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Crosstalk between the developing pancreas and its blood vessels: an evolving dialogue

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Abstract

Growth and development of embryonic organs goes hand in hand with development of the vascular system. Blood vessels have been known for centuries to supply nutrients and oxygen to all cell types in an organism, however, they have more recently been shown to provide specific cues required for the formation and functionality of a number of tissues. Here, we review the role of blood vessels during pancreas formation, from early specification of the initial pancreatic bud, to its growth and maturation. The overarching theme that emerges from the many studies carried out in the past decade is that the vasculature likely plays diverse and changing roles during pancreas organogenesis. Blood vessels are required for endocrine specification at the onset of pancreatic budding, while only a few days later, blood vessels suppress pancreatic branching and exocrine differentiation. In this review, we summarize our understanding to date about the crosstalk between the pancreas and its vasculature, and we provide a perspective on the promises and challenges of the field.

Keywords

blood vessel; endothelium; pancreas; epithelium; beta cell; endocrine

Introduction

Blood vessels are essential conduits of the circulatory system which function to transport nutrients and oxygen to all vertebrate organs, thereby maintaining their function and overall survival of the organism. More than a decade ago, a provocative set of findings suggested that blood vessels were also capable of influencing organ development and cell differentiation [1, 2]. In one study, signals from the newly formed dorsal aorta were shown

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to be required for pancreas development and for expression of insulin by endocrine progenitors [1]. Similarly, signals from endothelial cells (ECs) within the septum transversum were found to drive liver development [2]. Together, these studies introduced the idea that communication existed between early blood vessels and organ primordia, and raised the possibility that vascular ‘promotion’ of organogenesis was a widespread phenomenon.

Although compelling, within the framework of organogenesis studies, the idea was not completely novel, as it followed earlier observations in the developing and adult central nervous system, where neurogenesis was found to be closely associated with blood vessels [3, 4]. In the decade that has since followed, there have been a large number of subsequent studies in multiple systems, from the hematopoietic system to organs such as the pancreas, lung and liver, that have build upon this theme and further identified communication between tissues and their vasculature. These findings have pointed to a wide range of roles for ECs, ranging from apparently nurturing to suppressive roles [5–11].

The present challenge, and the subject of this review, is to take the whole of these studies and extract meaning. Longer term goals include identifying the signaling molecules that elicit responses in specific cell types, elucidating whether and how blood vessel growth, density or character influences tissue progenitors *in vivo*, identifying specific time points during organogenesis when these events occur, and extrapolating whether such signaling pathways can be manipulated therapeutically to improve either the regeneration or the directed differentiation of cells, including pancreatic beta cells.

While there have been many excellent and comprehensive reviews on the molecular underpinnings of pancreas development and beta cell ontogeny for additional details [12–18] (reviewed in Serup; Rieck, Bankaitis and Wright, this issue), here we provide a brief overview of the key developmental events in pancreatic growth and endocrine differentiation, and overlay the growing body of observations regarding how these steps are influenced by blood vessels.

Pancreas function and cell types

The pancreas is a essential glandular organ which regulates metabolic homeostasis via two parallel functions; one ‘exocrine’, governing the secretion of pancreatic juices into the gastrointestinal tract for digestion of food, and one ‘endocrine’, regulating the secretion of hormones, such as insulin and glucagon, into the bloodstream to control blood glucose levels. The bulk of the pancreas is comprised of grape-like clusters of exocrine acinar cells located at the tips of a branching, ramifying network of ducts. Embedded within this abundant exocrine tissue are scattered spheroid groups of endocrine cells, termed the ‘Islets of Langerhans’ by the German pathologist Paul Langerhans (1847–1888) who first identified them in 1869 and likened them to little ‘islands’. A human pancreas contains approximately one million islets that range in size from a few cells to a few hundred cells each.

Islets consist of five principal endocrine lineages, including insulin-producing beta cells, glucagon-producing alpha cells, somatostatin-producing delta cells, pancreatic polypeptide-

producing PP cells and ghrelin-producing epsilon cells. In rodents, beta cells make up the bulk of the islet, with alpha cells and other endocrine cell types forming a mantle around the islet periphery. In humans, this organization is less clear, as alpha cells are found within the islet core. It has been proposed that human islets may consist of many smaller ‘rodent-like’ endocrine aggregates or of folded ‘plates’ of alpha and beta cells lined by blood vessels [19]. Nevertheless, in all vertebrates, endocrine cells of mature islets release their hormone contents into dense networks of tightly associated capillaries, which connect them to the rest of the body.

