All-Trans Retinoic Acid Induces Differentiation of Ducts and Endocrine Cells by Mesenchymal/Epithelial Interactions in Embryonic Pancreas

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Retinoids during the embryonic period act as a mesenchymal inducer in many organs, including kidney, lung, central nervous system, and gut. Retinoic acid (RA) demonstrates insulinotropic effects in adult pancreas, but only a limited study has elucidated its role in pancreatic organogenesis. In this study, we have analyzed the existence of RA-signaling machinery in embryonic pancreas and evaluated its role using in vitro tissue culture experiments. Here we show the presence of endogenous retinaldehyde dehydrogenase 2 (RALDH2), the most effective RA-synthesizing enzyme, RA-binding proteins, and RA receptors (RARs) in embryonic pancreatic tissue. RALDH2 is expressed exclusively in the mesenchyme. Exogenously added all-trans-retinoic acid (atRA) in tissue culture experiments stimulated differentiation of endocrine and duct cells and promoted apoptotic cell death of acinar tissue. Furthermore, we demonstrate that atRA upregulates the PDX-1 expression. Taken together, our data suggest that atRA-mediated mesenchymal/epithelial interactions play an important role in determining the fate of epithelial cells via regulation of the PDX-1 gene, leading to the proper formation of the endocrine versus exocrine component during pancreatic organogenesis. Diabetes 52: 76–84, 2003

Several studies have pointed to the role of mesenchymal/epithelial interactions in pancreatic development and suggested the importance of diffusible mesenchyme-derived factors (1–3). However, efforts to purify these mesenchymal factors showed very limited success, leading researchers today to focus on a candidate approach for describing these factors. It is generally believed that long-lasting cross-talk between the endoderm and the mesoderm progressively commits cells to a specific fate during endoderm development (4,5). Development of the dorsal pancreas is tightly controlled by signals derived from notochord in the early stages (6). In later stages, mesenchyme-derived factors control proliferation and differentiation of the pancreatic progenitor cells and determine the proportion of endocrine versus exocrine tissue (7,8). The implication of signals from mesenchyme in the control development of exocrine tissue is well documented, but in the development of endocrine tissue, it is still highly contentious (9).

Understanding such underlying developmental processes and characterizing the factors necessary for pancreatic endocrine cell proliferation and differentiation can open the door to devise new therapeutic strategies against the incurable disease diabetes.

Vitamin A (retinol) is an essential component of diet and is important during early embryonic development (10). All-trans retinoic acid (atRA), one of the most active and physiological members of the retinoid family, has a wide spectrum of biological activities, such as cell growth, differentiation, and morphogenesis, and also acts as an important signaling molecule for mesenchymal/epithelial interactions in the development of kidney, lung, central nervous system, and gut (11–14). Considering the role of retinoids as an insulinotropic factor (15,16) or its deficiency related to the cause of type 1 diabetes (17,18), we hypothesized that RA plays an important role in endocrine cell differentiation during pancreas development. To confirm the presence of atRA and to establish its role in endocrine pancreas development and differentiation, we 1) analyzed endogenous expressions of RA synthesizing enzyme, RA receptors (RAR), and binding protein in pancreatic rudiments at various stages of development using RT-PCR and immunohistochemistry and 2) cultured embryonic day (e) 11.5 pancreatic rudiments in different concentration of exogenous atRA and evaluated its role by microscopic phenotype, immunohistochemical analysis, and electron microscopy.

Our results show that at e11.5, atRA is endogenously and exclusively present in pancreatic mesenchyme, evident by mRNA and protein expression of retinaldehyde dehydrogenase 2 (RALDH2) enzyme. This endogenous atRA may act in autocrine and/or paracrine fashion, as RARs are present in both epithelia and mesenchyme. Our in vitro model demonstrates that, in the presence of exogenous atRA, pancreatic rudiments differentiate into ducts and endocrine cells and inhibit acini. Furthermore,
atRA upregulates PDX-1, an important transcription factor in pancreatic development. These data suggest the important roles of atRA in determining the cell fate of pancreatic progenitor cells, leading the proper formation of endocrine versus exocrine pancreas during organogenesis.

RESEARCH DESIGN AND METHODS

Embryonic tissue procurement. All animal experiments were performed in accordance with the guideline for animal experiments of Kyoto University. Male and female ICR mice were mated overnight. The presence of a vaginal plug the next morning was indicative of pregnancy, and noon of that day was designated as day 0.5. Dorsal embryonic pancreata were harvested on e11.5, e12.5, e14.5, and e16.5 using micromanipulation techniques described previously (19). Two types of tissue were procured from e11.5 rudiments for in vitro culture, epithelia only (PE) and epithelia with mesenchyme (PEM).

