Cross-talk between Bone Morphogenetic Protein and Transforming Growth Factor-β Signaling Is Essential for Exendin-4-induced Insulin-positive Differentiation of AR42J Cells*

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A key goal of cellular engineering is to manipulate progenitor cells to become β-cells, allowing cell replacement therapy to cure diabetes mellitus. As a paradigm for cell engineering, we have studied the molecular mechanisms by which AR42J cells become β-cells. Bone morphogenetic proteins (BMPs), implicated in a myriad of developmental pathways, have not been well studied in insulin-positive differentiation. We found that the canonical intracellular mediators of BMP signaling, Smad-1 and Smad-8, were significantly elevated in AR42J cells undergoing insulin-positive differentiation in response to exendin-4 treatment, suggesting a role for BMP signaling in β-cell formation. Similarly, endogenous BMP-2 ligand and ALK-1 receptor (activin receptor-like kinase-1) known to activate Smads 1 and 8 mRNAs were specifically up-regulated in exendin-4-treated AR42J cells. Surprisingly, Smad-1 and Smad-8 levels were suppressed by the addition of BMP-soluble receptor inhibition of BMP ligand binding to its receptor. Here, insulin-positive differentiation was also ablated. BMP-2 ligand antisense also strongly inhibited Smad-1 and Smad-8 expression, again with the abolition of insulin-positive differentiation. These results demonstrate a previously unrecognized key role for BMP signaling in mediating insulin-positive differentiation through the intracellular Smad signaling pathway. In short, BMP signaling may represent a novel downstream target of exendin-4 (glucagon-like peptide-1) signaling and potentially serve as an upstream regulator of transforming growth factor-β isoform signaling to differentiate the acinar-like AR42J cells into insulin-secreting cells.

Type 1 diabetes is an insulin deficiency state due to pancreatic destruction of β-cells caused by autoimmunity. Several approaches to treat diabetes are being pursued, such as islet cell transplantation, pancreatic transplantation, and genetic manipulation. However, a key alternative strategy is cellular engineering to manipulate progenitor cells to become β-cells, allowing cellular therapy to cure diabetes. As a paradigm for cell engineering, we have used exendin-4 treatment of AR42J cells, a fairly plastic acinar cell carcinoma-derived cell line, as a model for studying the role of bone morphogenetic protein (BMP)2 signaling in the induction of insulin-positive differentiation. Exendin-4, a peptide from Helodermatidae venom, is a novel insulinotropic agent and a long-acting analogue of glucagon-like peptide-1 (GLP-1). It interacts with endocrine pancreatic islet GLP-1 receptors, inducing a stimulatory effect on insulin secretion. Over the past few decades significant progress has been made in our understanding of the biological function of BMPs, which have been found to regulate a myriad of developmental and differentiation process in the embryo, including epithelial-mesenchymal interactions, cell fate specification, dorsoventral patterning, and apoptosis as well as the secretion of extracellular matrix components (1–5).

BMPs are one of the multifunctional cytokines from the transforming growth factor-β (TGF-β) superfamily. The TGF-β isoforms proper (TGF-β1, -β2, and -β3) are also included in this superfamily. The canonical pathway for the pleiotropic biological effects of BMPs is signaling through the type I (activin receptor-like kinases ALK-2, ALK-3, and ALK-6) and type II serine/threonine kinase receptors (BMP receptor type II and the activin receptors ActR-IA and ActR-IIb) and their downstream effectors, known as Smad-1, Smad-5, and Smad-8 (6–9). Upon ligand stimulation, these receptor-regulated Smads become phosphorylated by activated type I receptor kinases and form heteromeric complexes with the shared common mediator Smad-4. Subsequently, these Smad complexes translocate into the nucleus, where they regulate the transcription of target genes (10, 11).