The destruction of insulin-producing beta cells results in the life threatening disease ‘Type I Diabetes Mellitus’. As a result, there has been great interest in the ontogeny of pancreatic cell lineages in the hopes that regenerative or replacement therapies might be developed [20, 21]. Understanding how to ‘make’ a beta cell has become a holy grail of the field. As a result, the focus of much current work has been to elucidate intrinsic mechanisms that dictate cell fate decisions, such as transcriptional networks and epigenetic modifiers [15, 22]. However additional questions have inevitably arisen on many different fronts: Can beta cells arise from other endocrine cell types, such as alpha cells? Does islet architecture matter to ultimate function? How much power do extrinsic signals hold on beta cell fate or proliferation? And, relevant to this review, do blood vessels influence the specification, differentiation or expansion of endocrine cells?

From the ‘get-go’: pancreatic epithelium and aortic endothelium

The pancreatic endodermal epithelium emerges and grows in close association with blood vessel endothelium. Briefly, in the mouse, specification of the pancreas occurs prior to closure of the primitive gut, around embryonic day 8.5 (or E8.5). At this time, developing blood vessels take shape immediately dorsal to the single layer of cuboidal pre-pancreatic endoderm, via *de novo* coalescence of endothelial precursors, or *vasculogenesis* [23] (Figure 1A). These vessels, which run the length of the anterioposterior axis and directly contact the endoderm, are termed the paired ‘dorsal aortae’ and represent the first embryonic vascular structures [24].

The first morphological sign of pancreas development occurs shortly after E9.0, as a thickening of the pre-pancreatic epithelium, both on the dorsal and ventral sides of the foregut-midgut juncture, which occurs as a result of columnarization of endodermal epithelial cells [25]. Transcriptional changes are also taking place in this early pancreatic tissue, with onset of the expression of pancreatic transcription factors, including *Pdx-1*, *Ptf1a* and *Ngn3* [26–29]. During these early cellular and molecular events, the dorsal pancreatic bud is in direct contact with the paired aortae, as they approach each other and fuse at the midline (around E9.0), while the ventral bud contacts the vitelline veins [1]. Later, after E9.5, intervening mesenchyme will displace the aortae dorsally, as it surrounds the growing bud, facilitating the formation of a net-like plexus of fine capillaries that will surround the entire gut tube epithelium, including the growing pancreatic bud (Figure 1B).

Primary and secondary transitions: changing association of pancreatic epithelium and its vasculature

The physical relationship of the pancreatic epithelium and its endocrine cells with its associated blood vessels changes in interesting ways over developmental time. Following initial specification, endocrine differentiation occurs in two major ‘waves’ of endocrine differentiation (reviewed in Rieck, Bankaitis and Wright, this issue). The first of these is called the ‘primary transition’ and occurs from approximately E9.5 to E12.0 [12, 30, 31]. During this time, the pre-pancreatic epithelium undergoes rapid stratification, thereby building a multilayered bud consisting of a pool of ‘protodifferentiated’ cells [25, 32]. Primitive ducts form within this epithelium following localized cell polarization and apical constriction, which results in formation of numerous microlumens that fuse with each other to create a three dimensional network of lumens [25, 32, 33]. Endocrine differentiation during this initial period is relatively limited and consists mostly of glucagon-expressing cells [34]. Intriguingly, the epithelium of the pancreatic bud at this stage is strikingly avascular, with blood vessels surrounding but not penetrating the stratified epithelium [8] (Figure 1C). It is only as the pancreatic epithelium begins to branch that vessels penetrate the epithelium [8], either actively by angiogenesis or passively as the epithelium remodels (Figure 1D).

