Mechanisms of Development xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Mechanisms of Development

journal homepage: www.elsevier.com/locate/mod

The global gene expression profile of the secondary transition during pancreatic development

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Abbreviations: 2210010C04Rik, RIKEN cDNA 2210010C04 gene; 5330417C22Rik, RIKEN cDNA 5330417C22 gene; A1AT, alpha1-antitrypsin; Ago1, argonaute RISC catalytic component 1; Amy2a5, amylase 2a5; Amy2b, amylase, alpha 2B; AP1/JUN, jun proto-oncogene; Bmp, bone morphogenetic protein; CAM, cell adhesion molecule; CD49f, Integrin subunit alpha 6 (Itga6); Cela1, chymotrypsin-like elastase family member 1; ChIP-seq, chromatin-immunoprecipitation sequencing; Cldn10, Claudin 10; Cpa1, carboxypeptidase 1; Drosha, drosha, ribonuclease type III; E, embryonic day; ECM, extracellular matrix; Edaradd, ectodysplasin-A receptor (EDAR)-associated adapter protein; EGF, epidermal growth factor; EMT, epithelial–mesenchymal transition; En1, engrailed homeobox 1; FACS, fluorescence activated cell sorting; FGF, fibroblast growth factor; Fgf1, fibroblast growth factor 1; Fgf10, fibroblast growth factor 10; Fgf15, fibroblast growth factor 15; Fgf20, fibroblast growth factor 20; Fgf23, fibroblast growth factor 23; Fgf6, fibroblast growth factor 6; Fgf7, fibroblast growth factor 7; Fgf8, fibroblast growth factor 8; Fgf9, fibroblast growth factor 9; Foxa2, Forkhead-Box-Protein A2; FVF^{-}, Foxa2-Venus negative; FVF^{+}, Foxa2-Venus positive; Fzd9, frizzled homolog 9; Fzd10, frizzled homolog 10; G6pc2, glucose-6-phosphatase, catalytic, 2; Gata6, GATA binding protein 6; Gck, glucokinase; Gdf10, growth differentiation factor 10; Ghr, growth hormone receptor; Ghrl, ghrelin/obestatin prepropeptide; Gip, gastric inhibitory polypeptide; Gipr, gastric inhibitory polypeptide receptor; Gli3, GLI-Kruppel family member GLI3; Glp1, glucagon-like peptide 1; Glp1r, glucagon-like peptide 1 receptor; GO, gene ontology; Grb7, growth factor receptor bound protein 7; GRNs, gene regulation networks; GWAS, genome-wide association study; H3K27ac, histone 3 lysine 27 acetylation; H3K4me1, histone 3 lysine 4 monomethylation; H3K4me3, histone 3 lysine 4 trimethylation; Hdac1, histone deacetylase 1; Hes1, hairy and enhancer of split 1; Hey1, hairy/enhancer-of-split related with YRPW motif 1; Hh, Hedgehog; Hhex, hematopoietically expressed homeobox; High mag, higher magnitude; HNF, hepatocyte nuclear factor; Hnf1a, hepatocyte nuclear factor 1, alpha; Hnf1b, hepatocyte nuclear factor 1, beta; Hnf4a, hepatocyte nuclear factor 4, alpha; Hnf4b, hepatocyte nuclear factor 4, beta; Iaap, islet amyloid polypeptide; Igf1, insulin-like growth factor 1; Igfbp4/5, insulin-like growth factor binding protein 4/5; Ihh, Indian hedgehog; Il11ra2, interleukin 11 receptor, alpha chain 2; Ins1, insulin 1; Ins2, insulin 2; Insrr, insulin receptor-related receptor; iPSC, induced pluripotent stem cells; Isl1, ISL LIM homeobox 1; Jag1, jagged1; Lum, Lumican; MAF, v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog; MAFB, v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B; Med12, mediator complex subunit 12; Med23, mediator complex subunit 23; Mist1/Bhlha15, basic helix-loop-helix family, member A15; MODY, maturity-onset diabetes of the young; MPC, multipotent progenitors; NeuroD1, neurogenic differentiation 1; Ngn3, Neurogenin 3; Nkx2-2, NK2 homeobox 2; Nkx6.1, NK6 homeobox 1; Npy, neuropeptide Y; Nr5a2, nuclear receptor subfamily 5; Onecut1/Hnf6, one cut homeobox 1; Onecut3, one cut domain, family member 3; Osr1, odd-skipped related 1; Otx2, orthodenticle homeobox 2; Pax4, paired box 4; Pax6, paired box 6; PC, principal component; Pcsk1-3, proprotein convertase subtilisin/kexin type 1–3; Pdgfc, platelet derived growth factor C; Pdx1, pancreatic duodenal transcription homeobox 1 factor; Pgf, placental growth factor; Ptf1a, pancreas specific transcription factor, 1a; Pyy, peptide YY; RA, retinoic acid; Rbm47, RNA binding motif protein 47; Rxrg, retinoic acid receptor gamma; Scgn, secretagogin, EF-hand calcium binding protein; Sdf1, stromal cell-derived factor 1; Serpina10, serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10; Serpina1a, serine (or cysteine) preptidase inhibitor, clade A, member 1A; Serpina1b, serine (or cysteine) preptidase inhibitor, clade A, member 1B; Serpina1d, serine (or cysteine) preptidase inhibitor, clade A, member 1D; Serpina1e, serine (or cysteine) preptidase inhibitor, clade A, member 1E; Sfrp1, secreted frizzled-related protein 1; Shh, sonic hedgehog; Six3, sine oculis-related homeobox 3; Slc2a2, solute carrier family 2 (facilitated glucose transporter), member 2; Slc30a8, solute carrier family 30 (zinc transporter), member 8; Slc38a3, solute carrier family 38, member 3; Smarca2, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2; Smarca4, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4; Smarcc1, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1; Smarcc2, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2; Smarcd1, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1; Smarcd1, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1; Smarce1, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1; Snai1/2, snail family zinc finger 1/2; Sox11, SRY (sex determining region Y)-box 11; Sox9, SRY (sex determining region Y)-box 9; Srpx2, sushi-repeat-containing protein, X-linked 2; Sst, somatostatin; Syt13, synaptotagmin 13; Syt6, synaptotagmin 6; Syt7, synaptotagmin 7; Syt8, synaptotagmin 8; Sytl1, synaptotagmin-like 1; Sytl4, synaptotagmin-like 4; T1/2D, type 1/2 diabetes; Tbx19, T-box 19; Tbx6, T-box 6; Tcf7l1, transcription factor 7-like 1; Tcf7l2, transcription factor 7-like 2; Tenc1, tensin like C1 domain-containing phosphatase; TF, transcription factor; TFBS, transcription factor binding site; Tgfß, transforming growth factor, beta 1; Thbs2, thrombospondin 2; Tmem171, transmembrane protein 171; TSS, transcriptional starting site; Twist1, twist family bHLH transcription factor 1; Vdr, vitamin D receptor; Vwa5b2, von Willebrand factor A domain containing 2B2; Wnt, wingless; Wnt1, wingless-type MMTV integration site family, member 1; Wnt10a, wingless-type MMTV integration site family, member 10A; Wnt10b, wingless-type MMTV integration site family, member 10B; Wnt2, wingless-type MMTV integration site family, member 2; Wnt3a, wingless-type MMTV integration site family, member 3A; Wnt4, wingless-type MMTV integration site family, member 4; Wnt5a, wingless-type MMTV integration site family, member 5A; Wnt6, wingless-type MMTV integration site family, member 6; Zeb1/2, zinc finger E-box binding homeobox 1/2.

