Expression of Type II Activin Receptor Genes During Differentiation of Human K562 Cells and cDNA Cloning of the Human Type IIB Activin Receptor

By Kristiina Hildén, Timo Tuuri, Marja Erämaa, and Olli Ritvos

Recent studies have indicated that activin A/erythroid differentiation factor is a physiologic hematopoietic growth and differentiation factor mainly for cells of the erythroid lineage. We studied the expression of the two type II activin receptor mRNAs during the differentiation of K562 erythroleukemic cells, which are known to be induced toward the erythroid lineage in response to activin or toward the megakaryoblastic lineage by phorbol myristate acetate (PMA). The cDNA of the human activin receptor type IIB (hActR-IIB) was cloned and sequenced from two RNA sources, the K562 cells and the human fetal brain, which is, of the tissues screened by Northern blot analysis, the most abundant source of ActR-IIB RNA. The cDNA encodes a predicted 512 amino acid protein containing an extracellular ligand binding domain, a hydrophobic transmembrane domain, and an intracellular serine/threonine kinase domain. The amino acid sequence is 99.2% and 98.4% homologous in the coding region to the previously described mouse and rat ActR-IIBs, respectively, and 65% identical to the other human activin serine/threonine kinase receptor, hActR-II. The alternative splicing events in the juxtamembrane region previously reported for the respective mouse receptor were not observed during the processing of K562 cell and human fetal brain RNA. Northern analysis showed that the 10- and 2.5-kb transcripts of hActR-IIB are more abundantly expressed than the 6.0- and 3.0-kb transcripts of hActR-II in K562 cells. No changes in the steady-state levels of hActR-II and IIB mRNAs were detected upon differentiation of K562 cells by activin A or by PMA. Similarly, the receptor mRNA levels remained constant in HL-60 cells induced to either monocyte/macrophage or granulocyte-like cells by PMA or dimethyl sulfoxide, respectively. Thus, the mRNA expression levels of both receptors apparently do not correlate with the differentiation status of these cells.

ACTIVINS WERE originally discovered as gonadal polypeptide hormones that stimulate follicle-stimulating hormone (FSH) secretion in cultured pituitary cells. There are three activins (A, B, and AB) that are homo/heterodimers of two closely related β subunits (βA, βB, and βAβB).1 Erythroid differentiation factor (EDF) was first found in the culture fluid of phorbol myristate acetate (PMA)-treated human monocytic leukemia cells (THP-1) based on its ability to induce erythroid differentiation of murine Friend erythroleukemia cells.2 Later, it turned out to be identical to activin A, because it was encoded by the same mRNA as the βA subunit of activin A.3 Activin A subsequently was shown to induce hemoglobin synthesis in human K562 erythroleukemia cells.4 Moreover, it enhances the growth of normal erythroid precursor cells both in vitro and in vivo.5,6 Activin A and its mRNA are expressed in the bone marrow and spleen of adult rodents.5,6 Activin A is also produced by murine and human bone marrow stromal cells in culture.10,11 Furthermore, it was recently shown to be secreted by human peripheral blood monocytes.11,12 These findings suggest that activin A acts as a natural regulator of erythropoiesis in the bone marrow.

The biologic effects of activin are expected to be mediated through type I and type II receptors.13 Although the structure of activin receptor I is unclear, two homologous type II activin receptors, ActR-II and ActR-IIB, have been recently characterized by cDNA cloning. ActR-II and ActR-IIB are transmembrane proteins with an extracellular ligand-binding domain and an intracellular kinase domain structurally related to several known serine/threonine kinases.14,15 K562 cells and other normal and malignant human myeloid cells possess specific binding sites for activin on their surface.16-19 However, it is not known which activin receptor subtypes are expressed in these cells. We now report the cDNA cloning of the human activin receptor IIB by reverse transcription-polymerase chain reaction (RT-PCR) from K562 cell RNA. We also studied whether the expression of hActR-II and hActR-IIB mRNAs is regulated during induced differentiation of K562 cells and HL-60 promyelocytic leukemia cells and determined the distribution of these mRNAs in midgestational human fetal tissues.