The secondary transition is characterized by rapid growth of the embryonic pancreas and onset of massive endocrine differentiation after E12.5. During this period, the unusual multilayered pancreatic epithelium begins a progressive resolution back to a single layer, resulting in more conventional epithelial tree-like network of ducts. As the bud epithelium resolves and the pancreatic tree takes shape, blood vessels intercalate between forming branches (Figure 1E). It was recently noted that these vessels remain physically associated with the trunk of the branching epithelium (central ductal-endocrine rich region), at the core of the growing bud, but become segregated away from the peripheral tips that will give rise to the acini [8]. This is likely driven by regionalized expression of the angiogenic cue VEGF-A, which is expressed at higher levels in the central trunk epithelium than in the newly formed exocrine tips [8]. The final conformation of the pancreatic and vascular trees in the mature organ is the close intercalation of their mono-layered tubular branches and dense vascular beds form within islets, which remain connected to larger vessels that run along the organ (Figure 1F). Therefore, the physical association of blood vessels with epithelial cells is fundamentally different during the first and second transitions.

Interestingly, endocrine neogenesis displays distinct mechanisms of cellular ontogeny during these two embryonic periods. Endocrine cells that emerge during the primary transition are primarily alpha cells, which often emerge or ‘bud out’ of the epithelium in groups (Ray MacDonald, personal communication). By contrast, the vast majority of endocrine cells that emerge following the secondary transition undergo EMT and delaminate individually from central regions of the epithelium [35]. These latter ‘secondary wave’ endocrine cells mostly differentiate into β -cells [36] and progressively coalesce into clusters, increasing in size and organizing into islets from late embryonic to postnatal stages. The basis for this difference is not understood. It is conceivable that the differential presence of

blood vessels during the first versus secondary transition impacts endocrine differentiation. Alternatively, it might be a consequence of the different inherent organization of the epithelium. Future studies will be required to address these possibilities.

Blood vessels promote early pancreas development

The close physical association of the pre-pancreatic endoderm and the overlying developing dorsal aorta in mouse has long been noted [16, 37]. However, the influence of this blood vessel on pancreas development was first demonstrated using embryological dissections and tissue recombinations [1]. Manually explanted E8.25 endodermal epithelium was embedded in matrigel and either grown alone or recombined with other embryonic tissues (i.e. notochord, neural tube or aortic endothelium). Following six days of culture, isolated endoderm explants, or those recombined with non-endothelial tissues, showed little growth or beta cell differentiation. By contrast, when aortic endothelium was present, endodermal cells displayed significant pancreatic growth and expression of both *Pdx-1* and insulin in scattered cells. In a reciprocal experiment, 'aortaleless' frog embryos were generated via the manual removal of endothelial progenitors from the embryonic lateral plate mesoderm. In these embryos, the aorta failed to form and differentiation of pancreatic endocrine cells, in turn, was also blocked. Lastly, in a third and final experiment, over-expression of vascular endothelial growth factor A, or VEGF-A, under the *Pdx-1* promoter instructed scattered cells within the stomach epithelium to acquire a pancreatic fate and express insulin. These ectopic insulin-expressing cells were notably correlated with areas of hypervascularization. Together, these results suggested that blood vessels were more than suppliers of nutrients and facilitators of gas exchange, because they acted as signaling entities during pancreas morphogenesis and beta cell differentiation.

Subsequent studies confirmed an inductive role for blood vessels in pancreatic specification and identified downstream targets [38]. Using *Flk-1*^{-/-} (*VEGFR2*^{-/-}) mutants embryos, which lack all blood vessels, it was shown that the dorsal pancreas initiated normally, but failed to expand and differentiate. The pancreatic epithelium in these embryos expressed *Pdx-1* during early budding, but this expression failed to be maintained and eventually extinguished. The downregulation of *Pdx-1* was independent of cell death [39]. These results suggested that signals from aortic endothelium were critical for the maintenance of the progenitor cell fate and for further endocrine differentiation. In addition, *Flk1*^{-/-} pancreatic epithelium showed a complete absence of *Ptf1a* gene expression, which is required by progenitors to acquire and maintain their pancreatic fate [29]. Lack of *Ptf1a* expression in the mutant epithelium was rescued in tissue recombination assays when it was cultured in the presence of aortic endothelium [38] indicating that signals from the embryonic dorsal aorta to the pancreatic endoderm were sufficient to induce *Ptf1a* expression and hence rescue pancreatic growth.