Tissue culture. E11.5 rudiments (PE or PEM) were grown under various culture conditions in 0.4-μm Millipore filter inserts containing 150 μl collagen I gel (Vitrogen; Cohan, Palo Alto, CA) or basement-rich gel (Matrigel; Collaborative Research, Bedford, MA) and placed in a standard 24-well plate under sterile conditions. Each well was filled with 500 μl Dulbecco’s modified Eagle’s medium F-12 (Gibco BRL, Grand Island, NY) containing 10% fetal bovine serum and 1% antibiotic/antimycotic solution (10,000 units/ml penicillin G, 10,000 μg/ml streptomycin sulfate, and 20 μg/ml amphotericin B [Gibco BRL]). Three different final concentrations (1, 10, and 100 μM) of atRA (Sigma) were supplemented later in the media and replaced every 48 h. Control tissues were cultured in collagen I or Matrigel with media and collected in glass slides. One of two consecutive sections was analyzed (to avoid counting the same cell twice) by immunohistochemistry for the given antigen. Only immunoreactive cells with visible nucleus were counted. The sum of β- and α-cells was regarded as total endocrine cells. For ductal/acinous cells, three different sections were randomly selected and counted by point counting using Densitograph software version for Macintosh. The results were obtained by quantifying at least four pancreatric rudiments in each category. These were compared and analyzed by ANOVA, and P < 0.05 was considered statistically significant.

Electron microscopy. Tissues were fixed in modified Karnovsky’s fixative (2% paraformaldehyde, 1.5% glutaraldehyde in 0.1 mol/1 cacodylate buffer pH 7.4) for 1–4 h, postfixed in 1% osmium tetroxide for 1 h, dehydrated in graded ethanol, and embedded in Epoxy. Thick sections were cut 0.5–1 μm and stained in toluidine blue. Thin sections were overlaid in copper grid, stained by uranyl acetate and lead citrate, and viewed with a Hitachi electron microscope. RT-PCR. RNA was extracted from pooled e11.5 epithelia and mesenchyme separately, e12.5 PEM, e14.5 PEM, e16.5 PEM, and e18.5 PEM using the SV Total RNA isolation kit (Promega, Madison, WI), and cDNA was prepared from 1 μg RNA by using RT and random hexamer (Advantage RT-for-PCR kit; Clontech, Palo Alto, CA) according to the manufacturer’s instructions. PCR was carried out with a mixture consisting of 100 ng cDNA, 20 pmol each of forward and reverse primers, 2 mmol/l dNTP mix, 10× PCR buffer (100 mmol/l Tris-HCl [pH 8.8], 500 mmol/l KCl, 0.8% NP40) with 15 mmol/l MgCl2, and recombinant Taq DNA polymerase (MBI fermentas, Vilnius, Lithuania). β-Actin was used as internal control, cDNA prepared without RT was used as negative control, and adult mouse liver for RARs (RARα, RARβ, and RARγ) and chicken ovalbumin upstream promoter transcription factor (COUP-TF II), and adult mouse lung for RARs and RARγ were used. The expression of mRNAs coding for RARs were analyzed using Microm HM 505E cryostat. Most of the sections were pretreated with 1 mol/l HCl at 37°C for 4–7 days under sterile conditions. Each well was filled with 500 μl Dulbecco’s modified Eagle’s medium F-12 (Gibco BRL, Grand Island, NY) containing 10% fetal bovine serum and 1% antibiotic/antimycotic solution (10,000 units/ml penicillin G, 10,000 μg/ml streptomycin sulfate, and 20 μg/ml amphotericin B [Gibco BRL]). Three different final concentrations (1, 10, and 100 μM) of atRA (Sigma) were supplemented later in the media and replaced every 48 h. Control tissues were cultured in collagen I or Matrigel with media alone or media with DMSO (the diluent for atRA). In addition, in a different series, the same three concentrations of atRA were added only on the 6th day of culture and harvested after 24 h. All of the tissues were cultured in 5% CO2 at 37°C for 4–7 days.