Recently, we have reported that exendin-4-induced differentiation of the β-cell-like phenotype in AR42J cells requires TGF-β isoform (TGF-β1, -β2, and -β3) signaling initially in the form of Smad-2 and followed by Smad-3. Smad-3 appears to play a secondary role in suppressing further increase in insulin mRNA and may facilitate β-cell-like maturation (12). Given the frequent interplay of function between TGF-β isoform signaling and BMP signaling, here we studied the possible role of BMP signaling in exendin-4-induced differentiation of AR42J cells into insulin-positive cells.

We observed a hierarchy of BMP signaling to TGF-β isoform signaling. Taken together with previous findings, our results suggest that GLP-1 stimulation of insulin-positive differentiation in AR42J cells acts first via BMP ligands and Smads, followed by TGF-β isoform signaling.

MATERIALS AND METHODS

Reagents and Kits—Exendin-4 was obtained from Sigma-Aldrich. The RNeasy mini kit, the Sensiscript reverse transcriptase kit, and the QiAquick gel extraction kit were all from Qiagen (Valencia, CA). AmpliTaq Gold with GeneAmp 10× PCR Buffer and MgCl2 solution was from Applied Biosystems (Foster City, CA). The F-12K nutrient mixture (Kaighn’s modification) was from Invitrogen.
Antibodies/Ligands—BMP-soluble receptor 1B (BMP-R1B) antibodies from Sigma-Aldrich were at a concentration of 3 μg/ml, which is defined as the effective concentration for inhibiting alkaline phosphatase production. In additional experiments, BMP-2 (obtained from R&D Systems, Minneapolis, MN) was measured by its ability through three different doses (10 pg/ml, 100 pg/ml, and 1 ng/ml) to rescue or augment the system. TGF-β1, purchased from Sigma-Aldrich, was added at a final concentration of 10 ng/ml, which was chosen with reference to the previous synergistic effect data as those required to enhance TGF-β activity.

Morpholino Oligos—Antisense morpholino oligos complementary to Smad-1 (5'-AGGATGGACGACATGACTGGCACTC-3'), ALK-1 (5'-CTTCCGAAAATCCCAGGTCATG-3'), BMP-2 (5'-TCGACTTTTAGAGACCCGACTCC-3'), and missense control oligos were obtained from Gene Tools (Philomath, OR). The scrape delivery method was performed, and the scraped cells along with morpholino oligos were incubated over the weekend in a 5% CO2 incubator, which gives the best result in our system.

List of PCR Primer Sequences—Given here are the PCR primer sequences for the following genes: β-actin, 5'-CTAAGGCCAACCGT-GAAAAG-3' (left primer) and 5'-ACCCCTCATAGATGGGCAC-3' (right primer); insulin II, 5'-GGGAGCGTGGATTCTTCTAC-3' (left primer) and 5'-CCTTCGGAAAATCCCCAGGGTCATG-3' (right primer); Pdx-1, 5'-GAAATCCACCAAAGCTCACG-3' (left primer) and 5'-GAATTCCTTCTCCAGCTCCA-3' (right primer); Pax-4, 5'-CTCGAATTGCCCAGCTAAAG-3' (left primer) and 5'-CCCGAGACTCGATTGATA-3' (right primer); Pax-6, 5'-TCAGCTTTGCTCAGTGAATGTT-3' (left primer) and 5'-ACTCACACATCCGTTGGCA-3' (right primer); Smad-1, 5'-CGGAACTGCAACTACCACCA-3' (left primer) and 5'-GACTGCGCCAGTAGCTGAGC-3' (right primer); Smad-5, 5'-AACTTTCACCATGGCTTCCA-3' (left primer) and 5'-CCAGAAGCTGAGCAAACTCC-3' (right primer); and Smad-8, 5'-GTATCATCGCCAGGATGCAGTCA-3' (left primer) and 5'-TGTGGGGAGCCCATTAGT-3' (right primer).