Recent work in the chick confirms an early role for blood vessel signals to the developing pancreas, and suggests that, at least in this system, the influence of the endothelium on pancreatic specification occurs even before blood vessels are formed [10]. Kume and colleagues found that CXCR4 receptor-expressing angioblasts migrate to the pre-stomach and pre-pancreatic endodermal region, recruited by the gut endoderm that expresses the

ligand CXCL12. Angioblasts then induce *Pdx-1* expression in the pre-pancreatic domain. CXCL12 over-expression experiments resulted in an expansion of the pancreatic *Pdx-1* domain and in ectopic attraction of angioblasts to the CXCL12 expressing region. Conversely, blockage of the CXCR4/CXCL12 signaling pathway by use of the CXCR4 inhibitor AMD3100 resulted in blocking dorsal aorta formation and in a concomitant decrease in *Pdx-1* and insulin expression. While these experiments clearly show that CXCR4 mediates chemotactic migration of blood vessel progenitors, the nature of the endothelium-derived inductive signal(s) that promote a pancreatic fate in the endoderm still remain(s) a mystery. Nonetheless, a growing number of studies suggest a positive role for the endothelium on early stages of pancreatic development.

Blood vessels inhibit later pancreas branching

In apparent contradiction to the suggested 'nurturing' role of the vasculature, studies in the last year have shown that blood vessels play very different roles later during pancreatic development. Three key studies have emerged demonstrating a surprising vascular suppression of pancreatic growth by inhibition of epithelial branching and exocrine differentiation, demonstrating the need for a delicate balance to be struck between endothelium and epithelium during pancreatic expansion [6, 8, 9].

In the first of these three recent studies, pancreatic explant cultures were experimentally hypervascularized using transgenic tissue overexpressing VEGF-A under the *Ptf1a* or Elastase promoter [8]. Significant loss of exocrine differentiation was observed, as well as loss of blood vessel enrichment at the pancreas core. Conversely, partial blood vessel ablation using *Pdx-1-Cre; Vegfa-loxP* embryos showed increased exocrine differentiation in areas with a decrease in vascular density. Treatment of wild-type explants with the VEGFR signaling inhibitor SU5416 for 2 days led to increased expression of the exocrine differentiation genes *Ptf1a*, *Rbpj*, *Rbpjl*, and the notch target genes *Hey-1* and *Hey-2*. In addition, the endocrine progenitor marker *Sox9*, the pro-endocrine marker *Ngn3*, and the endocrine transcription factor *Pax6* were all significantly decreased. When explants were cultivated with an excess of VEGF-A, a decrease in exocrine proliferation was observed, as well as a reduction in *Ptf1a* and *Hey-1* expression. However, the expression of *Rbpj*, *Rbpjl*, *Hey-2*, *Sox9*, *Ngn3* and *Pax6* in these explants was not significantly changed indicating that an increase in the amount of blood vessels is not sufficient to increase endocrine differentiation. These gain- and loss-of-function experiments, in conjunction with the regionalized physical association of blood vessels with the central ducto-endocrine epithelium, suggest a negative influence of the endothelium on exocrine differentiation and a positive influence on endocrine differentiation.

The inhibitory influence of blood vessels in pancreatic growth was also observed in G-coupled receptor shingosine-1-phosphate receptor 1 null (*SIP1*^{-/-}) mice [9]. Indeed, *SIP1*^{-/-} animals displayed both increased vascular density and decreased proliferation of *Pdx-1* expressing cells resulting in an overall reduction of the pancreas at the beginning of secondary transition. Mesenchymal gene expression, by contrast, remained unchanged. While authors did not identify defects in endocrine or exocrine differentiation, early embryonic mortality of *SIP1*^{-/-} mutant embryos by E12.5 (prior to pancreatic branching and

the secondary transition) may have precluded assessment of later differentiation defects. In an effort to investigate whether excess vessels in the *SIP1*^{-/-} pancreata were the cause of the observed defects, blood vessels were ablated in E11.5 cultured pancreatic buds using the VEGF receptor 2 (VEGFR2) inhibitor 1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluorophenyl]-3-(2-fluoro-phenyl)-urea (or quinolin-urea). However, similar to VEGF-deleted pancreas, increased exocrine differentiation was observed, supporting the idea that blood vessels restrain expansion of the pancreatic epithelium.

