Developmental Biology 378 (2013) 83–93

Contents lists available at SciVerse ScienceDirect

Developmental Biology

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

Smad signaling pathways regulate pancreatic endocrine development

Yousef El-Gohary [a,1], Sidhartha Tulachan [b,1], Ping Guo [a], Carey Welsh [c], John Wiersch [a,d], Krishna Prasadan [a], Jose Paredes [a], Chiyo Shiota [a], Xiangwei Xiao [a], Yoko Wada [e], Marilyn Diaz [f], George Gittes [a,(*)]

[a] Department of Surgery, Division of Pediatric Surgery, Children's Hospital of Pittsburgh, One Children's Hospital Drive, 4401 Penn Ave., Pittsburgh, PA 15224, USA
[b] Department of Internal Medicine, St. Elizabeth Health Center, 1044 Belmont Avenue Youngstown, OH 44501, USA
[c] Department of Pediatrics, Division of Neonatology, Children's Hospital of Pittsburgh, One Children's Hospital Drive, 4401 Penn Ave., Pittsburgh, PA 15224, USA
[d] Department of Surgery, School of Medicine, University of Texas Health Sciences Center at San Antonio, 7703 Floyd Curl Dr, Mail Code 7840, San Antonio, TX 78229, USA
[e] Division of Clinical Nephrology and Rheumatology, Niigata university graduate school of medical and dental sciences, 1-757, Asahimachi-dori, Chuo-ku, Niigata 9518510, Japan
[f] Laboratory of Molecular Genetics, D3-01, 111 TW Alexander Drive, NIEHS/NIH, RTP, NC, 27709, USA

A R T I C L E I N F O

Article history:
Received 21 November 2012
Received in revised form
4 March 2013
Accepted 4 April 2013
Available online 17 April 2013

Keywords:
Pancreas
TGF-β
Smads
Embryonic

A B S T R A C T

Expansion of the pancreatic endocrine cell population occurs during both embryonic development and during post-natal pancreatic growth and regeneration. Mechanisms of the expansion of endocrine cells during embryonic development are not completely understood, and no clear mechanistic link has been established between growth of the embryonic endocrine pancreas and the islet cell replication that occurs in an adult animal. We found that transforming growth factor-beta (TGF-β) superfamily signaling, which has been implicated in many developmental processes, plays a key role in regulating pancreatic endocrine maturation and development. Specifically, the intracellular mediators of TGF-β signaling, smad2 and smad3, along with their inhibitor smad7, appear to mediate this process. Smad2, smad3 and smad7 were all broadly expressed throughout the early embryonic pancreatic epithelium. However, during later stages of development, smad2 and smad3 became strongly localized to the nuclei of the endocrine positive cells, whereas the inhibitory smad7 became absent in the endocrine component. Genetic inactivation of smad2 and smad3 led to a significant expansion of the embryonic endocrine compartment, whereas genetic inactivation of smad7 led to a significant decrease in the endocrine compartment. In vitro antisense studies further corroborated these results and supported the possibility that interplay between the inhibitory smad7 and the intracellular mediators smad2/3 is a control point for pancreatic endocrine development. These results should provide a better understanding of the key control mechanisms for β-cell development.

© 2013 Elsevier Inc. All rights reserved.

Introduction

The pancreas is an endodermally derived organ consisting of two morphologically and functionally distinct tissues, the exocrine and endocrine pancreas. During embryonic growth, the pancreas goes through three stages of development. The first is a relatively undifferentiated stage where pancreatic morphogenesis is initiated with endodermal evagination. The second stage involves epithelial branching morphogenesis, which also includes the delamination of differentiating islet progenitors from the basement membrane. The final stage begins with the formation of acinar cells at the apices of the ductal structures. During these stages, the endocrine compartment undergoes amplification during two distinct waves of differentiation. The primary wave (pre-E13.5) is followed by the secondary wave of differentiation from the ducts (E13.5-E16.5)(Edlund, 2001; Prasadan et al., 2002). Specific factors governing this endocrine expansion are a subject of active study.

