic tissue engineering initiatives.

2. INTRODUCTION

The cohesive nature of the islets of Langerhans, a highly specialized aggregate of cells, suggests that cell-cell and cell-matrix interactions are critical for survival, development and function of the endocrine pancreas. Although numerous receptors and cellular molecules have been shown to facilitate islet survival and function, of particular importance are the integrins. A superfamily of cell adhesion receptors which bind to the extracellular matrix (ECM), cell-surface and soluble ligands, integrins regulate a plethora of cellular programs including proliferation, migration, differentiation, survival and function (1-8).

In the field of islet biology, a large body of evidence supports the notion that interactions between the

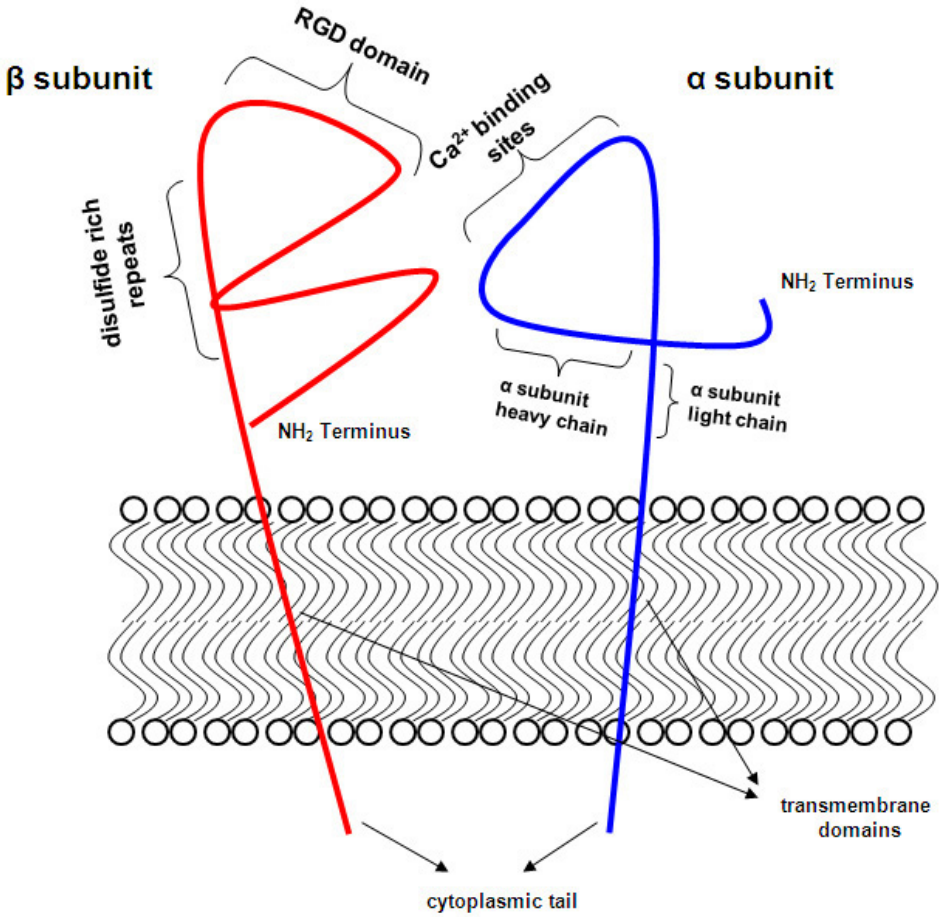

Figure 1. A schematic diagram illustrating motifs of the α and β integrin subunits, which are critical for their function. Adapted from references 111-114.

ECM and integrins provide a physical substratum for the spatial organization of cells and their downstream signaling pathways play critical roles in islet hormone regulation. Particular importance has been placed on the β1 subfamily of integrins, as they orchestrate the majority of islet cellular changes, and heavily influence alterations in hormonal gene expression (9,10). While possessing diverse ligand binding capabilities, the β1 integrin and its associated α subunits have repeatedly been shown to maintain islet architecture, by preventing anoikis and conferring a degree of stability (9,10,11).

The ability of integrins to coordinate events of cellular morphogenesis and regulate tissue homeostasis makes them ideal receptors to study and manipulate in islet biology, for greater remedial purposes such as islet transplantation and pancreatic tissue engineering. In this review, we focus on relationships between integrins and their respective ECM, which regulate processes of pancreatic morphogenesis, support islet viability, survival and function. Furthermore, we contend that the manipulation of these interactions may be beneficial in designing islet-based therapies including islet transplantation and the bioartificial endocrine pancreas, for the treatment of diabetes.

3. INTEGRINS AND EXTRACELLULAR MATRIX

3.1. Integrin family of receptors

Integrin transmembrane receptors are heterodimeric, composed of distinct α and β subunits. To date, 18α and 8β subunits have been identified, generating 24 distinct integrin receptors (1-4). The distinct structure of these heterodimers allows them to function as integrators of exterior and interior environments of a cell as they possess extracellular domains which bind to external ligands and cytoplasmic domains which facilitate interaction with the actin cytoskeleton and other affiliated proteins (1) (Figure 1). As a result, integrins exhibit unique and dynamic signaling capabilities, which allow them to coordinate and integrate extracellular events and intracellular changes (1,3,5,8). These bidirectional mechanisms described as "outside-in" and "inside-out" signaling, include the activation of numerous intracellular molecules which propagate conformational changes from cytoplasmic tails, across the membrane, toward ligand binding regions. This results in increased expression of integrins at the cell membrane as well as changes in receptor affinity and avidity which cause further alterations such as the