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http://dx.doi.org/10.1016/j.mod.2015.11.004

Please cite this article as: Willmann, S.J., et al., The global gene expression profile of the secondary transition during pancreatic development, Mechanisms of Development (2015), http://dx.doi.org/10.1016/j.mod.2015.11.004

ARTICLE INFO

Article history:
Received 19 June 2015
Received in revised form 26 November 2015
Accepted 27 November 2015
Available online xxxx

Keywords:
Pancreas
Endocrine
Exocrine
Ductal
Organogenesis
Foxa2

ABSTRACT

Pancreas organogenesis is a highly dynamic process where neighboring tissue interactions lead to dynamic changes in gene regulatory networks that orchestrate endocrine, exocrine, and ductal lineage formation. To understand the spatio-temporal regulatory logic we have used the Forkhead transcription factor Foxa2-Venus fusion (FVF) knock-in reporter mouse to separate the FVF^{+} pancreatic epithelium from the FVF^{-} surrounding tissue (mesenchyme, neurons, blood, and blood vessels) to perform a genome-wide mRNA expression profiling at embryonic days (E) 12.5–15.5. Annotating genes and molecular processes suggest that FVF marks endoderm-derived multipotent epithelial progenitors at several lineage restriction steps, when the bulk of endocrine, exocrine and ductal cells are formed during the secondary transition. In the pancreatic epithelial compartment, we identified most known endocrine and exocrine lineage determining factors and diabetes-associated genes, but also unknown genes with spatio-temporal regulated pancreatic expression. In the non-endoderm-derived compartment, we identified many well-described regulatory genes that are not yet functionally annotated in pancreas development, emphasizing that neighboring tissue interactions are still ill defined. Pancreatic expression of over 635 genes was analyzed with the mRNA in situ hybridization Genepaint public database. This validated the quality of the profiling data set and identified hundreds of genes with spatially restricted expression patterns in the pancreas. Some of these genes are also targeted by pancreatic transcription factors and show active chromatin marks in human islets of Langerhans. Thus, with the highest spatio-temporal resolution of a global gene expression profile during the secondary transition, our study enables to shed light on neighboring tissue interactions, developmental timing and diabetes gene regulation.

1. Introduction

Diabetes is an epidemic disease and caused 4.9 million deaths worldwide in 2014 (IDF Diabetes Atlas 2014). Type 1 (T1D) and type 2 diabetes (T2D) are triggered by autoimmune destruction of insulin-producing β-cells or by acquired insulin resistance with steady decline of functional β-cell mass, respectively. Current treatments significantly improve life quality of patients, however, they do not provide full glycemic control leading to long-term micro- and macro-vascular complications. New treatment strategies aiming at restoration of β-cell mass could normalize blood glucose control and eliminate disease complications (Bonner-Weir and Weir, 2005).