Cell Culture and Treatment—AR42J cells, purchased from American Type Culture Collection (Manassas, VA), were grown in Kaighn’s modification of Ham’s F-12K medium with 2 mM l-glutamine, 250 μg/ml amphotericin, 100 units/ml penicillin, 100 μg/ml streptomycin, and 20% fetal bovine serum at 37 °C under a humidified condition of 95% air and 5% CO2. Cells were plated at a density of ~10^4 cells/ml in 12-well plates. Morpholino antisense or missense control was added separately to culture media at 20 μM. Cells were then cultured with exendin-4 at doses of 1, 5, and 10 pM for 3 days.
Reverse Transcription PCR (Non-quantitative)—Total RNA was extracted from cells and treated with DNase. RNA was subjected to reverse transcription. cDNA was then amplified by PCR for 40 cycles. All PCR products were separated by electrophoresis in 2% agarose gel. The PCR cycles were as follows: initial denaturation at 95 °C for 10 min followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and final extension at 72 °C for 10 min.

SYBR Green Real Time Quantitative PCR—PCR amplifications were performed using a Bio-Rad iCycler (Hercules, CA) sequence detection system. Reactions were performed in a 50-μl reaction mixture that included 10× AmpliTaq Gold buffer, 25 mM MgCl₂, 2.5 mM dNTPs, 10× SYBR Green, AmpliTaq Gold polymerase, distilled H₂O, DNA template, and 10 μM each primer. Amplification was performed by initial polymerase activation for 10 min at 95 °C and 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s, and elongation for 30 s at 72 °C.

Western Blot Analysis—Proteins were separated on a 10% Tris-HCl Ready Gel (Bio-Rad), transferred onto nitrocellulose membranes, and incubated with a β-actin antibody (Abcam, Cambridge, MA) at a dilution of 1:5000, a Smad-1 antibody (Upstate Biotechnology) at 4 μg/ml, an ALK-1 antibody (Abcam) at 0.2 μg/ml, or a BMP-2 antibody (Abcam) at 2 μg/ml overnight at 4 °C. After incubation, the membranes were washed twice for 15 min in washing buffer (phosphate-buffered saline and 0.05% Tween 20) and incubated with a secondary anti-mouse (β-actin/BMP-2), anti-rabbit (Smad-1), or anti-goat (ALK-1) antibody coupled to horseradish peroxidase (Vector Laboratories, Burlingame, CA) for 1 h at room temperature. The membranes were then washed three times for 15 min in washing buffer, and immunoreactivity was normalized by chemiluminescence (Amersham Biosciences ECL Plus kit, RPN2132) according to the manufacturer’s instructions.

RESULTS AND DISCUSSION

Involvement of BMP Smads in Insulin-positive Differentiation—We found previously (12) that TGF-β isoforms and their Smads (-2 and -3) were essential for mature β-cell formation from AR42J cells treated with exendin-4 (12). Thus, we also wished to study a potential role for BMP signaling in the induction of this insulin-positive differentiation. Because Smad-1, Smad-5, and Smad-8 are receptor-regulated Smads in the BMP signaling pathway, we first examined the possible involvement of those Smads in the insulin-positive differentiation of AR42J cells induced by exogenous exendin-4. Interestingly, Smad-1 and Smad-8 mRNA levels were greatly elevated in response to exendin-4 treatment, whereas Smad-5 mRNA levels dropped precipitously from the base line (Fig. 1). These changes are reminiscent of those seen for Smad-3 and Smad-2, respectively (12).