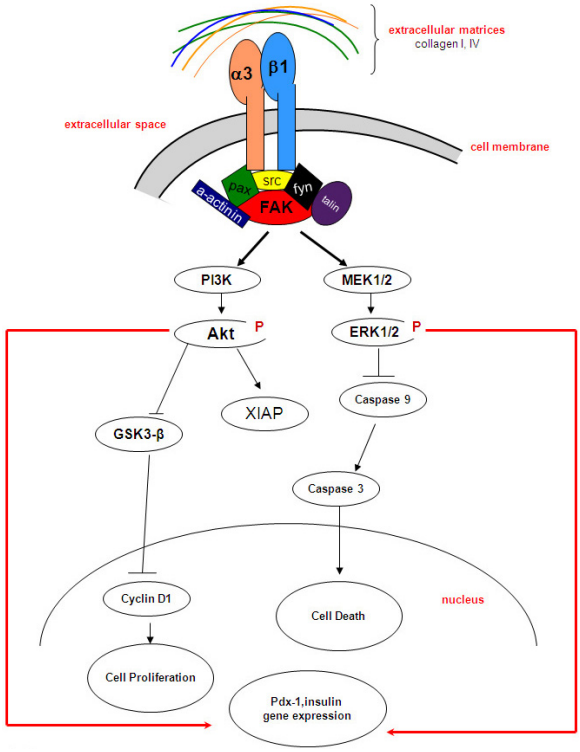

Figure 2. α3β1 Integrin signaling in pancreatic beta cells. Cell adhesion to collagen I or IV leads to the activation of FAK, AKT and ERK1/2 stimulating survival and transcription of beta cell genes. Adapted from references 56,115.

redistribution of integrins and clustering, facilitating ECM binding (3,5).

Integrin receptors also demonstrate a considerable amount of overlap in their ligand binding specificities (1,8). The redundancy in integrin-ECM interactions suggests that specific receptor-matrix relations elicit highly specialized signaling functions and that a certain degree of compensation by other integrins or receptor types still allow for the development of normally functioning tissues. Recent developments of murine loss of function phenotypes in either constitutive or cell type-specific models have been especially important to our understanding of integrin/ECM signal transduction pathways which affect development and maintenance of tissues (6). These models display a wide range of phenotypes, from either early lethality to apparently normal mice, reinforcing the notion that hierarchies must exist among the integrin family of receptors, and that compensation between integrins or other receptor types allow for development of normally functioning tissues.

3.2. Extracellular matrix proteins (ECM)

Ligands of integrin receptors, the ECM, was initially viewed as a scaffold required for arranging cells within connective tissue, and providing physical support to tissues and organs. The ECM has now been redefined as a highly dynamic, compliable and flexible structure, capable of altering cell morphology, gene expression and tissue function (12,13).

ECM proteins are present in every tissue but are mostly concentrated in connective tissue and basement membranes (BM). A heterogenous mixture of water and polysaccharides as well as either collagens, proteoglycans, non-collagenous glycoproteins or elastins, the ECM organizes itself into a three-dimensional tissue-specific meshwork and constantly undergoes processes of remodeling controlled by ECM synthesis and degradation (12,13).

In addition to providing a physical framework for cells within a tissue, the ECM also influences cell function in two general ways. Firstly, ECM behaves as a storage site for multiple components including growth factors, cytokines, chemokines and enzymes and enables their mobilization and subsequently their interaction with specific receptor types on cell surfaces (12-15). Secondly, the ECM interacts with cellular receptors affecting cell adhesion and migration, triggering downstream signaling cascades which, in turn, regulate gene expression required for cell differentiation, proliferation and survival (12-15).

The wide spectrum of ECM proteins and their respective receptors makes way for great diversity for cell-matrix interactions. Moreover, the large variety of ECM composition contributes largely to the properties and function of each tissue and organ. Recent advances in knowledge about cell-ECM interactions have stimulated researchers to manipulate these relationships for organ regenerative purposes as well as tissue engineering.

3.3. Integrins/ECM and signaling transduction

Like the majority of cellular receptors, the activation of an integrin, mediated by adhesion, triggers downstream signaling cascades, by the induction of calcium fluxes, tyrosine and serine/threonine kinases and changes in RhoGTPases, which subsequently, cause changes in cell proliferation, differentiation and survival (5). The focal adhesion complex is a major group of proteins which undergoes activation as a result of cell adhesion through integrin receptors (16). Integrin-ECM interactions at focal contacts cause clustering of integrins and the recruitment of signaling molecules and actin filaments to the cytoplasmic domain of the receptor (5,8). These plaques are essential for integrins to establish connections to numerous signaling molecules including focal adhesion kinase (FAK), c-Src, phosphoinositide3-kinase (PI3-kinase), RhoGAP, paxillin, talin, p130CS, integrin-linked kinase and phosphorylated Caveolin-1 (17). It is now well known that integrin-mediated signals confer anti-apoptotic properties, via PI3-kinase and Akt signaling and stimulate cell cycle progression through extracellular signal-regulated kinase (ERK) and cyclinD1 activation (Figure 2) (18).