MATERIALS AND METHODS

Cell cultures and induction of differentiation. Human K562 and HL-60 cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum and mouse NIH-3T3 fibroblasts were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% newborn calf serum. 2 mmol/L L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humified 95% air-5% CO₂ atmosphere. For megakaryoblastic differentiation, K562 cells were induced with increasing concentrations of PMA (0.01 to 10 ng/mL; Sigma Chemical Co, St Louis, MO; dissolved in dimethyl sulfoxide [DMSO]). For erythroid differentiation, K562 cells were treated with increasing concentrations of recombinant human activin A (0.1 to 30 ng/mL; a gift from Drs Y. Eto, H. Shibai, K. Shiota, and M. Takahashi, Ajinomoto Co, Kawasaki, Japan). HL-60 cells were induced to either monocyte/macrophage or granulocyte-like cells by 10 ng/mL PMA or 1.2% DMSO, respectively. For RNA preparation. Total RNA was isolated from K562 and HL-

From the Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland.

Submitted July 26, 1993; accepted December 1, 1993.

Supported by the Finnish Cancer Society, the Medical Research Council of the Academy of Finland, Helsinki University Research Funds, the Jenny and Antti Wihuri Foundation, the Ella and Georg Ehrenrooth Foundation, the Oskar Oflund Foundation, the Sigrid Juselius Foundation, the Orion Research and Science Foundation, and the Nordisk Insulin Foundation.

Address reprint requests to Kristiina Hildén, BS, Department of Bacteriology and Immunology, PO Box 21 (Haartmaninkatu 3), SF-00014 University of Helsinki, Helsinki, Finland.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1994 by The American Society of Hematology.
Table 1. Sequences of Primers Used for RT-PCR in This Study and Lengths of the Respective cDNA Fragments

<table>
<thead>
<tr>
<th>Fragment (length in bp)</th>
<th>Sense Primer</th>
<th>Antisense Primer</th>
<th>Nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>ActR-II (939)</td>
<td>1 TCTCTGCTGTGCGAGAAGGCAA</td>
<td>2 TGCCGACATCTCCCTGCAA</td>
<td>301 to 1259</td>
</tr>
<tr>
<td>ActR-II (554)</td>
<td>3 GAAACATGACGACCCGCTC</td>
<td>4 GGCGGAGAGCCGCGGTC</td>
<td>4 to 540</td>
</tr>
<tr>
<td>ActR-II (741)</td>
<td>5 CCTAATGGAATGGAATGCTCAT</td>
<td>6 TTCTTCAGCTCACGTGGAG</td>
<td>847 to 1588</td>
</tr>
<tr>
<td>ActR-II (156)</td>
<td>7 TGGGAAAGAGCAGAAGAACCAC</td>
<td>8 CTCAATGAAATAGTGCGTGT</td>
<td>108 to 663</td>
</tr>
<tr>
<td>α-Globin (422)</td>
<td>9 CCCGCAACGACAGGGCGAT</td>
<td>10 AGCCAGCCGAACTCGAT</td>
<td>53 to 476</td>
</tr>
<tr>
<td>TGF-β-1 (560)</td>
<td>11 GAGAGTCGAGAACGGGAGCC</td>
<td>12 AGCAAGAAGACGGGTTCAT</td>
<td>1112 to 1821</td>
</tr>
<tr>
<td>β-Actin (263)</td>
<td>13 CCGAGCCGAGGCGGTAG</td>
<td>14 TCAAAACATGATGGCGT</td>
<td>34 to 296</td>
</tr>
</tbody>
</table>

60 cells and human fetal tissues by the guanidinium isothiocyanate/CoCl2 method. Tissues from human fetuses of gestational ages vary-
60 cells and human fetal tissues by the guanidinium isothiocyanate/RNA was isolated from total RNA of K562 cells by a PolyATtract 
Clinic by approvement of the hospital's ethical committce and by 
consent of the mother undergoing legal abortion. Polyadenylated 
ing from I was used for extracting cytoplasmic RNA from NIH-3T3 fibroblasts 
and, for dot blot hybridization, from K562 cells.