FIGURE 3. Effect of BMP-soluble receptors on exendin-4-treated AR42J cells. Insulin II mRNA (A) increases were blunted in the presence of BMP-R1B-soluble receptors (A). Similar effects were seen on PDX-1 (B) and Pax-4 mRNA (C), suggesting that BMP isoform signaling was essential for β-cell maturation. The soluble receptors had an inhibitory effect on Smad-1 (D), Smad-3 (F), and Smad-8 (H). However, no apparent effect on Smad-2 (E) and Smad-5 (G) was seen (means ± S.D., n = 4).
FIGURE 4. Role for TGF-β type 1 receptor ALK-1 in mediating exendin-4-induced insulin differentiation. Insulin II mRNA (A) levels were totally inhibited by ALK-1 antisense, even below the base line. Similar effects on PDX-1 (B) and Pax-4 (C) were seen. Interestingly, Smad-1 (D) and Smad-8 (H) mRNA levels were also suppressed, but little effect was seen on Smad-5 (G). The TGF-β isoform target Smad-2 (E) was unaffected, whereas elevations in Smad-3 (F) were inhibited (means ± S.D., n = 4). A Western blot for ALK-1 in exendin-4 treated cells with ALK-1 antisense and missense (I) confirms protein knock-down (n = 4).
FIGURE 5. Effect of BMP-2 antisense on insulin-positive differentiation. Insulin II mRNA (A) was eliminated by BMP-2 antisense. Similar suppression was seen for PDX-1 (B) and Pax-4 mRNAs (C). Marked suppression of the BMP-2 downstream Smads, Smad-1 (D) and Smad-8 (H), was also seen. Exendin-4-induced suppression of Smad-2 (E) and Smad-5 (G) had minimal effect, but the suppression of Smad-3 mRNA (F) had great effect (means ± S.D., n = 4). A Western blot for BMP-2 in exendin-4 treated cells with BMP-2 antisense and missense (I) confirms sequence-specific effect of the antisense (n = 4).
Role of BMP Ligands and BMP Receptors in Exendin-4-induced β-Cell Differentiation—Simple PCR was performed to screen for BMP ligands as well as potential type I receptors (Fig. 2). ALK-1 is a type I TGF-β superfamily receptor that is thought to activate only Smad-1 and Smad-5 and transmits BMP-like signals (even though it is thought to serve as a type I receptor for TGF-β isoforms) (9, 18). ALK-3 and ALK-6 (also termed BMPR-IA and BMPR-IB, respectively) are type I receptors for BMPs that activate Smad-1, Smad-5, and Smad-8. BMP-2, BMP-4, and BMP-7 ligands bind with high affinity to ALK-3 and ALK-6, whereas BMP-7 only binds to the ALK-2 receptor, which then activates Smad-1, Smad-5, and Smad-8 (6–8).

The key result was that ALK-1 was up-regulated from undetectable at baseline to strongly positive in the exendin-4-treated AR42J cells. ALK-2, ALK-3, and ALK-6 were present at baseline and then decreased with the exendin-4 treatment. BMP-2 was negative at baseline and then up-regulated with the exendin-4 treatment. BMP-4 and BMP-7 were expressed in both baseline and exendin-4-treated cells. It is possible that BMP-2, which turns on in response to the stimulus, could then form a heterodimer with BMP-4 or BMP-7 and become stimulatory, possibly signaling through ALK-1. BMP-4 or BMP-7 alone may be inhibitory. (Fig. 2)

We then used BMP-soluble receptors as BMP ligand inhibitors to study a potential role for endogenous BMP signaling in exendin-4-induced insulin-positive differentiation of AR42J cells. BMP soluble receptors inhibited insulin expression and also blocked PDX-1 and Pax-4 expression similarly as in our previous findings with TGF-β-neutralizing antibodies (Fig. 3 A–C) (12). Then, mRNA levels of Smad-1, Smad-2, Smad-3, Smad-5, and Smad-8 in the BMP-soluble receptor-treated cells were tested (Fig. 3, D–H). There was suppression of Smad-1 and Smad-8 mRNA, implying that the soluble receptors had blocked BMP downstream signaling (Fig. 3, D and H). Prevention of the normal rise in Smad-3 mRNA that occurs in response to TGF-β isoform signaling (Fig. 3F) suggests that BMP signaling is acting upstream of TGF-β