We have previously investigated the role of TGF-β isoform signaling in early pancreatic organogenesis, demonstrating that the developing pancreas expresses TGF-beta type II receptor (TBR-II) and type I receptor. TBR-II localizes to ducts at later stages of pancreatic development(Crisera et al., 1999). Blocking TGF-β signaling in the embryonic pancreas, using a transgenic mouse expressing a dominant-negative TGF-β-type-II-receptor (DNTβRII) led to an increased number of endocrine cells arising from the embryonic ducts. The enhanced endocrine expansion was most prominent at E16.5, which corresponded to the normal peak of secondary wave endocrine differentiation (Tulachan et al., 2007). Whether adult pancreatic ducts can recapitulate the embryonic mode of development to give rise to new β-cells remains hotly

(*) Corresponding author. Fax: +1 412 692 5824.
E-mail addresses: yousef.gohary@gmail.com (Y. El-Gohary), george.gittes@chp.edu (G. Gittes).
[1] Both these authors contributed equally to the paper.

0012-1606/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ydbio.2013.04.003

debated(Bonner-Weir et al., 2008; El-Gohary et al., 2012; Furuyama et al., 2011; Inada et al., 2008; Kopp et al., 2011; Solar et al., 2009).

In vivo studies of the role of TGF-β ligands in development have been difficult since mice deficient in TGF-β2 and TGF-β3 developed severe embryonic developmental defects and 100% embryonic lethality(Kaartinen et al., 1995; Sanford et al., 1997); therefore, the role of TGF-β2 and TGF-β3 has been difficult to assess in vivo. Furthermore, disrupting the TGF-β1 gene leads to severe multifocal inflammatory diseases, thus confounding analyses of different tissues (Shull et al., 1992). Using a DNTβRII transgene avoided some of the problems associated with deletion of TGFβ ligands, and shed some light on a possible role for TGF-β signaling in regulating pancreatic endocrine expansion and maturation during development(Bottinger et al., 1997; Tulachan et al., 2007).

Canonical TGF-β signaling involves ligand binding to the TGF-β-receptor-type-II, a serine/threonine kinase receptor. Subsequently the type II receptor recruits and phosphorylates the type I receptors, which in turn activate downstream smad2/3 transcription factors that mediate TGF-β-regulated gene expression (Massague, 1998; Shi and Massague, 2003). Smad6 and smad7 are the inhibitory smads(Park, 2005; Yan et al., 2009). Smad6 is thought to inhibit specifically those smads that are downstream of BMP signaling, i.e. smads 1, 5, and 8(Massague and Gomis, 2006; Park, 2005), whereas smad7 seems to be more globally active against all receptor-activated smads (smads 1, 5, 8, plus smad2 and smad3). Thus, smad7 is the only inhibitory smad that inhibits smad2 and smad3, which are the TGF-beta and activin signaling smads(Park, 2005; Yan et al., 2009).

To investigate in more depth the mechanism of enhanced endocrine expansion in the DNTβRII mice, we specifically targeted the intracellular mediators of TGF-β signaling, smad2 and smad3, by analyzing smad2 conditional (Smad2$^{fx/fx}$) (Ju et al., 2006) and smad3 global mutant (exon2 deletion) mice (Datto et al., 1999). Smad2$^{fx/fx}$ mice were crossed with a pdx1-cre-ERT mouse to create tamoxifen-inducible smad2 conditional mutants for the pancreas. Ablation of smad2 or smad3 led to a significant increase in endocrine cell numbers at E18. Furthermore, we generated a smad7$^{fx/fx}$ knock-in mouse, conditionally deleting smad7 in the pancreas by crossing it with pdx1-cre-ERT mice. We saw a severely diminished number of hormone^{+} cells at E18.

These results implicate an important role for smad2 and smad3 in the expansion of the pancreatic endocrine compartment, and implicate smad7 as a key regulator of this expansion, likely through suppression of smads 2 and 3. These results should provide a better understanding of the key control mechanisms of β-cell development, and may provide a mechanistic link between developmental neogenesis of pancreatic endocrine cells and pancreatic islet cell replication.