Immunohistochemistry. Harvested tissues were fixed in 4% paraformaldehyde for 4 h then cryoprotected overnight in 30% sucrose, embedded in Tissue Tek OCT compound, frozen in liquid nitrogen, and cut into 6-μm sections using Microm HM 505E cryostat. Most of the sections were pretreated with peroxidase blocking reagent (Dako, Carpenteria, CA) to minimize nonspecific binding to endogenous peroxidase enzymes. Primary antibodies to the following antigens were used at the indicated dilution: insulin (guinea pig anti-swine; Dako, Kyoto, Japan); glucagon (rabbit anti-human; Dako) 1:600, cytokeratin MNF 116 (mouse anti-human; Dako) ready to use, carbonic anhydrase II (anti-human; Dako, Tokyo, Japan) 1:100, β3 integrin (mouse anti-human; Dako) according to the manufacturer’s instructions. PCR reactions were performed as follows: 1 cycle of 94°C for 5 min; then 40 cycles of 94°C for 30 s, annealing temperature (AT) for 30 s, 72°C for 30 s; and finally 1 cycle of 72°C for 7 min. Products of amplification were separated on 2% agarose gel and photographed after ethidium bromide staining. Each positive result was confirmed by repeating at least three times. We selected primers using published criteria (20), and AT for each primer set was calculated using conventional formulas. Primer sequences used are listed as forward then reverse 5’ to 3’.

β-actin primers 5’GGGCTGCTGATGACTCCG3’ and 5’GCTGGAGGTTGGCACAGGGC3’ amplify a product of 612 bp; RARα primers 5’CATGTTGCGAGAGTTGAGTACCC3’ and 5’TACACCTGTTCTCTCGATTCG3’ amplify a product of 367 bp; RARβ primers 5’TGGACAGACAGTACACG3’ and 5’GAAAAGGCTTGGTACAC3’ amplify a product of 155 bp; RARγ primers 5’GCCTCCTGGGTCCTACAG3’ and 5’ATGATACATTGTGTTCGGG3’ amplify a product of 155 bp; RXRα primers 5’TCAACCTCACAGTGTCGTC3’ and 5’AAAAACCAATGTTGTCG3’ amplify a product of 174 bp; RALDH2 primers 5’TTCGAGAGCTGACCTGCCAC3’ and 5’TCTGAGAAGCTCCGTCA3’ amplify a product of 147 bp; Brdu primers 5’TGGTAGAAGCTTGGACTG3’ and 5’TCTGAGAAGCTCCGTCA3’ amplify a product of 155 bp; and RXRβ primers 5’GCTGGAGGTTGGCACAGGGC3’ amplify a product of 200 bp. Brdu primers 5’TGATGAGAAGCTTGGACTG3’ and 5’TCTGAGAAGCTCCGTCA3’ amplify a product of 147 bp; and RXRβ primers 5’TGATGAGAAGCTTGGACTG3’ and 5’TCTGAGAAGCTCCGTCA3’ amplify a product of 155 bp; and RXRβ primers 5’TGATGAGAAGCTTGGACTG3’ and 5’TCTGAGAAGCTCCGTCA3’ amplify a product of 147 bp.

RESULTS

Endogenous expression of retinoids in pancreatic rudiments. We analyzed the mRNA and protein expressions implicated in retinoid metabolism. The expression of RA synthesizing enzyme RALDH2 mRNA was detected exclusively in mesenchyme at e11.5 (Fig. 1 I). This also held true at the posttranscription level, but the expression level was very low compared with that in the mesenchyme of stomach at e11.5 and 13.5 (Fig. 1 IIC and IF). The RALDH2 mRNA was also determined in PEM rudiments throughout the rest of the developmental period (Fig. 1 I). The expression of mRNAs coding for RARs were analyzed next in e11.5 rudiments. RARα was expressed in epithelium, with very low expression in mesenchyme; RARγ was expressed only in the mesenchyme; and RARβ expression was not detected at all. The RA binding protein CRABP II was detected only in mesenchyme, and strong expression of RARs suppressor receptor COUP-TF II was seen in mesenchyme with very low expression in epithelia (Fig. 1 I). PDX-1 was used as a pancreatic epithelia marker in
both RT-PCR and immunohistochemistry, demonstrating a good separation of epithelium and mesenchyme in these procedures (Fig. 1 I and IIB and E). 