RNA and DNA blotting and hybridization. For Northern blots, 
10 μg of total RNA or 3.9 μg of polyadenylated RNA were run in 
1.5% agarose gel and transferred on Hybond N nylon membranes 
(Amersham) according to standard methods. For dot blots, 3 μg of cytoplasmic RNA was denatured in 7.5% formaldehyde and 6× 
SSC (1× SSC: 0.15 mol/L NaCl, 0.15 mol/L sodium citrate [pH 
7.0]) at 60°C for 30 minutes, and then spotted onto nylon membranes 
using 96-well MiniMold I device (Schleicher and Schuell, Keene, 
NH). For Southern blotting of amplified cDNAs, the samples were run 
in 4% NuSieve agarose (FMC Bio Products, Rockland, ME) 
gels, stained with ethidium bromide, and photographed under UV 
light. The samples were then transferred on Hybond N nylon mem-
branes. The RNA and DNA were cross-linked to the membranes 
by UV irradiation and baking for 1 to 2 hours at 80°C.

For generating single-stranded cDNA probes, 30 linear amplification 
cycles (94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 
1 minute) were performed. The 8 μL labeling mixtures contained 
linearized plasmid DNA in an equivalent to 5 ng of cDNA 
DNA of interest; 1 μmol/L specific 3' end antisense primer; 50 μmol/L 
oligo d(T), primer (Boehringer Mannheim, Mannheim, Germany), 2 μL of 5× RT 
buffer (BRL), the four dNTPs (at 0.5 mmol/L final concentration for 
each; Perkin-Elmer Cetus Corp, Norwalk, CT), and 5 U human 
placental RNAse inhibitor (Amersham, Buckinghamshire, UK). The 
primers for amplification of human ActR-II and ActR-IIIB were de-
signed according to published rodent ActR-II sequences.14,15,26 
The amplification primers used in this study are detailed in Table 1. The 
50 μL PCR volume contained 1 μL of the RT reaction mixture, 62.5 
mmol of specific 3' and 5' primers, and other commercial GeneAmp 
PCR-kit reagents according to the Perkin-Elmer Cetus instructions.

Initial denaturation occurred at 94°C for 2 minutes, followed by 45 
cycles performed as follows: denaturation at 94°C for 30 seconds, 
amnealing at 55°C for 30 seconds, and extension at 72°C for 1 minute 
and 30 seconds. The PCR products were purified with a Magic 
PCR Preps DNA Purification System (Promega) and subcloned into 
pCR1000, pCR11 (InVitrogen, San Diego, CA), or pGEM-T vectors 
(Promega). Dideoxy chain termination sequencing of double-
stranded plasmid template clones was performed using [α-32P]-dCTP 
(40 μCi; Amersham). As a control of even loading in dots and gel lanes, 
we used the mouse β-actin double-stranded DNA probe, which was 
labeled by random-priming with the use of a kit purchased from 
Boehringer Mannhein. Probes were purified with NucTrap columns 
(Stratagene, La Jolla, CA) and used at 1 to 3 × 10⁶ dpm/mL in 
hybridization solution containing 50% formamide, 6× SSC, 5× Den-
hardt's solution, 100 μg/mL salmon sperm DNA, 100 μg/mL yeast 
RNA, and 0.5% sodium dodecyl sulfate (SDS). Dot, Northern, and 
Southern blots were hybridized 16 hours at 42°C and washed three 
times for 20 minutes with 1× SSC, 0.1% SDS at 55°C. Autoradiogra-
phy was performed on Kodak X-Omat AR film (Eastman Kodak, 
Rochester, NY) with intensification screens at −70°C. Autoradio-
graphic signals were quantified with the use of a transmission densi-
tometer (X-Rite Co, Grand Rapids, MI).