Materials and methods

Transgenic animals and genotyping

All the animal experiments were performed in accordance with guidelines established by the University of Pittsburgh Institutional Animal Care and Use Committees. Smad3-exon 2 null mutant mice were obtained from Jackson Laboratories (stock 003451), originally made by Luis Parada UT Southwestern. Transgenic mice expressing Smad2$^{fx/fx}$ were generous gifts from Dr. Erwin P. Bottinger, Mt. Sinai School of Medicine. All transgenic mice were crossed with Pdxcre-ER™ (Gu et al., 2002) (Mouse Models of Human Cancers Consortium, MMHCC). Non-pancreatic tissues from the embryos and adult mice were used for genotyping with the Extract-N-Amp PCR mix (Sigma, St. Louis, MO) kit and PCR probe that is specific for the transgene. Pancreata were isolated by micro-dissection from the transgenic embryos, as well as from the littermate controls.

Tamoxifen injection

The cre-ER™/LoxP system, tamoxifen (Sigma, St Louis, MO) was dissolved at 20 mg/ml in corn oil (Sigma) and was administered into adult mice intraperitoneally. For embryonic studies, pregnant females received a single dose 2 mg per 40 g body weight intraperitoneally at E10.5 and embryonic pancreases were subsequently harvested at different time points. The pancreas were then harvested and fixed in 4% paraformaldehyde overnight in 4 °C then placed in 30% sucrose overnight in 4 °C for cryoprotection. Tissues were then embedded in Tissue-Tech O.C.T compound and then frozen for sectioning.

Histology and immunohistochemistry

Harvested tissues were fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose overnight at 4 °C, then embedded in Tissue-Tech O.C.T compound and frozen in −20 °C. 6–8 μm-thick frozen sections were cut at −23 °C in a cryostat and mounted on gelatin-coated glass microscope slides (Superfrost Plus, Fisherbrand). For immunostaining, optimal dilutions and controls were used for each antibody used. Insulin guinea pig anti-swine 1:500 (Dako, Carpinteria, CA), glucagon rabbit monoclonal 1:2000 (Linco), amylase rabbit anti-human 1:400 (Sigma, St. Louis, MO), smad7 rabbit polyclonal IgG 1:50 (Santa Cruz biotech, CA), PDX-1 rabbit polyclonal 1:1400 (generous gift from Prof. Chris Wright, Vanderbilt University Medical School, Nashville, TN), PDX-1 goat polyclonal 1:1000 (Abcam), *Dolichos biflorus* agglutinin FITC conjugated (DBA), which binds to lectins present on ductal cells, 1:50 (Vector Laboratories, CA), anti-bromo-deoxyuridine (BrdU) rat monoclonal antibody 1:400 (Abcam), anti-bromo-deoxyuridine rat mono-cloncal antibody 1:100 (Novus Biologicals). Primary antibodies were incubated for 2 h at room temperature or at 4 °C overnight. Biotinylated Vectastain ABC kit or AMCA/CY3/FITC fluorescent conjugated donkey secondary antibodies were used for 1.5 h at room temperature. Immunoperoxidase was detected by DAB kit (Dako, Carpintaria, CA) or AEC (Sigma, St. Louis, MO) and fluorescently labeled samples were imaged using a fluorescent microscope. Tissue sections were viewed on an upright Axio Imager Z1 microscope. Images were captured with the AxioCam MRc5 and processed using AxioVs40 V4.8.2.0 software or with an inverted Olympus Fluoview 1000 confocal microscope to confocally image the tissue sections. The pictures were generated by overlay of the colors followed by merging of all color channels into one.

Bromo-deoxyuridine (BrdU) incorporation and cell counting

Pregnant mice were injected with BrdU (Sigma, St. Louis, MO) 200 mg/Kg intraperitoneally 4 h before harvesting the embryos The pancreas is then fixed in 4% paraformaldehyde overnight in 4 °C then placed in 30% sucrose overnight in 4 °C. Tissues were then embedded in Tissue-Tech O.C.T compound and then frozen for sectioning. Antigen retrieval was done on the slides by treating with sodium citrate (10 mM, pH 6.0), heating it in the microwave at low power for 15 min and then subsequently treating it with 2 M HCl for 35 min, followed by overnight incubation with primary antibodies. To quantify number of BrdU positive cells in embryonic wild-type and transgenic pancreases, the whole frozen pancreas was sectioned 6–8 μm-thick at −23 °C in a cryostat and mounted on gelatin-coated glass microscope slides (Superfrost

Download English Version:

https://daneshyari.com/en/article/10932181

Download Persian Version:

https://daneshyari.com/article/10932181

Daneshyari.com