Available online at www.sciencedirect.com

SCIENCE DIRECT

Experimental Cell Research 308 (2005) 114 – 122

Experimental Cell Research

www.elsevier.com/locate/yexcr

Convergence of bone morphogenetic protein and laminin-1 signaling pathways promotes proliferation and colony formation by fetal mouse pancreatic cells

Fang-Xu Jiang*, Leonard C. Harrison

Autoimmunity and Transplantation Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville 3050, Australia

Received 20 October 2004, revised version received 21 March 2005
Available online 23 May 2005

Abstract

We previously reported that bone morphogenetic proteins (BMPs), members of the transforming growth factor superfamily, together with the basement membrane glycoprotein laminin-1 (Ln-1), promote proliferation of fetal pancreatic cells and formation of colonies containing peripheral insulin-positive cells. Here, we further investigate the cross-talk between BMP and Ln-1 signals. By RT-PCR, receptors for BMP (BMPR) (excepting BMPR-1B) and Ln-1 were expressed in the fetal pancreas between E13.5 and E17.5. Specific blocking antibodies to BMP-4 and -6 and selective BMP antagonists partially inhibited colony formation by fetal pancreas cells. Colony formation induced by BMP-6 and Ln-1 was completely abolished in a dose-dependent manner by blocking Ln-1 binding to its α_6 integrin and α-dystroglycan receptors or by blocking the Ln-1 signaling molecules, phosphatidyl-inositol-3-kinase (P13K) and MAP kinase kinase-1. These results demonstrate a convergence of BMP and Ln-1 signaling through P13K and MAP kinase pathways to induce proliferation and colony formation in E15.5 fetal mouse pancreatic cells.

Keywords: BMPs; Development; Epithelial colony; Laminin-1; Pancreas; Signaling

Introduction

Understanding how extracellular signaling molecules regulate pancreatic β-cell development will be necessary to achieve the goal of regenerating β cells to cure type 1 diabetes. To this end, we used a serum-free culture system to demonstrate that bone morphogenetic proteins (BMPs), together with laminin-1 (Ln-1), promote proliferation of low-density fetal pancreatic cells and formation of colonies containing insulin-positive cells [1,2]. BMPs, members of the transforming growth factor β (TGFβ) superfamily, have divergent effects depending on cell type, stage of cell differentiation and the presence of other factors [3]. For example, BMPs alone can promote embryonic stem (ES) cell differentiation into non-neural cells [4], whereas in collaboration with leukemia inhibitor factor they can induce inhibitor of differentiation gene (Id) expression and block differentiation of ES cells [5]. BMP-4 and -8B synergistically induce primordial germ cell formation from epiblasts in mice [6], whereas BMP-2 and -7 exert opposite effects on renal branching morphogenesis [7].

The interactions of BMPs with their cell surface receptors (BMPRs) are regulated by a diverse group of extracellular BMP antagonist proteins containing one or more cysteine-rich (CR) domains that bind BMPs and prevent their interactions with BMPRs. BMP antagonists comprise at least noggin, chordin, follistatin, ventroptin, twisted gastrulation (Tsg) and members of the DAN/cerberus family [8]. Noggin and chordin bind a wide range of BMPs, whereas follistatin, ventroptin and Tsg bind specifically BMP-4 [9–11]. The role of these diverse regulatory molecules in islet cell development is largely unknown.

* Corresponding author. Fax: +61 3 9347 0852.
E-mail address: jiang@wehi.edu.au (F.-X. Jiang).

0014-4827/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2005.03.041

BMPs bind to type 1 and 2 BMPRs. The type 1 BMPRs comprise of BMPR-1A, BMPR-1B and activin receptor (ActR)-1A; the type 2 BMPRs comprise BMPR-2, ActR-2A and ActR-2B. BMP dimers initiate signaling by binding cooperatively to both type 1 and type 2 receptors. The type 2 receptors, constitutively active serine/threonine kinases, determine signaling specificity [12] and transphosphorylate the characteristic Gly–Ser segment in the type 1 receptor. The activated type 1 receptor then recruits and phosphorylates downstream signaling molecules of the Smad family. Activated Smads 1, 5 and 8 form a complex with the common Smad4 and translocate to the nucleus to regulate the transcription of various target genes.

