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p120 Catenin is required for normal tubulogenesis but not epithelial integrity in developing mouse pancreas

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ABSTRACT

The intracellular protein p120 catenin aids in maintenance of cell–cell adhesion by regulating E-cadherin stability in epithelial cells. In an effort to understand the biology of p120 catenin in pancreas development, we ablated p120 catenin in mouse pancreatic progenitor cells, which resulted in deletion of p120 catenin in all epithelial lineages of the developing mouse pancreas: islet, acinar, centroacinar, and ductal. Loss of p120 catenin resulted in formation of dilated epithelial tubules, expansion of ductal epithelia, loss of acinar cells, and the induction of pancreatic inflammation. Aberrant branching morphogenesis and tubulogenesis were also observed. Throughout development, the phenotype became more severe, ultimately resulting in an abnormal pancreas comprised primarily of duct-like epithelium expressing early progenitor markers. In pancreatic tissue lacking p120 catenin, overall epithelial architecture remained intact; however, actin cytoskeleton organization was disrupted, an observation associated with increased cytoplasmic PKCζ. Although we observed reduced expression of adherens junction proteins E-cadherin, β-catenin, and α-catenin, p120 catenin family members p0071, ARVCF, and δ-catenin remained present at cell membranes in homozygous $p120^{f/f}$ pancreases, potentially providing stability for maintenance of epithelial integrity during development. Adult mice homozygous for deletion of p120 catenin displayed dilated main pancreatic ducts, chronic pancreatitis, acinar to ductal metaplasia (ADM), and mucinous metaplasia that resembles PanIN1a. Taken together, our data demonstrate an essential role for p120 catenin in pancreas development.

Introduction

Pancreatic development proceeds from a cluster of endodermal epithelial cells that give rise to a highly specialized, heterogeneous endocrine and exocrine organ. The early pancreatic bud is enveloped by mesenchyme, which is required for pancreas development and is thought to provide inductive signals for the specification of various cell types (Golosow and Grobstein, 1962; Landsman et al., 2011). Beginning at E13.5, the mouse pancreas changes rapidly during the 'secondary transition,' which is marked by dramatic increases in endocrine cell numbers and acinar cell differentiation (Rutter et al., 1968). Endocrine cells delaminate from the embryonic epithelia, coalesce into early islets, and migrate throughout the tissue (Pictet et al., 1972). The developing pancreas arborizes to generate a highly branched network of exocrine tissue consisting of acini capping the tips of terminal ducts and extending to the main pancreatic duct (Puri and Hebrok, 2007; Villasenor et al., 2010).

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Epithelial integrity is essential for proper organogenesis during development. Adherens junctions are an integral part of the maintenance of tight cell–cell adhesion in epithelial tissues. Adherens junction proteins serve roles in tissue homeostasis, embryonic development, tissue morphogenesis, and tumorigenesis (Hartsock and Nelson, 2008; Perez-Moreno and Fuchs, 2006). The core of the adherens junction in epithelial tissues is comprised of E-cadherin, β-catenin, p120 catenin, and α-catenin. Through homophilic, Ca^{2+}-dependent interactions, extracellular E-cadherin associates with E-cadherin molecules of adjacent cells (Shapiro and Weis, 2009). β-catenin binds to the catenin-binding domain of intracellular E-cadherin and α-catenin, which associates with the actin cytoskeleton. p120 catenin stabilizes epithelial cell adherens junctions through its interaction with the juxtamembrane domain of E-cadherin molecules (Ishiyama et al., 2010). Cadherin–catenin complexes are rapidly turned over in the absence of p120 catenin, demonstrating a crucial role for p120 catenin in cadherin stability (Davis et al., 2003). p120 catenin in the cytoplasm modulates the activities of small Rho family GTPases by inhibiting RhoA and activating Rac1 and Cdc42, which together influence cytoskeletal dynamics and cell migration (Anastasiadis et al., 2000; Noren et al., 2000).

p120 catenin is a member of the larger catenin gene family that is comprised of three subfamilies; p120, beta, and alpha. The p120 subfamily contains 7 members which include p120 catenin, ARVCF, δ-catenin, p0071, and plakophilins 1–3 (Zhao et al., 2011). Like p120 catenin, ARVCF, δ-catenin, and p0071 are capable of binding to the juxtamembrane domain of cadherin molecules in adherens junctions through their central Armadillo repeat domains, while plakophilins 1–3 primarily function in linking the intermediate filaments of cells through desmosomes. p120 catenin, ARVCF, and p0071 are expressed ubiquitously and as multiple isoforms. Expression of δ-catenin is thought to be restricted almost entirely to the nervous system (Mariner et al., 2000; Pieters et al., 2012). The biological interplay between p120 catenin and its family members is incompletely understood, especially during development.