RESULTS

We amplified ActR-II and ActR-IIIB cDNAs by RT-PCR using pairs of different primers designed according to known 

![Fig 1. Schematic representation of the human activin receptor IIßb cDNA and location of primers used for PCR cloning. Straight lines are untranslated sequences and boxes represent the open reading frame. The extracellular domain (II), the transmembrane region (II), and the intracellular kinase domain (II) are shown. Primers no. 1 through 6 (see Table 1) synthesized according to the mouse ActR-II sequence were used to amplify the indicated portions of the human sequence from K562 cell or human fetal brain cDNAs.](image-url)
ACTIVIN TYPE II RECEPTORS IN K562 CELLS

rodent ActR-11s sequences (Fig 1 and Table 1). Amplification of K562 cell cDNA with primers no. 1 and 2 yielded a 959-bp DNA product with 90.1% nucleotide sequence homology to the mouse ActR-IIB. This fragment was used as a hybridization probe to screen Northern blots of human fetal tissue RNA samples.

Figure 2B shows that ActR-IIB mRNAs are variably expressed in several human fetal tissues, with the expression level being highest in the brain. Both the ActR-IIB and ActR-II mRNAs appear to be highly expressed in developing human neural tissues (cerebrum and spinal cord; Fig 2A and B). Moderate expression levels of both ActR-IIB and ActR-II are detected in muscular tissues such as skeletal muscle, heart, and stomach. Low but detectable expression of ActR-IIB is also observed in several glandular tissues (kidney, salivary, and pancreas) and hematopoietic tissues (spleen, liver, thymus, and bone marrow). The adrenal, which consists of both glandular (cortex) and neural (medulla) tissue, also expresses ActR-IIB. Although ActR-IIB and ActR-II are coexpressed in several human fetal tissues, ActR-IIB shows a wider tissue distribution than ActR-II. Taken together, the differences in the expression levels of ActR-II and ActR-IIB indicate that both genes are controlled in a tissue- and gene-specific manner during human development.

For obtaining the full reading frame of hActR-IIB, we used human fetal brain cDNA to amplify RT-PCR clones representing the lacking 5’ and 3’ sequences (Figs 1 and 3). The human amino acid sequence is 99.2% and 98.4% homologous to the previously described mouse and rat ActR-IIBs, respectively.15,26 The human ActR-IIB, like the respective mouse type IIB activin receptor, appears to be a 512 amino acid transmembrane protein with an extracellular ligand binding domain and an intracellular signalling domain with predicted serine/threonine specificity. The human sequence differs by 5 amino acids from the corresponding mouse sequence. Three substitutions in the signal peptide, one in the ligand binding domain, and one in the C-terminal region are found (Fig 3). The mouse ActR-IIB gene encodes four different activin receptor isoforms, which are produced by alternative mRNA splicing.15 Two of the ActR-IIB isoforms differ from each other by the inclusion of a 24 amino acid alternatively spliced segment in the cytoplasmic juxtamembrane domain. A second alternative splicing event generates two additional receptor isoforms that lack an 8 amino acid proline-rich cluster in the external juxtamembrane region. The receptor isoform that includes both segments is designated ActR-IIB2. The isoforms that include only the first segment or the second segment are designated ActR-IIB3 and ActR-IIB4, respectively, and the isoform that lacks both segments is designated ActR-IIB5. The ActR-IIB2 isoform is predominant in the mouse26 and the presently reported human cDNA resembles this isoform. During the characterization of several human ActR-IIB cDNA clones we did not encounter any isoforms other than ActR-IIB2. Similar alternative splicing events that generate different splicing variants in the juxtamembrane region of the corresponding mouse receptor may not occur during the processing of the human transcript. To test this hypothesis, we performed RT-PCR on K562 cell and human fetal brain polyadenylated RNAs using primers no. 1 and 4 (Table 1) flanking the region of the cDNA encompassing the alternative spliced regions of the respective mouse sequence. Figure 4 shows that only a fragment corresponding to the ActR-IIB2 isoform can be amplified from K562 cell and human fetal brain RNA, whereas, under similar experimental conditions, the four mouse splicing variants are detected in NIH-3T3 fibroblast RNA corresponding to the results reported in mouse Balbc 3T3 cells by Attisano et al.15 In NIH-3T3 cells, the ActR-IIB2 isoform is clearly more abundantly expressed than the ActR-IIB3, ActR-IIB4, and ActR-IIB5 variants, which also corresponds to earlier results with Balbc 3T3 cells.15 Thus, we conclude that no alternative splicing events occur in the juxtamembrane region of the receptor during the processing of the human ActR-IIB K562 cell and fetal brain mRNAs.