**FIGURE 6.** Insulin-positive differentiation in response to exogenous BMP-2 after treatment with BMP-2 antisense. Insulin mRNA (A) levels in exendin-4 treatment with increasing doses of exogenous BMP-2 rescue and BMP-2 antisense treatment are shown. PDX-1 (B) and Pax-4 (C) levels were affected similarly as those of insulin II. Levels of Smad-1 (D), Smad-3 (F), and Smad-8 (H) mRNA were all rescued in parallel to insulin II, suggesting that BMP-2 is a key regulator of insulin through these Smads. Down-regulation of Smad-2 (E) and Smad-5 (G) expression was unaffected by BMP-2 (means ± S.D., n = 4). MS, missense; AS, antisense.
isoform signaling. In Smad-2 and Smad-5, the decrease that normally occurs with exendin-4 was not affected (Fig. 3, E and G).

ALK-1 is an activator of BMP Smads, although it can bind TGF-β isoforms and Müllerian inhibiting substances. We used ALK-1 morpholino antisense and blocked insulin-positive differentiation and the accompanying PDX-1 and Pax-4 up-regulation (Fig. 4, A–C). Elevations in Smad-1 and Smad-8 mRNA were again inhibited (Fig. 4, D and H), whereas Smad-5 mRNA levels were barely affected (Fig. 4G). Interestingly, Smad-2 suppression was unaffected (Fig. 4E), but elevations in Smad-3 mRNA levels were blocked (Fig. 4F). These results suggest that ALK-1 morpholino blocks BMP Smad activation, which then leads secondarily to inhibition of the elevation of Smad-3 mRNA levels. These results would again imply that TGF-β isoform signaling is downstream of BMP signaling. To confirm the effects of the morpholino ring antisense on Smad levels, Western blotting was performed in morpholino ring antisense-treated and sense-treated controls, confirming sequence-specific effects in target Smads by antisense (Figs. 4I, 5I, and 7H).

To test a potential role for the endogenous ligand BMP-2 in insulin-positive differentiation as suggested by the screening PCR in Fig. 2, BMP-2 morpholino antisense was used. Here again we saw complete suppression of insulin II, PDX-1, and Pax-4 mRNA (Fig. 5, A–C), consistent with the soluble BMP receptor data shown earlier. For the Smads, there was a strong suppression of Smad-1 and Smad-8 mRNA levels (Fig. 5, D and H), but no effect on Smad-2 and Smad-5 suppression (Fig. 5, E and G). Smad-3 was suppressed back to baseline (Fig. 5F), which is again consistent with TGF-β isoform signaling being downstream of BMP signaling.

**Rescue Effects of Exogenous BMP-2 on Insulin-positive Differentiation—** Because BMP is known to act through different receptors and can exert differ-
ent effects at different concentrations, a rescue-type experiment was performed with three different doses of exogenous BMP-2 after BMP-2 antisense treatment to determine the potential for either a rescue effect or a supraphysiologic effect and to confirm the specificity of the BMP antisense experiments. The rescue dose of 10 pg/ml BMP-2 showed a complete rescue even beyond that of the missense controls, suggesting that endogenous BMPs are likely to be important. However, the lowest tested dose of 1 pg/ml BMP-2 does not get a rescue effect (data not shown). Interestingly, higher concentrations did not have an effect either. The loss of effect at higher and lower doses does suggest that the BMP-2 effect is a specific and important endogenous effect rather than a simple pharmacologic effect (Fig. 6).