**Culture in three-dimensional gel systems and exogenous atRA treatment.** First, e11.5 PE rudiments were cultured in a three-dimensional gel system of collagen I or matrigel as controls. PE did not survive in collagen I gel but developed into cystic ductal structures when cultured in matrigel (Fig. 2A, C, and E). When exposed to exogenous atRA, the rudiments survived but failed to show overt differentiation in collagen I gel, but those cultured in matrigel showed very early duct differentiation with increase in endocrine cells (Fig. 2B, D, and F). The increase in endocrine cells was quantitative and statistically significant ($P < 0.05$; Fig. 5A).

PEM control rudiments underwent various morphological changes redolent of normal development in vivo when cultured in these two gel systems. They showed active cell proliferation as shown by BrdU staining (Fig. 4G). At day 4 of culture, endocrine cells were present as solitary cells, PDX-1 expression was seen both in acinar and endocrine cells, and acinar cells began to appear. By day 7, endocrine cells began to coalesce (Fig. 3D and G), acinar differentiation became more pronounced (Fig. 4D), and PDX-1 expression was seen almost only in insulin-producing cells (Fig. 3F). Ductal differentiation was seen more in matrigel specimen, probably owing to the presence of insoluble basement membrane components (e.g., laminin, type IV collagen, and heparan sulfate proteoglycans). These observations are analogous to the findings described in the previous experiment (19).

PEM rudiments treated with exogenous atRA showed increase in the expression of PDX-1 and the number of endocrine cells in a dose-dependent manner (Fig. 3E, F, H, I, K, and L). PDX-1 expression was detected in the ducts and endocrine cells at day 4 but almost only in insulin-producing cells after 7 days of culture (Fig. 3K and L). Most of the differentiated endocrine cells were in islet orientation (i.e., spheroidal in shape with insulin-producing cells occupying the center), few were seen as isolated solitary in or emerging from the ducts and coexpressed with PDX-1 (Figs. 3L and 6E and F). The maximum numbers of endocrine cells were seen at the 10 $\mu$mol/l concentration of atRA, and the proportion was more than double the control rudiments. The differences were significant at the 95% confidence level with $P < 0.0001$. However, no significant differences were found between the proportions of endocrine cells in rudiments cultured in the matrigel or collagen I gel system treated with exogenous atRA (Fig. 5B). Similarly, the pervasiveness of cystic structures increased commensurately with increasing doses of atRA (Figs. 3B and C and 5D). These cystic structures were confirmed to be ducts by cytokeratin and CA II staining (Fig. 6A and B). On the contrary, acinar differentiation became sparse in rudiments treated with increasing doses and completely arrested at a higher (100 $\mu$mol/l) dose of atRA as validated by $\alpha$-amylase staining (Figs. 4C and F and 5D). At 100 $\mu$mol/l, BrdU-positive proliferating cells were seen mainly in ducts, protodifferentiated endocrine cells, and a few fully differentiated endocrine cells (Fig. 4J–L).

**Electron microscopy of atRA-treated pancreas.** The controls showed mostly acinar components, identified by pyramidal epithelial cells containing a large number of secretory granules compatible with zymogen granules in the apical cytoplasm, and crowded with closely spaced
parallel cisternae of granular endoplasmic reticulum in the basal region. However, in rudiments treated with atRA, large numbers of duct cells were prevalent and were identified by their typical simple epithelium, uniformity in cellular and nuclear size, and more condensed nuclear chromatin. The cytoplasm was scanty with poorly developed cytoplasmic organelles. The apical surface projected short microvilli, and lateral surfaces of neighboring duct cells exhibited more interdigitations (Fig. 6C). The endocrine cells were identified by size and shape of endocrine granules and were different from acini or duct cells. A few of the duct cells also exhibited some endocrine granules in their cytoplasm. The ultrastructural findings were compared with previously published articles (21,22) and were compatible with the immunohistochemical findings shown above.

**atRA pulse treatment showed overexpression of pro-apoptotic protein and PDX-1 in acinar cells.** Analysis of cultured pancreas with atRA added on day 6 of culture showed profound dysmorphogenesis of acinar components (Fig. 7E). These cells demonstrated overexpression of pro-apoptotic protein Bax in their cytoplasm (Fig. 7G) with increase in ssDNA-positive nuclear fragmentations (Fig. 7H). However, the endocrine cells were almost free from the apoptotic effects of atRA. There was almost no expression of antiapoptotic protein Bcl-2 or Bcl-xL in atRA-treated rudiments (data not shown), and no obvious signs of Bax or ssDNA expression were noted in the controls (Fig. 7C and D). Many PDX-1 overexpressing acinar cells as well as endocrine cells with preislet morphology were seen in atRA-treated samples (Fig. 7F). The quantitative study demonstrated the significant increase in the number of PDX-1–positive cells (Fig. 7).