In addition to traditional signaling mechanisms, integrins can be also activated in the presence of soluble growth factors or ligands for other receptors, thereby influencing downstream cascades and mediating changes in

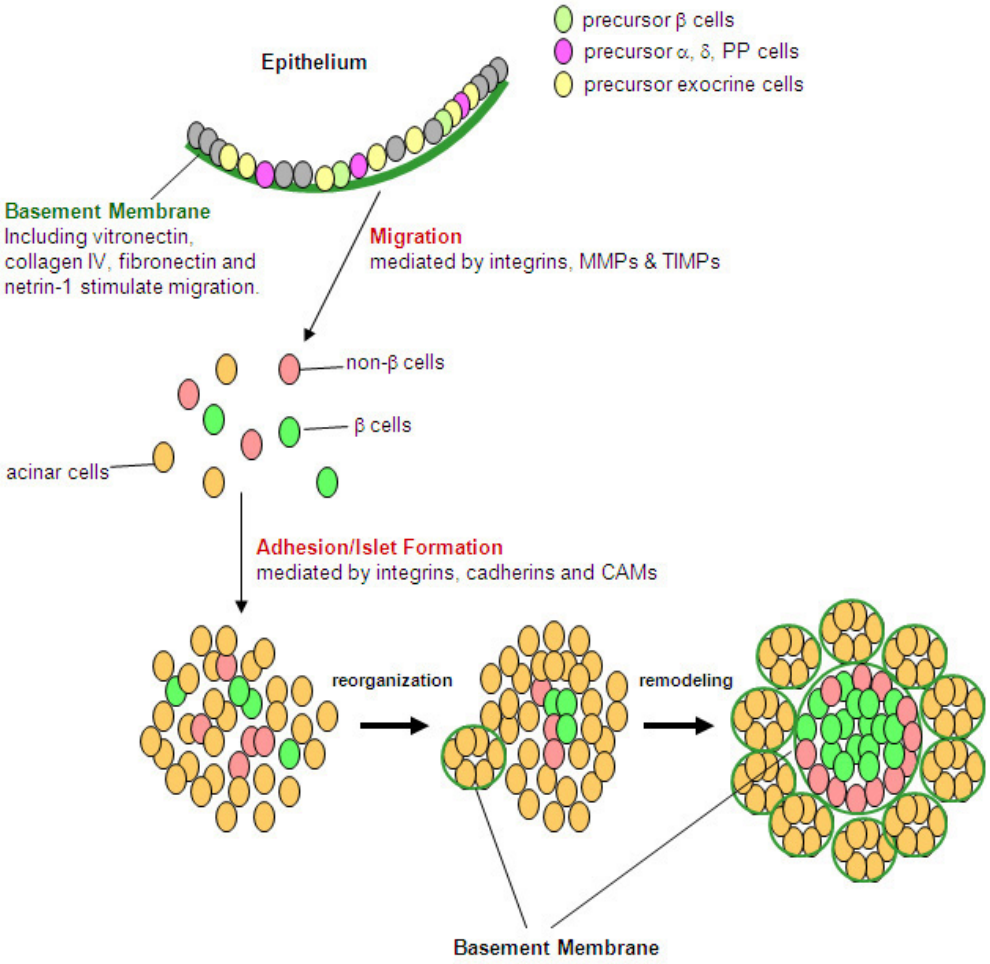

Figure 3. A schematic illustrating adhesive and migratory cycles leading to islet formation. Pancreatic precursors residing in the epithelium attach to and migrate through the basement membrane, leading to their differentiation into endocrine or exocrine cells. Specialized ECM and integrin interactions allow for adhesion and migration of endocrine cells to form the islets of Langerhans.

cell survival and function. Since development of several tissues can proceed normally without the presence of widely expressed integrins, it is highly likely that these receptors participate in a much larger network of signaling processes. In fact, studies have demonstrated that cellular responses to soluble growth factors including EGF, PDGF, LPA and thrombin require the adhesion of cells on substrates via integrins (18-21). Furthermore, it is well known that the activation of parallel pathways by integrins and growth factors, synergize at the level of phosphorylation of downstream signaling proteins (5,19). The ECM, therefore, can be thought of as a niche which simultaneously provides a physical substratum and houses numerous growth factors, cytokines and mitogenic signals, allowing for integrins to influence numerous cellular behaviours including survival and function.

4. INTEGRINS AND ECM IN ISLET FORMATION

ECM proteins and cell adhesion receptors have been well known to contribute to the organogenesis, morphogenesis and cytodifferentiation of several organ systems including the pancreas (22). Recent findings have demonstrated that integrin adhesion systems are implicated in numerous developmental stages and regulate diverse processes including cell positioning, tissue patterning, compartmentalization and cell polarity (22-26).

In the pancreas, many integrin-ECM interactions have been characterized in rodent and human systems as summarized in Tables 1 and 2. The migration of islet precursors from the ductal epithelium into the surrounding mesenchyme is governed by the activities of matrix metalloproteinases (MMPs) (Figure 3) (27). In fact, interactions between MMP2 and MMP9 and their inhibitors, tissue inhibitors of MPs (TIMPs), are largely responsible for processes of aggregation. Transforming growth factor-β (TGF-β) signaling also stimulates islet morphogenesis by enhancing MMP2 expression and activity, further supporting the role of MMPs in islet cell morphology and development (27,28). Epidermal growth factor receptor (EGFR) signaling acts as a positive regulator of MMP activity; EGFR mutant mice display a decrease in pancreas size due to reduced epithelial

branching and islet migration (29). Thus, matrix degradation is critical for remodeling, epithelial morphogenesis and migration of endocrine precursors.

Studies have identified the BM, rich in laminin, as a critical mediator of ductal and tubular morphogenesis and differentiation, giving rise to either endocrine or exocrine precursors (Figure 3). The epithelial-mesenchymal interface, established by the BM, is rich in biologically active components including collagens, heparin and chondroitin sulfate, laminin and fibronectin (30-36). Specifically, the role of laminin in the regulation of ductal morphogenesis from undifferentiated pancreatic epithelium has been well investigated (30,37-39). Organization and concentration of laminin-1 in the BM is critical for its development and subsequent influence over epithelial cell differentiation into either the endocrine or exocrine pancreas. Direct contact of pancreatic epithelium with laminin-1 allows for the formation of the exocrine pancreas, whereas those cells which are not involved in this interaction organize into endocrine cell clusters and eventually form islets by the end of gestation (30). Highly specialized experimental models in the rodent, where early pancreatic epithelium is grown under laminin-poor renal capsules, resulted in the default selection of the endocrine lineage, demonstrating that the absence of laminin is necessary for endocrine cell formation (30). *In vitro* experiments have further solidified the notion that interactions between laminin-1 and integrin receptors allow for epithelial cell differentiation.