Smad-1 Is Necessary in the Hierarchy of β-Cell Differentiation—Initially, ALK-1 mRNA was found to be up-regulated with exendin treatment in AR42J cells (Fig. 2). ALK-1 mainly works through the phosphorylation of Smad-1 and Smad-5, but not Smad-8. Because Smad-1 mRNA was up-regulated and Smad-5 down-regulated (Fig. 1), Smad-1 seemed to be a likely candidate in mediating ALK-1 effects. We found that there was a strong inhibition of insulin II, PDX-1, and Pax-4 mRNA with Smad-1 antisense (Fig. 7, A–C). In all cases, when insulin mRNA levels were reduced a concomitant block of the key pro-insulin transcription factors PDX-1 and Pax-4 was also seen. Interestingly, the typical changes in Smad-2 and Smad-3 mRNA levels seen with exendin-4 treatment were not seen with Smad-1 antisense (Fig. 7, D and E), consistent with our theory that BMP signaling is upstream of TGF-β isoform signaling. Additionally, even with TGF-β blockage, Smad-2 was always down-regulated previously whenever AR42J cells were exposed to exendin-4. The failure of Smad-2 mRNA expression levels to go down despite exendin-4 suggests that the mechanism for Smad-2 down-regulation is through BMP-mediated Smad-1 activation. With the Smad-1 antisense, although the Smad-8 mRNA levels were blocked (Fig. 7G) the Smad-5 mRNA levels did not go down (Fig. 7F), consistent with the possibility that there is a direct competition between Smad-1 and Smad-5 similar to that suggested earlier for Smad-2 and Smad-3 (12).

The Hierarchy between BMP and TGF-β Isoform Signaling—Loss of insulin-positive differentiation with Smad-1 antisense could be rescued with exogenous TGF-β1, suggesting that BMP or Smad-1 signaling is upstream of TGF-β isoforms (Fig. 8A–C). Also, these results suggest that a key role of Smad-1 is to activate, directly or indirectly, TGF-β isoform signaling. Smad-2 and Smad-5 were elevated with the Smad-1 antisense (Fig. 7D and F), but that effect was mostly reversed with the addition of exogenous TGF-β1 (Fig. 8, D and F).

Conclusion—Understanding the mechanisms of the TGF-β superfamily signaling cascade in the possible regulation of insulin-positive differentiation has high importance for our goal of engineering glucose-
Smad-8, in TGF-β signaling, is consistent with TGF-β and BMP Smads (12), and by immunostaining (14).

BMPs and TGF-βs have been strongly implicated in many embryologic and cell differentiation pathways. Jiang et al. (15) showed that BMP induces embryonic pancreatic epithelia to form insulin-positive cell colonies. Thus, we hypothesized that BMP signaling may play a role in the insulin-positive differentiation of AR42J cells. Our results support the idea that BMP-2 ligands play an important endogenous role in this process and that BMPs sit high on a hierarchy above TGF-β isoforms in the initiation of insulin-positive differentiation.

Previously, we quantified BMP Smads, namely Smad-1, Smad-5, and Smad-8, in TGF-β neutralizing antibody-treated AR42J cells and found that there were essentially no changes in any of the BMP Smads (12), which is consistent with TGF-β signaling being downstream of BMP signaling. Here, with the BMP soluble receptor we showed that the Smad-2 decrease that normally occurs with exendin-4 treatment was not affected, whereas the elevation in Smad-3 that occurs in response to exendin-4 was completely blocked. These results suggest a novel overlap of BMP and TGF-β isoform pathways with BMP upstream of TGF-β isoform signaling, which, in turn, appears to be specifically responsible for Smad-3 up-regulation. Based on these findings, we hypothesize that BMP may be necessary to stimulate these precursor cells to become endocrine-committed progenitor cells that then proceed further to differentiate into mature β-cells. Overall, there appears to be a synergistic interaction of BMP and TGF-β signaling to push AR42J cells toward an insulin-positive fate.

To understand the role of Smad-1 in mouse development, Robertson and co-workers (16) generated a Smad-1 null mutant mouse. They found an essential role for Smad-1-dependent signals in primordial germ cell specification (16), but because of early lethality there was no potential for analysis of pancreatic development. Our results with Smad-1 antisense and the rescue of Smad-1 antisense by exogenous TGF-β1 suggest that BMP signaling activates Smad-2 and Smad-3 pathways, possibly through the induction of the release of TGF-β isoforms from the cells in a paracrine or autocrine manner.