**DISCUSSION**

RA has been well acknowledged as a signaling molecule for mesenchymal/epithelial interactions of various organs (11–14), and here we have demonstrated its role in the development of pancreas, especially in differentiation of ducts and endocrine cells. Our study focuses on e11.5 pancreatic rudiments when the local mesenchyme is well developed and yet can be separated mechanically from epithelium. This gives a good ground to explore the type of signaling molecules involved in the interaction between these two surfaces.

Mesenchymal interaction could be due to secretion of inducing or transforming factors, production of extracellular matrix, and information exchanged through cell-to-cell contact during development. The ability of exogenous...
atRA to induce differentiation in both PE and PEM and its exclusive endogenous expression in mesenchyme suggests that it is one of the candidates of mesenchymal factors for endocrine differentiation. This hypothesis supports the permissive effect of mesenchyme on the development of the endocrine pancreas (23).

**Endogenous expression of retinoids suggest importance during pancreatic development.** RA is synthe-

FIG. 3. Induction of endocrine cells in atRA-treated PEM. E11.5 PEM were cultured in either collagen I gel (A–C and G–L) or matrigel (D–F). At day 7 of culture controls (A, D, G, and J) or atRA-treated 10 μmol/l (B, E, H, and K) and 100 μmol/l (C, F, I, and L). Hematoxylin and eosin staining (A–C); insulin (D–I); PDX-1:insulin double staining (J–L). Note the proliferation of cystic structures and induction of endocrine cells (in both gel systems) with increasing dose of atRA. Insulin cells are PDX-1 positive. Magnification ×100.

FIG. 4. Decrease of acini and BrdU-positive proliferating cells in atRA-treated PEM. A, D, and G: controls. B, E, and H: 10 μmol/l (C, F, and I–L) and 100 μmol/l atRA-treated rudiments at day 4 (A–C) and at day 7 (D–L). Amylase staining (A–F), BrdU staining (G–I), and insulin:BrdU double staining (J–L). Note the inhibition of acinar components with increasing dose of atRA. At 100 μmol/l concentration, acinar differentiation was completely arrested (C and F). Some of the ductal structures in atRA-treated rudiments exhibited positive amylase staining (white arrow heads). Therefore, the acino-ductal transdifferentiation may be the most likely cause of ductal induction. BrdU staining shows that the active proliferating cells are present in both control and atRA-treated rudiments. However, at a higher dose of atRA, the proliferative cells are confined mostly to ductal and endocrine compartment. J–L: Insulin:BrdU double staining shows active proliferating cells mostly in ducts (white arrow) and endocrine cells at protodifferentiated (green arrow head) or fully differentiated (green arrow) stage. Magnification ×100 in A–J, ×200 in J, and ×400 in K and L.
sized from retinaldehyde mostly by NAD-dependent dehydrogenase (24). Among the three dehydrogenases, RALDH2 exhibits the greatest substrate specificity, and its distribution provides the most accurate guide to the localization of atRA in the embryonic tissues (25,26). Our highly sensitive RT-PCR and immunohistochemistry results show that RALDH2 is expressed solely in the mesenchyme at e11.5 (Fig. 1 I and II C and F) and in whole pancreas throughout the rest of the developmental period. This result is similar to the findings that RALDH2 is widely expressed in mesenchyme in developing gut including the foregut (27,28).

The expression of CRABP II in mesenchyme also illustrates the dominant role of mesenchyme in retinoid metabolism; in addition, its expression contributes to the synthesis of RA because it has been shown to be associated with cells that synthesize RA (29,30). CRABPs may have some role in development of islets because they are localized in adult islets (31), and transgenic mice overexpressing CRABP I die prematurely as a result of tumor originating from islet of Langerhans (32).

Retinoid signaling is transduced by two families of nuclear receptors, RAR (\(\alpha, \beta, \gamma\)) and RXR (\(\alpha, \beta, \gamma\)) isoforms. All-trans form is the natural ligand for the RAR, and 9-cis form is the natural ligand for RXR, although the latter binds to both receptor families (26). A previous article by Kadison et al. (33) showed that at e12.5, RAR\(\alpha\) expression is seen only in mesenchyme and RAR\(\beta/RAR\gamma\) in epithelia by Western blot analysis, but our highly sensitive RT-PCR results at e11.5 show few differences. Here we have tried to be meticulous by using absolute epithelial marker PDX-1 and appropriate positive controls to derive our conclusions. Therefore, these discrepancies could be due either to the method and time of analysis or to the quality of samples. The expression of RARs in both epithelia and mesenchyme raises the possibility that en-
endogenous atRA acts in both an autocrine and a paracrine manner through these receptors. However, the strong expression of RAR repressor COUP-TF II in mesenchyme suggests that endogenous RA primarily prefers paracrine action because most of the autocrine actions may be repressed by these orphan receptors in mesenchyme (34).