Northern analysis of K562 cell polyadenylated RNA showed 10-kb and 2.5-kb transcripts for hActR-IIB (Fig 5). A very weak 2.1-kb hybridization signal is also observed with the ActR-IIB probe (Fig 5). For ActR-II, 6.0- and 3.0-kb transcripts were detected (Fig 5). The expression levels of both ActR-IIB and ActR-II are relatively low in K562 cells when compared with, eg, human fetal brain tissue. Although no exact quantitative determination of ActR-IIB and ActR-II mRNA levels was performed, ActR-IIB appears to be more abundantly expressed than ActR-II in K562 cells.
Human activin receptor type IIb cDNA and the translated amino acid sequence. Underlined amino acids and nucleotides denote differences compared with the mouse activin receptor type IIb sequence. Nucleotides at the 5' end corresponding to primer no. 1 and the nucleotides at the 3' end corresponding to the sequence complementarity for primer no. 6 are underlined.

**Comparison of the Activin Receptor**

Based on the comparison of hybridization signals obtained after multiple exposure times of the Northern blots, we studied whether ActR-IIIs mRNA levels are regulated during differentiation of K562 cells. Activin regulates erythroid differentiation of K562 cells by inducing α, γ, and ε globin gene expression and hemoglobin production. We observed that activin A increases α-globin mRNA expression in K562 cells in a concentration- (Fig 6A) and time- (not shown) dependent manner. However, neither ActR-II nor ActR-IIIB mRNA levels were affected by activin A treatment as studied by dot blot (Fig 6B and C) or Northern blot (not shown) hybridizations. On the other hand, the tumor promoter PMA induces megakaryoblastic differentiation and TGF-β, mRNA expression in these cells. We found that, although PMA induces TGF-β, mRNA expression in a concentration-dependent manner (Fig 6D) in K562 cells, it had no effect on ActR-II and ActR-IIIB mRNA levels (Fig 6E and F). Figure 5 shows that the relative expression levels of the two major transcripts of both ActR-IIIB and ActR-II were not altered by induced differentiation. Furthermore, we detected that ActR-IIIB expression in human HL-60 promyelocytic leukemia cells was not regulated by PMA or DMSO, which induce these cells to either monocyte/macrophage or granulocyte-like cells (not shown).

**DISCUSSION**

The present study was undertaken to characterize which type II activin receptor genes are expressed in human erythroleukemia K562 cells. We detected both ActR-II and ActR-IIIB expression in these cells but no regulation of either receptor mRNAs during induced erythroid or megakaryoblastic differentiation.
The specific transcripts were visualized by hybridizing the filter containing mRNA expression in human and mouse juxtamembrane region of the mouse type IIB activin receptor sequentially with 32P-labeled ActR-IIB2. Arrowheads indicate the location of specific mRNA transcripts detected with ActR-IIB, a-globin, and ActR-II cDNAs. Differentiation was observed. We isolated a cDNA encoding the full reading frame of human ActR-IIB and found no alternative splicing variants for its mRNA. Both the type II and IIB activin receptors show a wide tissue distribution pattern in the developing human fetus.

The full-length hActR-IIB cDNA sequence was derived from several partial overlapping cDNA clones generated by RT-PCR from K562 cell and human fetal brain RNA. Like the type II activin receptor, the sequence of hActR-IIB shows extremely high, with more than 98% homology between its rodent counterparts. Also, the ActR-IIB homologues described for Xenopus laevis are more than 80% identical in amino acid sequence with their mammalian counterparts. Because alternative splicing events in the X. laevis sequence of hActR-IIB are identical in amino acid sequence with their mammalian counterparts, this suggests that cells bearing ActR-IIB1 and ActR-IIB2 isoforms may have higher sensitivity to activin than those harboring type IIB1, IIB4, or II receptors. Thus, our study suggests that the human ActR-IIB RNA is processed to encode the high-affinity ActR-IIB2 receptor, but further expression studies of the cDNA have to be performed to confirm the binding properties of the human ActR-IIB2.