It was interesting that the BMP-soluble receptor did not block Smad-2 down-regulation, whereas Smad-1 antisense did. These results suggest that BMP ligand-independent pathways, which may also activate Smad-1, may play a more important role in the decrease of Smad-2 levels. We found previously that Smad-2, which is highly expressed in untreated AR42J cells, was necessary for insulin-positive differentiation.

A role for Smad-5 down-regulation in insulin-positive differentiation is also unclear. The Hammerschmidt group (17) has shown distinct roles for Smad-1 and Smad-5 during dorsoventral patterning of the zebrafish embryo. Their data suggest that Smad-1 acts later than Smad-5 and is itself a transcriptional target of Smad-5-mediated BMP-2 signaling (17). Thus, the decrease in Smad-5 may disinhibit or otherwise allow Smad-1 to up-regulate and/or become activated.

REFERENCES

1. Hogan, B. L. (1996) Curr. Opin. Genet. Dev. 6, 432–438
2. Arora, K., O’Connor, M. B., and Warrior, R. (1996) Am. N. Y. Acad. Sci. 785, 80–97
3. Tiso, N., Filippi, A., Paula, S., Bortolussi, M., and Argenton, F. (2002) Mech. Dev. 118, 29–37
4. Weaver, M., Yingling, J. M., Dunn, N. R., Bellusci, S., and Hogan, B. L. (1999) Development 126, 4005–4015
5. Monsoro-Burq, A. H., Duprez, D., Watanabe, Y., Bontoux, M., Vincent, C., Brickell, P., and Le Douarin, N. (1996) Development 122, 3607–3616
6. Yamashita, H., Ten Dijke, P., Heldin, C. H., and Miyazono, K. (1996) Bone 19, 569–574
7. Aoki, H., Fujiw, M., Imamura, T., Yagi, K., Takehara, K., Kato, M., and Miyazono, K. (2001) J. Cell Sci. 114, 1483–1489
8. Nishimura, R., Hata, K., Ikeda, F., Matsubara, T., Yamashita, K., Ichida, F., and Yoneda, T. (2003) Front. Biosci. 8, s275–s284
9. Miyazawa, K., Shinomaki, M., Hara, T., Furuya, T., and Miyazono, K. (2002) Genes Cells 7,1191–1204
10. Shi, Y., and Massague, J. (2003) Cell 113, 685–700
11. Attisano, L., and Wrana, J. L. (2002) Science 296, 1646–1647
12. Yew, K. H., Prasadan, K. L., Preudet, B. L., Hembree, M. J., McFall, C. R., Benjes, C. L., Crowley, A. R., Sharp, S. L., Li, Z., Tulachan, S. S., Mehta, S. S., and Gittes, G. K. (2004) Diabetes 53, 2824–2835
13. Viljacic, P., Myburgh, D., Ryan, A. K., van Rossijen, M. A., Mummery, C. L., and Gupta, I. R. (2004) Am. J. Physiol. 286, F625–F633
14. Borxson, M., Hougaard, D. M., Nielsen, J. H., Tonrehave, D., and Larsson, I. L. (2003) Histochem. Cell Biol. 116, 263–267
15. Jiang, F. X., Stanley, E. G., Gonez, L. J., and Harrison, L. C. (2002) J. Cell Sci. 115, 753–760
16. Tremblay, K. D., Dunn, N. R., and Robertson, E. J. (2001) Development 128, 3609–3621
17. Dick, A., Meier, A., and Hammerschmidt, M. (1999) Dev. Dyn. 216, 285–298
18. Oh, S. P., Seki, T., Goss, K. A., Imamura, T., Yi, Y., Donahoe, P. K., Li, L., Miyazono, K., ten Dijke, P., Kim, S., and Li, E. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 2626–2631
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