The ECM also confers migratory properties essential for islet formation. Endocrine precursors located in pancreatic ducts, migrate through the basement membrane and cluster with the aid of integrin receptors during early stages of islet assembly (Figure 3) (40,41). Studies by Cirulli and colleagues have demonstrated that integrins αvβ3 and αvβ5 contribute to the migration of putative endocrine progenitor cells (42). The treatment of human fetal islet-like clusters with anti-αvβ3 and anti-αvβ5 antibodies resulted in the detachment of cells and three-dimensional clustering. Furthermore, when human fetal pancreatic fragments were transplanted into the kidney capsule of NOD/SCID mice along with RGD blocking peptides, a significant disruption in islet architecture and size was observed (42).

Another migratory cue which regulates islet cell development is netrin (43). A well known contributor of axon guidance, netrin is expressed in discrete pancreatic populations within fetal and adult ductal epithelia and exocrine tissue. Although it is not found in adult islets, it is localized to the basal surfaces of pancreatic cells. Integrins α6β4 and α3β1 (43) mediate the adhesion and migration of fetal pancreatic epithelial cells to netrin-1. The majority of cells which migrate on netrin-1 are Pdx-1$^+$, suggesting that netrin-1 supports the migration of pancreatic progenitors (43).

Once islets form, islet-ECM and cell-cell interactions become essential for proper coordination of events including development and maturation. Vitronectin and its association with several integrin receptors have been shown to contribute significantly to human fetal islet development. Its association with epithelial and insulin$^+$ cells combined with its restricted expression near progenitor populations, suggests that its synthesis and deposition is involved in the regulation of morphogenetic events needed for islet neogenesis (42). Moreover, *in vitro* experiments have identified relationships between vitronectin and integrins αvβ1 and αvβ5 as important mediators of human beta cell adhesion, spreading and motility (42). In particular, αvβ1 was responsible for migration of human fetal beta cells. The developmental loss of this integrin in adult beta cells resulted in the lack of migration, suggesting that this receptor is critical for motile processes required for fetal islet assembly. Given that expression of the αvβ1 integrin is lost in adult beta cells, it is most likely that this receptor aids in static cell-matrix interactions (42).

Numerous other studies have also demonstrated the importance of the β1 integrin in processes of remodeling and maturation (9,10,44). The spatial and temporal expression of the β1 integrin and its associated α subunits has been studied in the fetal and pre- and post-natal rat pancreas, indicating the importance of the β1 integrin family during development and suggesting that these heterodimers contribute substantially to islet restructuring (9). The β1 integrin was expressed in ductal populations as well as glucagon$^+$ and insulin$^+$ cell types in the rat pancreas (9). While expression levels of integrin α3β1 and α6β1 significantly increase early in development, α5β1 remains constant. The expression of these integrins decrease as rat pancreatic development progresses, possibly since the majority of pancreatic construction occurs by e18 with remodeling occurring until the first three weeks of postnatal life.

Similarly, in the human fetal pancreas, the importance of α3β1, α5β1 and α6β1 has been demonstrated, as these integrins exhibit specific expression patterns during progressive pancreatic development (10). Although the expression of all three integrin heterodimers was noted before the budding of glucagon$^+$ and insulin$^+$ cells from pancreatic ducts, α3β1 and α6β1 demonstrated significant increases in expression by 16-20 weeks of development. These studies suggest that the formation and maturation of the islets of Langerhans requires increasing α3β1, α5β1 and α6β1 expression to contribute adhesive and migratory properties (10).

Collagen IV and its interactions with integrin α1β1 has also been shown to support the adhesion and migration of human fetal and adult beta cells (45). The migratory response of fetal beta cells was much higher in comparison with adult beta cells, suggesting that this critical basement membrane protein provides the necessary motile processes required for islet neogenesis. The strong adhesion rate and maturational loss of migration, proposes that collagen IV is essential for maintenance of architecture and integrity in adult islets.

Co-localization studies of these integrins with specific ECM proteins have further solidified the

importance of integrin-ECM relationships in the development of the islets of Langerhans. Association of collagen IV, laminin and fibronectin with the β1 integrin family further reinforces that the multiplicity of this receptor is critical for islet development and biology (9,10).

Lastly, cell adhesion molecules (CAMs) and cadherins have also been shown to contribute to proper islet formation and maintenance of architecture (46,47). Cadherins E and N are expressed in areas of cell contact between beta cells and other cell types (46,47), while R-cadherin is prominently expressed only on beta cells (46,47). Neural cell adhesion molecule (N-CAM) is expressed on both beta cells and other cell types in the pancreas and is required for proper islet cell aggregation and organization into typical islet structures, with beta cells concentrated in the center and alpha cells remaining in the periphery (47,48).