The notion that a critical balance of blood vessel to epithelial mass needs to be ensured was further supported by Dor and colleagues [6]. In these studies, hypervascularization was induced by overexpression of VEGF-A in Pdx-1 expressing cells by a tetracycline inducible system (*Pdx-1-tTA mice*) and resulted in a decrease in pancreatic size. Both E12.5 pancreatic buds *in vivo* and embryonic explants of *Pdx-1-tTA* mice exhibited decreases in exocrine differentiation, as evidenced by reduced Cpa1-expressing epithelial 'tip' domains, as well as a decrease in the number of Ngn3-expressing cells and in Amylase expression. These data suggested that the overall reduction in organ size was due to significantly reduced tip formation and consequently to a decrease in the number of differentiated endocrine and exocrine cells. Conversely, ablation of blood vessels in explants with the quinolin-urea VEGFR2 inhibitor resulted in excess exocrine differentiation, as well as accelerated commitment of the pancreatic proto-differentiated cells to the endocrine fate [6]. These studies therefore unequivocally demonstrate that ECs suppress outgrowth and differentiation of exocrine cells during pancreas development.

To understand these findings in the context of previously identified positive EC signals found in pancreas specification [1, 10, 38], we propose that it is critical to consider the changing relationship of blood vessels to the pancreatic epithelium. When the pancreas first buds out, blood vessels surround, but do not penetrate, the stratified epithelium of the pancreatic bud (at E10.5). In contrast, during morphogenetic transformation and resolution of the epithelium, blood vessels invade and intercalate between resolving branches (E11.5-E15.5), coming into close proximity to the central pancreatic epithelium. The possibility therefore arises that blood vessels play different roles at different times due to their changing interface with the developing epithelium (Figure 2).

At early stages, where the progenitor pool needs to expand, blood vessels may promote a 'progenitor state' by expanding the proto-differentiated epithelium and inhibiting its further steps differentiation (including tip formation). However, at later stages, where endocrine, exocrine and ductal cell types differentiate, blood vessels inhibit exocrine differentiation along the central, blood vessel rich areas of the pancreas. It is harder to find the common theme between these studies regarding the role of vessels on endocrine differentiation, as one suggests promotion (necessity, but not sufficiency- [8], one suggests inhibition [6] and one suggests little to no role [9]. However, as a whole these studies nonetheless make the case that blood vessels influence pancreatic development and are therefore likely to play multiple roles during pancreatic growth. It is conceivable that at later time-points of development (i.e. late embryonic or postnatal stages) blood vessels might play still additional roles, as yet to be determined.

Crosstalk between blood vessels and pancreatic mesenchyme

In the studies outlined above, signals between blood vessels and pre-pancreatic epithelium are assumed to be exchanged directly, however there is evidence to suggest a 'relay' mechanism whereby vessels indirectly influence the endoderm via promotion of survival and growth of the surrounding mesenchyme [39]. The mesenchyme has long been known to be essential for driving epithelial growth and differentiation in the pancreas [40–44] and given that vessels arise and develop embedded within the pancreatic mesenchyme, this possibility is not altogether surprising.

Indeed, blood vessels appear to be essential to maintenance of the pancreatic mesenchyme. *Flk-1*^{-/-} pancreatic buds show a progressive loss of this mesenchyme due to increased apoptosis [39]. Dorsal pancreatic mesenchyme expresses the transcription factor Isl-1 [45] and is a rich source of many factors including the essential factor Fgf10, which drives pancreatic expansion and endocrine differentiation [42]. In recombination experiments, Isl-1⁺ mesenchyme was rescued when *Flk1*^{-/-} explanted pancreatic buds (endoderm and associated mesoderm) were co-cultured with aortic endothelium, suggesting that blood vessels send survival signals to preserve a healthy mesenchyme. Similarly, supplementation of Fgf10 in *Flk1*^{-/-} bud cultures proved sufficient to rescue lost endodermal *Ptf1a* expression [39]. Together, these results highlighted the essential cross-communication between neighboring tissues in the pancreas and identified a relay system between epithelium, mesenchyme and endothelium during early pancreatic development.