The insulin-producing β-cells are part of the endocrine pancreas, the islets of Langerhans, which also consists of glucagon-producing α-cells, somatostatin-producing δ-cells, ghrelin-producing ε-cells and pancreatic polypeptide-producing PP-cells (In't Veld and Marichal, 2010). These endocrine cells secrete hormones into the blood stream to regulate nutrient metabolism and glucose homeostasis. The exocrine compartment of the pancreas produces digestive enzymes from its acinar cells, which are then drained via a ductal system into the duodenum.

The Forkhead transcription factor Foxa2 regulates endoderm formation and epithelialization during gastrulation (Burtscher and Lickert, 2009). Foxa1 and Foxa2 are crucial upstream regulators of the pancreatic and duodenal homeobox 1 (Pdx1) transcription factor (Gao et al., 2008). Pancreas development starts with the patterning of the foregut endoderm and the specification of the pre-pancreatic field marked by Pdx1 (Jensen, 2004; Pan and Wright, 2011; Zorn and Wells, 2009). By E9.5, the first visible pancreatic epithelial buds emerge from the dorsal and ventral foregut and expand into the surrounding mesenchyme. Growth and expansion of the pancreatic buds are associated with the first wave of differentiation and appearance of glucagon-positive cells during the so-called primary transition (E9.5–12.5). By the end of the primary transition, these two buds rotate and fuse together and active growth and epithelial remodeling shape the future pancreas. During the secondary transition (E12.5–15.5), multipotent pancreatic progenitors become committed to the ductal, endocrine and acinar lineages. Several transcription factors such as Pdx1, Ptf1a, Hnf1a, Hnf1b, Hnf4a, Nkx6.1, and Sox9, as well as signaling molecules, such as FGF, EGF, Wnt, Bmp, Shh and Notch have been shown to coordinate commitment and differentiation of the pancreatic lineages concomitant with epithelial branching morphogenesis (Puri and Hebrok, 2010; Pan and Wright, 2011; Raducanu and Lickert, 2012; Pagliuca and Melton, 2013; Shih et al., 2013; Migliorini et al., 2014). Inductive instructions relayed from the surrounding mesenchyme as well as epithelial polarization contribute to the patterning of the pancreatic epithelium into distinct trunk and tip domains. The tip domain, at least for a certain period of time, is the supplier of multipotent progenitors that can generate the endocrine pool as well as ductal and acinar cells (Zhou et al., 2007). Endocrine lineage segregation is initiated by the induction of Neurogenin 3 (Ngn3), which marks the cells that are committed to an endocrine fate and will start migrating out from the ductal epithelium into the surrounding mesenchyme where they cluster together to form the islets of Langerhans (Gradwohl et al., 2000; Schwitzgebel et al., 2000). Cell delamination, asymmetric cell division and epithelial–mesenchymal transition (EMT) have been proposed to govern the endocrine precursors' delineation from the trunk epithelium, however, the mechanisms of pancreatic lineage segregation and neighboring tissue interactions are only beginning to be understood.

To systematically profile pancreas development, we captured the spatio-temporal global gene expression of the endoderm-derived epithelial and non-endodermal compartments during the secondary transition. Pancreatic organs were isolated, dissociated and sorted for epithelial (Foxa2-Venus fusion positive, FVF^{+}) and non-endodermal (FVF^{-}) populations and subjected for mRNA profiling on four consecutive days between E12.5–15.5. Extensive statistical analyses, using principal component analysis and linear regression modeling, clearly identified two distinct tissue compartments solely based on their transcriptional profile. Subsequent bioinformatical analyses, using pathway analysis of the profiles, underpin the importance of the established spatio-temporal genome-wide expression resource. We demonstrate how the provided data resource can be mined to advance mechanistic understanding of the secondary transition, neighboring tissue interactions, and diabetes gene regulation. For further validation, we analyzed and classified mRNA expression patterns using the Genepaint database (www.genepaint.org) of 635 regulated genes in embryos and pancreata at E14.5. This enabled us to identify almost all known regulators of pancreas development and to propose gene regulatory networks (GRNs) for genome-wide association study (GWAS)-annotated diabetes genes. We further analyzed publicly available chromatin-immunoprecipitation sequencing (ChIP-seq) data sets of human islets of Langerhans (Pasquali et al., 2014; Morán et al., 2012) and analyzed the regulation of newly identified genes by pancreatic transcription factors. Importantly, many potential regulators of development and disease were identified in the endodermal and non-endodermal compartments of the pancreas, which provides a high spatio-temporal resolution of the GRNs involved in pancreatic lineage allocation for future functional interrogation.

Please cite this article as: Willmann, S.J., et al., The global gene expression profile of the secondary transition during pancreatic development, Mechanisms of Development (2015), http://dx.doi.org/10.1016/j.mod.2015.11.004