5. INTEGRINS AND ECM IN ISLET SURVIVAL AND FUNCTION

Several studies have implicated integrin-matrix relationships as critical determinants of islet survival, proliferation, differentiation and function. Given that the ECM confers stability and support to islets, destruction of matrix proteins would undoubtedly increase cell necrosis and apoptosis. In fact, freshly isolated canine islets were shown to undergo apoptosis, due to loss of the perinsular-basement membrane (49). However, when cultured in or on matrix proteins such as collagen I and fibronectin, these islets exhibited improved survival. These islets were also shown to undergo a transdifferentiation process, characterized by a switch from islet to ductal phenotype indicating a loss in stability, as a result of perturbed islet-matrix relationships (50). Moreover, the aggregatory nature of pancreatic islets also contributes to the survival of individual islet cells. Cell sorting experiments, whereby human islets are dispersed into single cells resulted in reduced survival and increased apoptosis (51). However, matching ECM proteins to specific integrin receptors expressed on islet cells improved cell survival.

Perturbation studies using immunoneutralizing antibodies or siRNA have been valuable for the identification of key integrin receptors which mediate adhesion, spreading and proliferation when interacting with specific matrix proteins. Again, recent evidence has highlighted the importance of the β1 integrin family and its associated α subunits in the regulation of numerous islet cell activities. Both primary and transformed rat islet cells were shown to form aggregates resembling native islets when cultured on collagen I, *in vitro* (52). Interactions between matrices and the β1 integrin were further solidified when a decrease in adhesion and spreading were observed upon treatment of rat islet cells with anti-β1 integrin, and subsequently cultured on bovine corneal endothelial cell extracellular matrix (BCEM) (53). Isolated rat islets and human fetal islet epithelial clusters demonstrated similar decreases in adhesion and spreading when cultured on fibronectin, laminin and collagen I after treatment with an immunoneutralizing antibody for β1 (9,10). Blocking the β1 integrin resulted in increased apoptosis of beta cells in rat and human fetal primary cells, and reduced Pdx-1, insulin and glucagon gene expression. Taken together, these studies indicate the importance of the β1 integrin in mediating cellular function and conferring anti-apoptotic properties.

Relationships between α6β1 and 804G matrix (secreted by 804G epithelial cells) have also been shown to affect beta cell function *in vitro*. The increased insulin secretory response noted when rat beta cells adhered to this matrix was shown to be mediated through the α6β1 integrin (54). Furthermore, adhesion of cells to this matrix and treatment with insulin secretagogues allowed for increased expression of the integrin itself, suggesting that both "inside-out" and "outside-in" signaling mechanisms are active and critical for islet function. Laminin, the primary constituent of the 804G matrix, is thought to be responsible for the majority of positive effects on islet survival and function (55). Both beta cell spreading and insulin secretion in response to glucose stimulation, on 804G matrix, was significantly inhibited when cells were treated with anti-laminin-5 antibody and complementary results were noted with anti-β1 integrin treatments.

The above studies clearly demonstrate that integrin-matrix interactions are critical for the viability and function of islets, and that in most cases "inside-out" and "outside-in" signaling mechanisms are necessary for facilitating these events. Studies exploring changes in downstream signaling cascades have primarily identified FAK, MAPK and ERK-activation as critical mediators of islet cellular events (11,44,56). A decrease in insulin content and subsequent increase in insulin release as a result of human fetal beta cell adhesion to collagen IV and vitronectin was shown to be dependent on ERK signaling (57). Collagen IV was also shown to induce ERK-dependent insulin secretion in human adult beta cells (57). Moreover, laminin-5 and β1 interactions increased FAK and ERK1/2 phosphorylation (11). Metabolic changes triggering the release of intracellular calcium are also suspected to be involved in the functional changes noted in integrin-matrix interactions.

While the influential role of the β1 integrin in islet survival and function has been well established, information regarding the contributions of its associated α subunits is limited. Several studies have demonstrated high expression levels of integrins α3, α5 and α6 in islet cell populations in both rodent and human systems (9,10,53) – but their regulation of islet survival and function and their downstream signaling cascades has not been extensively studied. Recent evidence has shown that interactions between collagen I/IV and the α3 integrin is important for maintenance of viability along with Pdx-1 and insulin expression in both the INS-1 and primary human fetal islet cells (56). Moreover, perturbing the function of the α3 integrin resulted in decreases in FAK, AKT and ERK1/2 phosphorylation (Figure 2). These findings highlight: (i) that α integrin subunits also modulate islet cell survival and function and (ii) that they are capable of signaling through highly specialized downstream cascades, independent of

the β1 integrin. The majority of integrin-matrix interactions have been shown to potentiate islet survival and function; however, it is important to note that a loss in function can be caused by adhesion to certain ECM substrates. Human fetal and adult beta cells demonstrated significant decreases in insulin gene expression when cultured on collagen IV and vitronectin (57). Similarly, blockade of the β1 integrin in isolated rat islets, reduced cellular apoptosis and increased Akt phosphorylation (58).

6. ISLET TRANSPLANTATION

Diabetes mellitus, a metabolic disorder of either absolute or relative insulin deficiency, affects 194 million people today (59). Although exogenous insulin therapy is currently the most common form of treatment, normal physiological glycemic control can only be achieved by beta cell replacement. There are several advantages to islet transplantation, including a simple administration route which is minimally invasive and the possibility of repeating transplantation procedures without major patient discomfort (60). The Edmonton protocol, was initially successful, allowing for 80% insulin independence for one year (59,61). However, long-term function of islet grafts failed with less than 10% of patients maintaining insulin independence for 5 years (60). The possible reasons for poor long-term graft survival may include excessive cell death due to isolation, inadequate immunosuppression and beta cell toxicity resulting in the loss of long-term viability and islet mass (62) as well as limited islet supply, which also contribute to the current limitations of islet transplantation. Recent research has shown that certain immunosuppressive treatments greatly affect the long-term survival of islet grafts by inhibiting the natural process of neogenesis and negatively impacting beta cell survival, proliferation and differentiation (63). Although there are several issues which still need to be addressed through clinical and basic science research, there has been immense progress in the field of islet transplantation.