Ln-1 signaling is transduced by at least two types of receptors, the integrins and the non-integrin receptor, α-dystroglycan (α-DG). The integrins bind to the global modules 1–3 of laminin [13]. In addition to their ability to link cells to their extracellular microenvironment, integrins transduce cellular signals via association of their cytoplasmic domains with signal transduction cascades [14]. All known epithelial integrin receptors for laminin are among the isoforms composed of α_6, α_3, β_1 and β_4 subunits [15]. Blocking Ln-1 binding to α_6 integrin by monoclonal antibody or inhibiting α_6 integrin downstream signaling through phosphatidyl-inositol-3-kinase (PI3K) with wortmannin or through MAP kinase kinase 1 (MEK-1) with PD98059 promotes β-cell differentiation in vitro [16]. Ln-1 plays an important role in proliferation and/or differentiation of many other cell types. For example, it specifically induce β-casein gene expression in mammary epithelia [17] and neuron generation from retinal neuroepithelial cells [18]. The cross region of Ln-1 selectively promotes fetal lung epithelial cell proliferation, the outer globular region of the α1 and β1 chains mediates epithelial cell polarization, and the inner globular region of the β1 chain binds to heparin sulfate proteoglycan and stimulates lumen formation [19]. α-DG is a highly glycosylated peripheral membrane glycoprotein, initially identified in muscle [20] and subsequently in other tissues including the pancreas [21], which associates with a transmembrane protein, β-dystroglycan (β-DG), a component of the dystrophin–glycoprotein complex (DGC). α-DG binds to the global module 4 of laminin [13], whereas β-DG is connected with the F-actin cytoskeleton through dystrophin [22,23]. In non-muscle tissues including the pancreas, β-DG may directly interact with the actin cytoskeleton via its cytoplasmic tail [24]. We found that heparin, an inhibitor of Ln-1 binding to α-DG, impaired pancreatic β-cell differentiation, suggesting that α-DG is essential for the survival and differentiation of precursor cells in vitro [16].

Although we had evidence that Ln-1 transduces survival, proliferation and differentiation signals for β-cell precursors through α_6 integrins and α-DG [16], the role of these signals in growth and colony formation induced in concert with BMPs [1,2] was unknown. Here, we attempt to dissect how the signals responsible for proliferation and colony formation in fetal pancreas cells are transduced by employing specific blocking antibodies to BMPs and α_6 integrins, BMP antagonists and several types of inhibitors of surface receptors and downstream molecules of Ln-1 signaling.

Materials and methods

In vitro culture of fetal pancreas cells

Pancreata were dissected from embryonic day (E) 15.5 C57/B6 mouse fetuses and dissociated into single cells as described [2]. Briefly, dissected pancreas was digested with dispase (Becton Dickinson Bioscience, Bedford, USA), 1: 2 in RPMI with 10% fetal calf serum, for 60 min at 37°C in a shaking water bath. Cells were counted in a hemocytometer and viability determined by trypan blue dye exclusion. Each fetal pancreas yielded ~110,000 viable cells (112,699 ± 16,603, $n = 20$). Dissociated cells were plated in 8-well (for colony quantitation) chamber slides (Nunc, Naperville, USA) or in flat bottom 96-well (for proliferation assay) plates (Becton Dickinson Labware, Franklin Lakes, USA) at 925 cells/mm^2 in AIM V medium supplemented with N-2 (1:100, Gibco BRL Life Technologies, Gaithersburg, USA), 500 UI/ml penicillin and 500 μg/ml streptomycin. Ln-1 (160 μg/ml), purified from murine Engelbreth–Holm–Swarm tumour basement membrane (Becton Dickinson Bioscience), was overlayed onto cells in the presence of several commercially available BMPs, functional blocking antibodies, BMP antagonists or specific inhibitors, as indicated. Cultures were incubated in 10% CO_2 90% air at 37°C for up to 6 days. Phase contrast images of colonies were photographed with an Olympus IX70 digital camera.

Growth factors, antibodies and reagents

Recombinant human BMP-4, -5 and 6 and the BMP antagonists, recombinant mouse noggin/Fc chimeric protein, chordin and DAN were all purchased from R&D Systems (Minneapolis, USA). These factors, dissolved at a concentration of 10 μg/ml in mouse tonicity phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin, were added at the start of culture.

Monoclonal mouse anti-BMP-4 (clone 66119.11) and -6 (clone 74219.11) antibodies and both IgG2b and goat anti-BMP-5 immunoglobulins were from R&D Systems. Antibodies were used at recommended concentrations. Rat monoclonal anti-α_6 integrin antibody (clone: NKI-GoH3; IgG2a) that specifically blocks Ln-1 binding to α_6 integrins [25–27] was purchased from Research Diagnostics Inc (Flanders, USA). Monoclonal mouse anti-α-DG antibody (clone VIA4-1; IgM) was purchased from Upstate Biotechnology (Lake Placid, USA).

Heparin, which binds to the global module 4 of Ln-1 and prevents the interaction of Ln-1 with α-DG [28], and