Induction of endocrine and ductal differentiation but inhibition of acini by atRA. Our ex vivo observations demonstrated the endogenous presence of retinoids. How does this RA affect the pancreatic development and differentiation? To discern the answer, we cultured undifferentiated e11.5 pancreatic rudiments at different concentration of exogenous atRA. The results obtained upon atRA administration in the three-dimensional organ culture system strongly suggest that it plays an active role in the development of pancreas. Besides the regressive effect on acinar differentiation, both quantitative and significant augmentation of endocrine cells at the posttranscriptional level was appreciated. The increase in differentiation of ductal structures (confirmed by cytokeratin/CAII staining and electron microscopy) is also important because they are necessary for branching morphogenesis and for origin of endocrine cells. In our model, endocrine cells are seen to originate from newly differentiated ducts, which was validated by double staining of insulin and BrdU/PDX-1 (Fig. 6D–F). This finding resembles and supports the in vivo and in vitro model of endocrine pancreas development (35,36). Kadison et al. (33) showed that exogenous atRA and 9-cis RA have completely different ontogenic effects during pancreas differentiation. However, few comparative studies between RA isomers have shown 9-cis to be almost 10 times more potent than the all-trans form (37,38). Our results also suggest that atRA has a similar type of ontogenic effects as 9-cis RA at a higher dose.

Pancreatic lineage selection by atRA. With sharp changes in the fate of pancreas cultured under the influence of exogenous atRA, we tried to seek the mechanisms underlying the inhibition of acinar cells and induction of ducts or endocrine cells. Our results indicate that upregulation of PDX-1 by atRA plays the central role on these phenotypic selections. The possible underlying mechanism for inhibition of acini is via apoptosis. The PEM rudiments pulse treated with atRA 24 h before harvesting showed dysmorphogenesis of acinar cells and overexpression of the proapoptosis protein Bax in their cytoplasm with increase in anti-ssDNA positive DNA fragmented cells. The demonstration of apoptosis in these cells could be due to the direct effect of atRA itself or the upregulation of PDX-1, because persistent upregulation of PDX-1 destroys the acini population by fatty infiltration and apoptosis (39).

Acinoductal transdifferentiation is the most likely cause of ductal induction in atRA-treated rudiments (40). This hypothesis is supported by some amylase-positive ducts in atRA-treated rudiments and duct cells exhibiting increase in PDX-1 expression.

The mechanism for induction of endocrine cells may be due either to stimulating the expression of insulin mRNA levels probably by binding to the RARE located upstream of the insulin gene enhancer (41) or to the induction in endocrine transcription factors by atRA. RA has been implicated in foregut A-P patterning through expression of homeobox genes (42,43). The homeobox gene PDX-1 plays a critical role in development of pancreas (44,45). Recent
studies show that atRA induces the XIHbox-8 gene in *Xenopus* (46,47), the amphibian homologue of PDX-1. In our model, the effect of atRA on induction of endocrine cells seems to be partially mediated by the upregulation of PDX-1 gene. There is a growing list of experiments in different organs, cell lines, and stem cells, where the role of RA is as an inducer of pancreatic endocrine transcription factors. The role of Pax-6 in RA signaling of the developing eye (48), Xlim-1 (*Xenopus* equivalent to human Lim-1) mRNA induction by RA (49), and upregulation of Neuro D on exposure of RA (50) are a few of these examples. However, more studies are needed to clarify the detailed relationship between RA and these endocrine transcription factors during pancreas development.

In conclusion, the overall findings demonstrate that atRA is an important signaling molecule in the development of the embryonic pancreas and plays a great role in differentiation of endocrine and ductal cells, predominately through paracrine actions and upregulation of PDX-1 gene. Understanding the full picture of the developmental process of the endocrine pancreas is still incomplete, but we believe that identification of signaling molecules, information on their proliferative or inductive signals, and elucidating their roles with transcription factors will certainly unravel the greater details in the future.

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