6.1. Integrin-ECM interactions protect β-cell function following transplantation

The *in vitro* generation and expansion of islets with maintenance of viability and function is a possible alternative that can circumvent donor shortage limitations (64,65). Several expansion programs worldwide are currently investigating mechanisms of beta cell growth, by focusing on either beta cell neogenesis or replication (66-76). In this regard, the culture of islets on substrates which mimic natural microenvironments seems to be a logical prospect. Islet-ECM interactions have been shown to inhibit apoptosis and induce prolonged survival of beta cells after isolation (44,49). These interactions also allow for extensive manipulation for successful *in vitro* survival and proliferation.

A study by Bonner-Weir *et al.* (69) examined the proliferation and differentiation of human ductal tissue into islet cells on Matrigel, as well as their function *in vitro*. Findings of this study highlight two key areas of research: (i) matrix proteins have the ability to support and stimulate three dimensional organization of pancreatic precursors into islet cells, which in turn, facilitates survival and function and (ii) that the shortage in donor material can be alleviated by inducing ductal precursors to differentiate into an islet population through three dimensional assays. Long-term culture studies of human islets embedded in type I collagen maintained their architecture and high secretory capacities (77). Moreover, culturing isolated rat islets on a collagen I, II, IV and laminin hydrogel resulted in significant decreases in cell death, but no changes in insulin, glucagon and somatostatin expression were observed (78). A study by Montessano *et al.* (52) demonstrated the ability of dispersed neonatal rat islet cells to reorganize into islet-like organoids within a three dimensional collagen matrix (52). Islet cells seeded on collagen matrix remained in monolayer, while the addition of a second layer of collagen I resulted in a dramatic reorganization of islets. The typical architecture of islets *in vivo*, was re-established in this three dimensional model, as beta cells remained concentrated in the center of these organoids and alpha and delta cells were located in the periphery.

A relatively novel and interesting area of research is focused on using naturally occurring matrices (Table 2) to culture and induce the expansion of islets. The small intestinal submucosa (SIS), a cell-free matrix extracted from porcine intestines, is rich in collagens, glycoproteins, proteoglycans and glycosamineglycans (79,80). Its use as a scaffold in the remodeling and regeneration of a variety of other tissues suggests that it may be useful in potentiating islet survival and function. The *in vitro* tissue culture of isolated rat islets on SIS resulted in the maintenance of morphology, increased insulin secretion upon glucose challenge and a significant reduction in cell death, suggesting that the SIS confers both protection and support to islets (79-82). Moreover, intrinsic mechanical properties make this matrix highly compliable and histocompatible while its porous nature allows for the diffusion of cellular nutrients.

Recent islet isolation methods have significantly improved over the last three decades; however the current method of collagenase digestion still destroys the basement membrane which results in reduced islet viability and function (81). In order to improve current transplantation methods, an understanding of the peri-insular membrane, islet-exocrine interface and general distribution of matrix proteins in the islet and surrounding periphery is required.

6.2. Islet transplantation: important points of consideration

Expression patterns of the laminin, fibronectin and collagen family of proteins have been thoroughly examined in both human and rodent pancreas. Collagens I, IV and V are abundantly expressed in the peri-insular region of the human pancreas, while collagen V and VI expression is significant around adult islets (83). Moreover, collagen VI demonstrated consistent levels of expression in the islet-exocrine interface throughout the head, body and tail regions of the pancreas (84). Laminin was found to be expressed in acinar basement membrane and the surrounding ductal epithelium but no significant expression

was found surrounding adult murine islets (85,86). Interestingly, fibronectin expression was predominant underneath endothelial cells, in the perivascular regions, the islet periphery and intraglobular regions (42,86). The less studied proteoglycan, Lumican, was found to be localized in alpha cells (87). The corresponding integrin receptors reportedly expressed in islets include α3, α5, αV and β1 (88). In fact, β1, α2 and α6 were found in the parenchyma, whereas α3 was expressed in ductal cells. The laminin receptor α6 demonstrated high levels of expression in exocrine acini, while the expression of fibronectin receptors, α4 and α5, was primarily noted in the ECM surrounding ducts and vessels (88). After islet isolation, the disruption of islet-matrix relationships results in decreased integrin expression which may be due to perturbation of the peri-insular basement membrane. This critical information, provided by immunohistology studies of matrix and integrin expression, should be taken into consideration during islet isolation, and methods of preserving such relationships should be devised.

A recent finding by Nikolova *et al.* (89) demonstrated that β cells rely on adjacent capillary endothelial cells to produce basement membrane ECM proteins. As a result, a depletion of islet capillaries demonstrated lower levels of insulin gene expression and secretory granules (89). Developmental studies also showed that a rich vasculature is necessary for the induction of insulin in embryos, indicating that the ECM is involved in ensuring intact β cell function (90,91). *In vitro* assays conducted with several varieties of laminin demonstrated up to 2 fold increases in insulin gene expression. These results indicate that the existing dynamic between endothelial and hormone producing cells, which reside within the islet, must also be preserved in order to ensure success of islet transplantation procedures.

7. BIOARTIFICAL ENDOCRINE PANCREASS: PROBLEMS, PROMISE AND PROGRESS

The extensive research examining islet-matrix interactions lends itself to future therapies involving the derivation and use of encapsulated islets referred to as the bioartifical endocrine pancreas (BAP). The construct of this potential therapy requires the fulfillment of specific criteria including biocompatibility, adequate diffusional properties and easy retrievability (92-94). Moreover, the device must facilitate the maintenance of functional islets, which are typically embedded in either a biological or synthetic matrix and encapsulated within a semipermeable membrane, permitting the exchange of nutrients, glucose and insulin (94). An ideal therapy, the concept of the BAP makes possible the use of non-human donors, reducing limitations associated with tissue shortage. More importantly, the BAP is designed to by-pass natural immune reactions of the body, so that immunosuppressive therapies are not required.