Another set of mesenchymal factors that have been shown to influence pancreatic growth belong to the Bone Morphogenetic Protein (BMP) family (reviewed in Serup, this issue). In the chick embryo, BMP4 and BMP7 are expressed in the mesenchyme that surrounds the pancreatic epithelium, which in turn displays evidence of BMP responsiveness as it expresses phosphorylated Smad 1, 5 and 8 [46]. When BMP signaling in the chick pancreas was blocked using electroporation-based overexpression of Noggin, a BMP inhibitor, a reduction in pancreatic branching and precocious endocrine differentiation were observed. These embryos also showed defects in vascular remodeling, with major vessels developing abnormally. An effect on blood vessels is perhaps not altogether unexpected, as BMP signaling is known to influence the developing vasculature [47], however the vascular defects were dramatic, with failure of both normal dorsal aortae fusion at the midline and dorsal displacement, along with ventral positioning of the ophalomesenteric veins relative to the pancreatic bud. These results once again highlight cross-talk between the mesenchyme, epithelium and endothelium, and suggest a potential importance for vascular remodeling during pancreatic development (a possibility heretofore unexplored). In addition, the coupled vascular and pancreatic defects in these 'BMP-suppressed' embryos raise a variety of interesting but unanswered questions: Do large blood vessels provide different signals to the pancreatic epithelium than capillaries? Or conversely, is vascular remodeling secondarily impaired as a consequence of failed pancreatic branching? Understanding the coordination of blood vessel and epithelial development in the pancreas is still in its infancy and much remains to be investigated.

Seeking candidate endothelial signals

Although over a decade has gone by since blood vessels were first shown to influence pancreatic development and beta cell ontogeny, only a small number of specific candidate signals have been proposed to date. Initial co-culture experiments suggested that signals required for beta cell development arose from the endothelium itself, however since then signals arising from circulating blood within vessels, including blood-borne oxygen, have also both been proposed.

Plasma factors are candidate mediators of pancreatic development since the early 'unsheathed' dorsal aorta, lacking smooth muscle coverage and being known for its leakiness [48], contacts pancreatic endoderm. Semb and colleagues examined N-cadherin null embryos (*Cdh2*^{-/-}), which had been previously shown to lack dorsal pancreatic outgrowth due to absence of associated pancreatic mesenchyme [49, 50]. Strikingly, mesenchymal survival and expansion could be rescued upon restoration of cardiovascular function via cardiac-specific expression of N-cadherin in the heart. The requirement for proper vascular function (i.e. blood flow within the dorsal overlying the pancreatic bud) suggested that the critical mesenchymal-survival factor likely originated from blood. Explant studies were then used to demonstrate that the plasma factor sphingosine-1-phosphate (S1P) was able to promote dorsal pancreas formation in a manner dependent on the presence of mesenchyme. Indeed, S1P could rescue dorsal bud formation in *Cdh2*^{-/-} explants, possibly via promotion of mesenchymal survival, suggesting that blood vessels around the early pancreatic bud carry factors required for normal pancreas development.

An alternative possibility also put forth was that S1P might be acting on the endothelium itself, which expresses cognate Edg receptors, and that this signaling secondarily affects pancreas development. This is conceivable given that S1P has been shown to be required for maintenance of the integrity of blood vessels [51], which in turn are known to provide inductive signals necessary for pancreatic development. These results challenged the idea that endothelial-derived factors were involved, suggesting instead that blood-borne factors were important, but nonetheless contributed to the general notion that blood vessels influence pancreas development.

Another role of blood vessels that is difficult to ignore is their capacity to transport oxygen. Oxygen may yet represent an unexpected player in pancreatic development and endocrine differentiation [52]. *In vitro* experiments demonstrated that oxygen promotes endocrine differentiation, while reduction of oxygen levels inhibits it [53, 54]. In addition, it was observed that during the primary transition pancreatic epithelial cells expressed high levels of HIF1 α , a transcription factor expressed under hypoxic conditions, while HIF1 α levels decreased dramatically during the secondary transition. This temporal change in HIF1 α expression is likely a result of the increase in oxygen levels that occurs as blood vessels perfuse the epithelium. Endocrine neogenesis appears to correlate with low levels of HIF1 α and high levels of oxygen [53]. Use of the prolyl hydroxylase dimethyloxaloylglycine (DMOG), which stabilizes HIF1 α , suppressed endocrine differentiation in rat explants, supporting a positive role of oxygen and the HIF1 α signaling cascade in pancreatic endocrine differentiation. Furthermore, one study correlated the onset of endocrine

differentiation that occurs after E12.5 with a relatively sudden perfusion of blood within pancreatic capillaries of the embryonic pancreas [54].