To facilitate the cloning of hActR-IIB cDNA, which was expressed at relative low levels in K562 cells, we delineated the tissue distribution pattern of ActR-IIB mRNAs in human fetal tissues. We found that developing human neural tissues such as brain and spinal cord express high levels of ActR-IIB and therefore we used fetal brain RNA to obtain cDNA clones covering the whole reading frame of ActR-IIB. We also found that ActR-II mRNAs are abundantly coexpressed with ActR-IIB in human fetal neural tissues. This indicates a role for activin during the development of the nervous system. This conclusion is supported by the earlier demonstration of activin β1 and β2 mRNA expression in the embryonic rat brain. Recent reports from studies on nonmammalian vertebrates have shown the expression of ActR-II in chicken embryo neural tissues and ActR-IIB in Xenopus tissues undergoing neurulation. A role for activin in central differentiation was observed. We isolated a cDNA encoding the full reading frame of human ActR-IIB and found no alternative splicing variants for its mRNA. Both the type II and IIB activin receptors show a wide tissue distribution pattern in the developing human fetus.

The full-length hActR-IIB cDNA sequence was derived from several partial overlapping cDNA clones generated by RT-PCR from K562 cell and human fetal brain RNA. Like the type II activin receptor, the sequence of hActR-IIB shows extremely high, with more than 98% homology between its rodent counterparts. Also, the ActR-IIB homologues described for Xenopus laevis are more than 80% identical in amino acid sequence with their mammalian counterparts. Because alternative splicing events in the juxtamembrane region of the mouse type IIB activin receptor generate RNA transcripts for four different receptor isoforms, we examined whether this feature is also characteristic to the human ActR-IIB gene. However, based on our RT-PCR studies, we did not find any other receptor transcript isoforms than the ActR-IIB2 variant in K562 cell and human fetal brain RNA samples. The ActR-IIB2 variant appears to be the most prevalent receptor isoform in the mouse, and all ActR-II isoforms were reported in other species resembling ActR-IIB2 in structure. Although the biologic relevance of different isoforms is presently unclear, the ActR-IIB1 and ActR-IIB2 isoforms appear to bind activin with higher affinity than the ActR-IIB3 and ActR-IIB4 variants that have binding abilities comparable to that of ActR-II receptor. This suggests that cells bearing ActR-IIB1 and ActR-IIB2 isoforms may have higher sensitivity to activin than those harboring type IIB1, IIB4, or II receptors. Thus, our study suggests that the human ActR-IIB RNA is processed to encode the high-affinity ActR-IIB2 receptor, but further expression studies of the cDNA have to be performed to confirm the binding properties of the human ActR-IIB2.

To facilitate the cloning of hActR-IIB cDNA, which was expressed at relative low levels in K562 cells, we delineated the tissue distribution pattern of ActR-IIB mRNAs in human fetal tissues. We found that developing human neural tissues such as brain and spinal cord express high levels of ActR-IIB and therefore we used fetal brain RNA to obtain cDNA clones covering the whole reading frame of ActR-IIB. We also found that ActR-II mRNAs are abundantly coexpressed with ActR-IIB in human fetal neural tissues. This indicates a role for activin during the development of the nervous system. This conclusion is supported by the earlier demonstration of activin β1 and β2 mRNA expression in the embryonic rat brain. Recent reports from studies on nonmammalian vertebrates have shown the expression of ActR-II in chicken embryo neural tissues and ActR-IIB in Xenopus tissues undergoing neurulation. A role for activin in central differentiation was observed. We isolated a cDNA encoding the full reading frame of human ActR-IIB and found no alternative splicing variants for its mRNA. Both the type II and IIB activin receptors show a wide tissue distribution pattern in the developing human fetus.