The implantation of these devices may be into the blood supply or peritoneal cavity and are meant to mimic the behavior and function of a healthy pancreas (92-94). Designing a BAP is a very delicate procedure, as islets must be enveloped with homogenous and semi-permeable artificial membranes, without disruption of tissue morphology, integrity or functional competence. The BAP is an ideal device for the treatment of type I diabetes, but effectively controlling biomaterial and functional aspects has proven difficult in the development of an effective prototype. Nevertheless, several industrial and basic science laboratories are dedicated to developing the BAP into a plausible therapy for patients with insulin-dependent diabetes. There are three different encapsulated systems currently being used: (i) intravascular macrocapsules - perfusions chambers connected to the blood circulation, (ii) extravascular macrocapsules – diffusion chambers that are transplanted intraperitoneally or subcutaneously or (iii) extravascular microcapsules – encapsulation of one or few islets in a membrane (92,93,95). While all three prototypes have been investigated, the extravascular microcapsules have been studied at large.

7.1. Encapsulation: enhancing islet cell survival and function

It has been demonstrated by several research groups that the efficient function of islet cell types in the BAP is dependent on an effective matrix structure, either synthetic or biological. While the majority of studies have made use of synthetic matrix constituents, a few laboratories have reported the benefits of embedding islets in biologically active compounds, thus taking advantage of integrin-ECM interactions. A recent study by Edamura *et al.* (96) compared the effects of embedding isolated porcine islets in either Matrigel or type I collagen. The Matrigel prototype stimulated a greater increase in insulin production and efficiently reduced blood glucose levels when implanted, in comparison with the collagen I capsule, indicating that specific biological compounds are more suitable for the derivation of a BAP prototype (96). Another study was conducted by Salvay *et al.* (97), where streptozotocin-induced diabetic mice were implanted with microporous polymer scaffolds adsorbed with collagen IV, fibronectin, laminin-332 or serum proteins, containing 125 mouse islets. Mice which received the collagen IV-adsorbed graft, achieved euglycemia the fastest and their response to glucose challenge was comparable to normal mice (97). Fibronectin and laminin grafts also promoted euglycemia, but required more time (97). Finally, all three ECM protein grafts displayed normal cell-cell contact and intact islet architecture as well as increased vessel density within the graft when compared to serum-coated scaffolds, indicating that the incorporation of ECM proteins into a BAP prototype enhances long-term islet cell survival and function (97).

Seeding of islet cells into microporous scaffolds has also been shown to enhance the survival of implanted grafts and reverse diabetes in streptozotocin-induced diabetic mice (98). These microporous scaffolds possess a high surface area/volume ratio supporting cell adhesion, and increase nutrient transport by diffusion from neighbouring tissue. These scaffolds also allow for cell infiltration from surrounding tissue, thus enabling the full integration of the graft into the host which is especially important for re-establishment of the vascular network

Table 1. Summary of integrin receptors involved in islet development

Integrin	Role in islet development	References
α1β1	-adhesion/migration of beta cells	45
α3β1	-migration of pancreatic progenitors	43
	-maturation of rat/human islets	9, 10
α5β1	-maturation of rat/human islets	9, 10
α6β1	-pancreatic epithelial differentiation into ductal structures	54
	-maturation of rat/ human islets	9, 10
αvβ1	-migration for islet assembly	45
αvβ3	-migration of endocrine progenitors	42
αvβ5	-migration of endocrine progenitors	42
α6β4	- migration of pancreatic progenitors	43

Table 2. Summary of ECM matrix proteins involved in development, survival and function of islets

Extracellular matrix protein	Role in islet development, survival and function	References
Laminin	-epithelial cell differentiation into endocrine/ exocrine fate	30, 37
	-adhesion/spreading of rat/human islet clusters	9, 10
	-reduced islet cell death	78
Fibronectin	-adhesion/spreading of rat/human islet clusters	9, 10
	-reduced islet cell death	50
Collagen I	-adhesion/spreading of rat/human islet clusters	9, 10
	-reduced islet cell death	50
	-maintenance of islet architecture and function	50
	-reorganization of islets into organoids	53, 77, 78
Collagen II	-reduced islet cell death	78
Collagen IV	-adhesion/migration of human fetal beta cells	45
	-reduced islet cell death	78
Vitronectin	-adhesion/motility of human fetal/adult beta cells	42, 45
Netrin-1	-migration of pancreatic progenitors	43
804G matrix	-adhesion/spreading of beta cells	55
	-protects against apoptosis of beta cells	55
Bovine Corneal Endothelial Matrix	-adhesion/spreading of beta cells	54
Matrigel	-formation of three-dimensional ducts	69
	-budding of pancreatic epithelium into islet-like clusters	69
Small Intestinal Submucosa	-maintenance of morphology	79-81
	-reduced cell death	79-81
	-increased insulin secretion	79-81

within the prototype. Blomeier *et al.* (98) demonstrated richly vascularized islets in microporous scaffolds two weeks following their implantation. These islets highly expressed endocrine cell markers insulin, Pdx-1 and somatostatin (98). Finally, the implanted microporous scaffolds were able to achieve euglycemia in diabetic mice, indicating that this prototype enables the survival of islets and increases function (98).