In addition, recent work, revealed that the oxygen sensing protein Von Hippel-Lindau (VHL), while not required for endocrine differentiation and specification, was required for proper beta cell function and glucose homeostasis in pancreas-specific conditional deletion models [55–57]. These results strengthen the importance of oxygen in pancreatic function, however future studies need to determine whether other oxygen and/or its sensors might play a role during pancreas development and endocrine differentiation, as this is an interesting direction that needs to be further explored.

Given that only a handful of studies seeking vascular signals have been carried out, and that each study identified different required signals, we deem it highly probable that there exists more than a single blood vessel/blood-derived candidate, and propose that these signals are likely dynamic, changing over developmental time. At this point, however, the identity of the complete set of blood vessel signals that in combination, or along with other permissive signals from the mesenchyme or from epithelium itself, direct the step-wise progression of progenitors to beta cell fate, still remains elusive. Whether these signals are secreted, or require cell-to-cell contact, also remains unknown. The identification of the molecular characteristics of blood vessel signaling cue(s) is of great importance given its potential for the improvement of *in vitro* therapies and endoderm differentiation techniques.

Summary

Overall, studies of blood vessel signals during the early stages of pancreatic specification and endocrine differentiation suggest that blood vessels play a nurturing role that sustain and support the pancreatic primordium. They do so by directly signaling the endoderm [1, 10, 38] and the mesenchyme [39], and by also transporting secreted factors [49, 54], which are necessary for proper development of both the growing epithelial ductal tree, the endocrine cells that emerge from it, and the mesenchyme that sustains them. During later stages, blood vessels profoundly impact organ morphogenesis by restraining exocrine differentiation and pancreatic growth [6, 8, 9]. However, while the vasculature likely also influences endocrine differentiation following the secondary transition, this relationship remains unclear. We propose that, in part, the basis for the changing influence of the vasculature on the pancreatic epithelium lies in their differential interactions during development and in the changing responsiveness of the underlying pancreatic epithelium. The pancreatic epithelium does not ‘react’ the same to alterations in vascular density, at early versus later timepoints. However, it is also likely that signals from blood vessels change over time. It is therefore the next challenge to identify and functionally test the battery of changing extrinsic signals, including those from closely associated blood vessels, which influence the fate of this critical cell type: the beta cell.

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Highlights

- Blood vessels influence pancreas organogenesis and differentiation of its cell lineages.
- Aortic endothelial cells are required for early endocrine specification.
- Blood vessels suppress later pancreatic branching and exocrine differentiation.
- Understanding how blood vessels impact the developing pancreas and identifying candidate signals may improve replacement and regenerative therapies for diabetes.

Figure 1. Changing physical relationship of pancreatic epithelium and blood vessels during embryogenesis

Immunofluorescent views of the developing pancreas **A–E**) E-Cadherin (green); **B–F**) PECAM (red); **A**) laminin (white). In panel **A**, aortae are outlined with dotted red line. **A**) Paired dorsal aortae lie dorsal to the single layered endodermal epithelium in the pre-pancreatic region. **B**) Epithelium of the budding pancreas at E9.25 displays columnarization. Note capillary endothelial cells surround the pancreatic bud. **C**) Stratified epithelium of the E10.5 pancreatic bud is avascular. Endothelial cells form a plexus around the growing bud. **D**) As branching of the epithelium starts, blood vessels are observed to invade the bud. **E**) Epithelium of the expanding pancreatic bud at E13.5 has resolved and is largely monolayered. Note intercalated blood vessels between epithelial branches. **F**) Vasculature of adult islets. Note high density of vessels in islet and proximity of islets with large pancreatic vessels. *, islet.

Figure 2. Schematic showing model for evolving signals from blood vessels to developing pancreatic epithelium

A) Aorta lies immediately dorsal to the pre-pancreatic endoderm (yellow cells) and provides signals supporting specification of endocrine cell progenitors. Blood vessel signals have been proposed to come from endothelium or plasma (1), or alternatively to act via a relay signal by supporting pancreatic mesenchyme (grey cells) (2). **B)** Blood vessels surround the stratifying pancreatic epithelium as the pancreatic bud takes shape. It is possible that blood vessel signals support pancreatic development during this stage. (Presumed relationships not directly demonstrated by available studies are shown in grey). **C)** Blood vessels also surround the stratified bud at E10.5 and are likely to communicate with bud epithelium. **D)** Blood vessels have been shown to preferentially associate with trunk epithelium and to suppress branching epithelium and exocrine differentiation at later stages, following the secondary transition.