The full-length hActR-IIB cDNA sequence was derived from several partial overlapping cDNA clones generated by RT-PCR from K562 cell and human fetal brain RNA. Like the type II activin receptor, the sequence of hActR-IIB shows extremely high, with more than 98% homology between its rodent counterparts. Also, the ActR-IIB homologues described for Xenopus laevis are more than 80% identical in amino acid sequence with their mammalian counterparts. Because alternative splicing events in the juxtamembrane region of the mouse type IIB activin receptor generate RNA transcripts for four different receptor isoforms, we examined whether this feature is also characteristic to the human ActR-IIB gene. However, based on our RT-PCR studies, we did not find any other receptor transcript isoforms than the ActR-IIB2 variant in K562 cell and human fetal brain RNA samples. The ActR-IIB2 variant appears to be the most prevalent receptor isoform in the mouse, and all ActR-II isoforms were reported in other species resembling ActR-IIB2 in structure. Although the biologic relevance of different isoforms is presently unclear, the ActR-IIB1 and ActR-IIB2 isoforms appear to bind activin with higher affinity than the ActR-IIB3 and ActR-IIB4 variants that have binding abilities comparable to that of ActR-II receptor. This suggests that cells bearing ActR-IIB1 and ActR-IIB2 isoforms may have higher sensitivity to activin than those harboring type IIB1, IIB4, or II receptors. Thus, our study suggests that the human ActR-IIB RNA is processed to encode the high-affinity ActR-IIB2 receptor, but further expression studies of the cDNA have to be performed to confirm the binding properties of the human ActR-IIB2.

To facilitate the cloning of hActR-IIB cDNA, which was expressed at relative low levels in K562 cells, we delineated the tissue distribution pattern of ActR-IIB mRNAs in human fetal tissues. We found that developing human neural tissues such as brain and spinal cord express high levels of ActR-IIB and therefore we used fetal brain RNA to obtain cDNA clones covering the whole reading frame of ActR-IIB. We also found that ActR-II mRNAs are abundantly coexpressed with ActR-IIB in human fetal neural tissues. This indicates a role for activin during the development of the nervous system. This conclusion is supported by the earlier demonstration of activin β1 and β2 mRNA expression in the embryonic rat brain. Recent reports from studies on nonmammalian vertebrates have shown the expression of ActR-II in chicken embryo neural tissues and ActR-IIB in Xenopus tissues undergoing neurulation. A role for activin in central differentiation was observed. We isolated a cDNA encoding the full reading frame of human ActR-IIB and found no alternative splicing variants for its mRNA. Both the type II and IIB activin receptors show a wide tissue distribution pattern in the developing human fetus.
nervous system development is also supported by the findings that activin promotes neural cell survival in vitro and stimulates proliferation and inhibits terminal differentiation of several neural cell lines. Both ActR-II and ActR-IIB were relatively abundantly expressed in developing human muscular tissues, including the heart, skeletal muscle, and stomach. Others have also shown ActR-II expression in the developing mouse and chicken heart. In line with our results, Ohuchi et al have previously shown ActR-II expression in myotomes of developing chicken embryos. Our results are also supported by the experiments based on a mutant ActR-IIB inhibiting activin signalling that have shown that activin is required for the induction of muscular tissue in the developing Xenopus embryo. Relatively low but detectable expression levels of ActR-IIB were also found in glandular tissues such as the kidney, salivary, pancreas, and adrenal, as well as in fetal hematopoietic tissues including spleen, thymus, liver, and bone marrow, indicating wide distribution of the receptor during human development. A role for activin in the development of the kidney is also supported by a study showing ActR-II transcripts in chicken mesonephros. On the other hand, together with this study, the report of Roberts et al showing high expression of activin mRNA in salivary gland suggests a role for activin during the development of this tissue. Based on ample evidence on the role of activin as a hematopoietic regulator, it is not surprising that several human hematopoietic tissues express activin receptors. Taken together, both ActR-II and ActR-IIB show a wide distribution pattern in the midgestational embryo, supporting an important role of activin during human development.