Exposing isolated islets to a combination of biological and synthetic peptides is also advantageous. The addition of laminin peptides to a poly (ethylene glycol) capsule, preserved viability, reduced apoptosis and enhanced insulin secretion from MIN6 cells. Moreover, specific laminin sequences were able to further enhance the survival and function of these cells, indicating that the inclusion of specific peptides may also be useful when designing a BAP (99).

7.2. Encapsulation: the disadvantages

Although the studies described above demonstrate promising results, the long-term survival and function of islets following encapsulation is limited. The factors which impede the success of encapsulation for islet transplantation include: (i) biocompatibility, (ii) inadequate immunoprotection and (iii) hypoxia.

Inadequate biocompatibility is identified by pericapsular overgrowth on microcapsules that consist of fibroblasts and macrophages. Studies by Safely *et al. (*100) demonstrated inflammatory cell deposition around microencapsulated porcine islet xenografts in NOD mice 15 days following transplantation and that tissue overgrowth on the microcapsules is directly related to islet graft failure (100). Causes of this fibrotic growth include the biomaterials used to fabricate the BAP, as well as imperfect encapsulation. The search for materials with better diffusional properties and minimal cellular overgrowth has led to the consideration of mainly synthetic compounds (92,101,102), however research examining the biocompatibility of natural matrices is currently underway. The surface textures of BAP devices have also been shown to impact fibrotic and immune responses.

Although islets are protected from cellular engulfment in a BAP, they still remain permeable to small molecules of the immune system including NO, oxygen radicals, and inflammatory cytokines including IL-1β, TNF-α and IFN-γ from macrophages and T-cells involved in the inflammatory reaction (92,93,103-105). The use of specialized synthetic materials including polyetheretherketone (PEEK) for membranes, as well as pre-coating procedures with proteins has demonstrated a certain level of protection from cytokines including IL-1, while maintaining adequate insulin secretion upon glucose stimulation (92). Although a few studies show improvement to a certain extent, small immune molecules still destroy islets, leading to poor functioning of grafts.

Lastly, cell death as a result of hypoxia is another disadvantage of encapsulation. The islet isolation process destroys both intra-islet blood vessels and the surrounding supply, but a new vasculature can be established during the first few weeks of transplantation. Unfortunately, the presence of a barrier membrane prevents the formation of blood vessels in and around the islets, preventing revascularization of the isolated tissue (92). Consequently, tissue oxygen and nutrient requirements are not met and the metabolite removal process is greatly hindered, leading to hypoxic cell death of the core tissue (93). To circumvent hypoxia and its consequences, implanting a BAP into areas with high levels of vascular perfusion, has been examined by Knight *et al*. Islets were seeded into vascular chambers near the splenic or epigastric artery or vein, and allowed for normoglycemia by 7 weeks post-implantation (105). Although these results are encouraging, the implantation of a BAP near a vascular bed poses other threats including embolism and thrombogenesis, and thus may not be the best option. Several other approaches have been proposed to overcome oxygen transport limitations including the incorporation of oxygen carriers/transporters such as hemoglobin (106,107), as well as oxygen generator systems which electrolyze water to form oxygen and hydrogen within a BAP prototype (108). Finally, several laboratories have been dedicated to exploring mechanisms of neovascularization into potential implantation sites for the BAP, prior to transplantation of the graft, as a strategy to overcome hypoxia.

8. 3D-SCAFFOLDS

A relatively novel area of research within the encapsulation field is examining the differentiation of embryonic stem cells (ESC) within 3D scaffolds. A recent study by Lees *et al.* (108) examined the differentiation potential of hESCs following their encapsulation and implantation in between liver lobules of SCID mice. Interestingly, specific markers of the endoderm and pancreas were detected including GLP-1, IAPP and insulin (110). Moreover, a significant deposition of matrices laminin and collagens I and IV were observed within the capsule, along with endothelial progenitor cells, indicating the formation of a primitive pancreas-like structure (109). Similarly, encapsulated islet-like cell clusters isolated from fetal pigs underwent differentiation into viable and functional islets which normalized blood glucose levels in diabetic mice (110). These results indicate the potential use of progenitor cells in 3D scaffolds as a plausible prototype for the BAP.

9. SUMMARY AND PERSPECTIVES

Manipulation of ECM-integrin interactions is an attractive strategy for potentiating beta cell survival and function in either islet transplantation or the bio-artificial pancreas. Several research groups have reported that these relationships are beneficial for survival, proliferation and function of islet cells *in vitro*, by enhancing either neogenesis or replication. Unfortunately, the benefits of these relationships have not been shown to translate well into therapeutic approaches such as islet transplantation or the bio-artificial endocrine pancreas. The majority of challenges are associated with long-term survival and function of islets. Further studies are required to fully understand integrin-ECM interactions and apply this knowledge to create clinically-effective methods for diabetes treatment.

10. ACKNOWLEDGEMENTS

This work was supported by grants from the Natural Sciences & Engineering Research Council of Canada. Dr. Wang is supported by a New Investigator Award from Canadian Institutes of Health Research.

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Abbreviations: BAP: bioartifical endocrine pancreas; BM: basement membrane; e18: embryonic day 18; ECM: extracellular matrix; EGF: epidermal growth factor; LPA: lysophosphatidic acid; MIN6 cells: mouse insulinoma 6 cell line; PDGF: platelet derived growth; factor; SIS: small intestinal submucosa

Key Words: Extracellular matrix, Integrins, Development, Function, Bioartificial Pancreas, Review

Send correspondence to: Rennian Wang. Victoria Research Laboratories, Room A5-140, 800 Commissioners Road East, London, Ontario, N6C 2V5, Canada, Tel: 519-685-8500 ext. 55098, Fax: 519-685-8186, E-mail: rwang@uwo.ca

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