A number of studies have revealed critical yet diverse roles for p120 catenin in different organ systems (Bartlett et al., 2010; Davis and Reynolds, 2006; Elia et al., 2006; Kurley et al., 2012; Marciano et al., 2011; Oas et al., 2010; Perez-Moreno et al., 2006, 2008; Schackmann et al., 2013; Smalley-Freed et al., 2010; Smalley-Freed et al., 2011; Stairs et al., 2011; Tian et al., 2012). In p120 catenin conditional deletion studies, the results are highly tissue-specific and unpredictable. In terms of tissue-specific effects, the biological contribution of p120 catenin to pancreas development has not been studied. Here, we ablated p120 catenin selectively in developing pancreatic epithelium using an early Pdx1:Cre (hereafter C^{Pdx1}) driver. Expression of Cre in this line is ubiquitous in multipotent pancreatic progenitor cells as early as E8.5, and thus targets all epithelial lineages of the developing pancreas – acinar, islet, duct, and centroacinar (Gu et al., 2002). C^{Pdx1}; $p120^{f/f}$ pancreases displayed striking developmental anomalies. Loss of p120 catenin early in pancreatic development resulted in aberrant tubulogenesis, expansion of tubular epithelia, loss of acinar cell mass, disruption of islet localization, and inflammation within the embryonic pancreas. The expanded tubular epithelium was accompanied by the continued expression of Sox9 and Aldh1, suggesting a possible block in progenitor differentiation. In expanded ductal epithelia of C^{Pdx1}; $p120^{f/f}$ pancreata, actin cytoskeleton organization was disrupted, which was accompanied by an increase in cytoplasmic PKCζ, a modulator of cytoskeletal dynamics, suggesting a connection between p120 catenin loss and actin cytoskeleton organization mediated by PKCζ. Despite the loss of p120 catenin, epithelial cell–cell junctions appeared normal and defects in cell adhesion were not observed. Although epithelium lacking p120 catenin displayed a reduction in adherens junction members E-cadherin, β-catenin, and α-catenin, we show that p120 catenin family members ARVCF, δ-catenin, and p0071 are present at cell membranes in p120 catenin-null epithelia, suggesting that these proteins might serve to stabilize adherens junctions in the absence of p120 catenin. Adult homozygous $p120^{f/f}$ animals displayed dilated main pancreatic ducts, chronic pancreatitis, acinar to ductal metaplasia (ADM), and mucinous metaplasia that resembled Pancreatic Intraepithelial Neoplasia 1a (PanIN1a). Taken together, our data suggest a crucial role for p120 catenin in proper tubulogenesis and lineage specification during pancreatic development.

Materials and methods

Mice

All animal studies were approved by the Animal Care and Use Committee at Johns Hopkins University. Mouse strains used in this study were Tg(Pdx1-cre)89.1Dam (MGI ID: 2684317) (Gu et al., 2002), $Ctnnd1^{tm1Abre}$ (MGI ID: 3617486) (Davis and Reynolds, 2006), and $ROSA^{mT/mG}$ (MGI ID: 3716464) (Muzumdar et al., 2007). The mice were housed under a 14/10 h light/dark cycle with free access to food and water.

Genotyping

$Ctnnd1^{tm1Abre}$, Pdx1-cre, and $ROSA^{mT/mG}$ alleles were maintained by breeding heterozygous mice to C57BL/6J mice. Genotyping was accomplished by PCR or Transnetyx. Primers used to genotype for the $Ctnnd1^{tm1Abre}$ allele were p120 FP (5′-TTTTAGAGCCTCCCACATACAAGC-3′) and p120 RP (5′-TCAGCACCCACACAAAGGTTG-3′) as previously described (Davis and Reynolds, 2006). Primers used to genotype for the Pdx1-cre allele were Pdx1-FP (5′-GAACTGGGGAGGAAAAGGAG-3′) and Cre2-RP (5′-GATGAAGCATGTTTAGCTGG-3′). Primers used to genotype for the $ROSA^{mT/mG}$ allele were Rosa26r FP (5′-CTCTGCTGCCTCCTGGCTTCT-3′), Rosa26r RP (5′-CGAGGCGGATCACAAGCAATA-3′), and mTmG RP (5′-TCAATGGGCGGGGGTCGTT-3′). Primers used to determine the sex of neonatal mice were designed to amplify the ZFX and ZFY genes as previously described (Valer Carstea et al., 2007).

Histology/Immunofluorescence

Mouse pancreata were fixed in 10% Neutral Buffered Formalin or 4% Paraformaldehyde at 4 °C and embedded in paraffin for sectioning. Five micron sections were prepared for hematoxylin and eosin staining, alcian blue staining, immunofluorescence, and immunohistochemistry. Primary antibodies and other immunofluorescent reagents used in this study are listed in Table S1. Secondary antibodies were used at 1:250 and were from Jackson ImmunoResearch.

Embryonic tissues prepared for frozen sections were fixed in 4% Paraformaldehyde at 4 °C, subsequently incubated in 30% sucrose at 4 °C for cryoprotection, and embedded in OCT (Sakura Finetek 4583 CRYO-OCT Compound). Frozen sections were used for Phalloidin, CD49f, and Muc1 staining in Fig. 5A and α-catenin and p120 catenin staining in Fig. 6B. A citrate-based Antigen Unmasking Solution from Vector Laboratories (H-3300) was used for antigen retrieval for all immunofluorescent staining except in OCT embedded sections. Primary antibody epitopes were retrieved with a heat-mediated microwave antigen retrieval method. All sections were blocked in 10% FBS with 0.2% Triton-X 100 in PBS. Primary antibodies were incubated overnight in blocking buffer at 4 °C. Subsequently, secondary antibodies were incubated at RT

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