Our study shows that K562 cells express both type II and IIB activin receptor mRNAs. We found that, in these cells, ActR-IIB mRNAs are somewhat more abundantly expressed than those of ActR-II and that induced erythroid or megakaryoblastic differentiation of K562 cells did not affect their relative expression levels. For both ActR-IIB and ActR-II, expression of two major transcripts was observed. The exact nature of the human ActR-II 6.0- and 3.0-kb transcripts is unclear. However, if these transcripts are structural counterparts of the respective mouse mRNAs, they are likely to arise from the use of different polyadenylation signals during the transcription of ActR-II gene. The gene structure of mouse ActR-II has been recently reported and a transcription start site together with a polyadenylation signal giving rise to the 3.0-kb transcript have been identified. Moreover, in the original report of mActR-II expression cloning, a cDNA clone expanding beyond the 3.0-kb transcript polyadenylation signal was reported, suggesting that a longer transcript arises during mActR-II gene transcription, which is in line with the existence of the 6.0-kb transcript. It appears that in the mouse and rat tissues the relative expression levels of these two transcripts are regulated in tissue- and developmental-stage-specific manner, but for the human ActR-II we did not observe changes in the expression level of either transcript during induced differentiation of K562 cells. We presently report that ActR-IIB is expressed as two major transcripts of 10 and 2.5 kb. In contrary to ActR-II, there are not sufficient sequence data available on the 3' and 5' untranslated regions of the ActR-IIB gene from any species to predict whether these different transcripts arise from the presence of a precursor RNA in the samples used for Northern blot studies or whether different polyadenylation signals are used during the transcription of ActR-IIB.

Although we did not observe regulation of ActR-II or ActR-IIB mRNAs in K562 cells, several reports indicate that these mRNAs are regulated in other systems in a tissue- or cell-specific manner. In the present study, this is supported by the differences of expression levels of both receptors in distinct human fetal tissues. On the other hand, De Jong et al have observed that retinoic acid downregulates ActR-IIB levels without affecting ActR-II expression in human teratocarcinoma cells. Moreover ActR-II and ActR-IIB mRNAs are regulated in specific patterns during different stages of germ cell maturation in the seminiferous epithelium of the rat testis. Also, Shinozaki et al have shown that ActR-II is induced by gonadotropins in the rat ovary. Taken together, although we did not find regulation of ActR-II and ActR-IIB mRNAs in K562 cells, they are independently regulated by several factors in a tissue- or cell-specific manner in other systems.

In K562 cells, activin regulates both mitogenesis, by inhibiting cell growth, and differentiation-related phenomena such as hemoglobin production. The relative roles of different activin receptor types in the regulation of these events is presently unknown. TGF-β, which has a type I and II receptor system resembling that of activin, uses different receptor types for the regulation of mitogenesis and the production of extracellular matrix proteins. The type I TGF-β receptor mediates the induction of growth inhibition in target cells. On the other hand, type I receptor, which is also a serine/threonine transmembrane kinase receptor, appears to be responsible for effects of TGF-β on extracellular matrix. The availability of the cloned hActR-II and hActR-IIB will facilitate further studies aiming to delineate the relative roles of different activin receptor types in transducing the diverse regulatory effects of activin in target cells.

ACKNOWLEDGMENT

We thank Ritva Javanainen for her skilful technical assistance and Anja Mäki for oligonucleotide primer synthesis.

NOTE ADDED IN PROOF

After the communication of this report, Matsuzaki et al reported the sequence of a human serine/threonine kinase receptor, whose ability to act as a type I activin receptor was described by Attisano et al. This was also confirmed by Tsuchida et al who reported the respective sequence in the rat. The mouse TGF-β type I receptor reported by Eber et al is an homolog of the human and rat type I activin receptors and was recently shown to meet the criteria expected of an activin type I receptor. The nucleotide sequence data of hActR-IIB reported in this report will appear in the EMBL, GenBank, and DDBJ Nucleotide Sequence Databases under accession no. X77533.

REFERENCES

ACTIVIN TYPE II RECEPTORS IN K562 CELLS


40. Hashimoto M, Nakamura T, Inoue S, Kondo T, Yamada R,


56. Tsuchida K, Matthews LS, Vale WW: Cloning characterization of a transmembrane serine kinase that acts as activin type I receptor. Proc Natl Acad Sci USA 90:11242, 1993

Expression of type II activin receptor genes during differentiation of human K562 cells and cDNA cloning of the human type IIB activin receptor

K Hilden, T Tuuri, M Eramaa and O Ritvos