Assessment of the function of the $\beta_C$-subunit of activin in cultured hepatocytes

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Assessment of the function of the $\beta_C$-subunit of activin in cultured hepatocytes. *Am J Physiol Endocrinol Metab* 287: E247–E254, 2004. First published March 23, 2004; 10.1152/ajpendo.00390.2003.—We assessed the function of the $\beta_C$-subunit of activin in hepatocytes. We studied the effect of conditioned medium of Chinese hamster ovary (CHO) cell line stably expressing the $\beta_C$ gene (CHO-$\beta_C$) on growth of AML12 hepatocytes. We also examined the effect of recombinant activin C and transfection of the $\beta_C$-gene by using adenovirus vector. CHO-$\beta_C$ secreted activin C, a homodimer of the $\beta_C$, as well as precursors of the $\beta_C$. The conditioned medium of CHO-$\beta_C$ increased both [H]thymidine incorporation and the cell number in AML12 cells. It also supported survival of AML12 cells in a serum-free condition. Recombinant human activin C also increased both [H]thymidine incorporation and the number of AML12 cells. Transfection of AML12 cells with the $\beta_C$-subunit led to the stimulation of [H]thymidine incorporation. Analysis of the conditioned medium revealed that the $\beta_C$-subunit formed a heterodimer with the endogenous $\beta_A$, the formation of which was dependent on the amount of $\beta_C$ expressed. Recombinant activin C did not affect the binding of 125I-activin A to its receptor or follistatin. These results indicate that activin C stimulates growth of AML12 cells. The $\beta_C$-subunit modifies the function of the $\beta_A$-subunit by multiple mechanisms.

activin C; growth; apoptosis

ACTIVINS ARE MEMBERS of the transforming growth factor-$\beta$ (TGF-$\beta$) superfamily and elicit diverse actions in various types of cells (21, 29). Thus they regulate organogenesis and formation of the basic structure of solid organs during development (1, 20). After birth, activins function as tissue repair factors and are involved in wound healing and regeneration processes of various tissues (12, 15, 19). They also modulate the immune system (25) and regulate endocrine functions including the hypothalamic-pituitary-gonadal axis (29).

In the liver, the $\beta_A$-subunit of activin is expressed mainly in parenchymal cells (28), and nonparenchymal cells also express a certain amount of the $\beta_A$-subunit in normal conditions (8). The $\beta_B$-subunit of activin is also expressed in the liver, but the expression level is much lower compared with that of the $\beta_A$-subunit (8). Activin A, a homodimer of the $\beta_A$-subunit, is a potent inhibitor of DNA synthesis in hepatocytes (28). After partial hepatectomy, the expression of the $\beta_A$-subunit decreases rapidly but is then markedly upregulated 24 to 48 h after hepatectomy (28). Activin A produced in hepatocytes acts as an autocrine factor and tonically inhibits liver regeneration, as blocking the action of activin A accelerates liver regeneration (15). It is also critical in maintaining a constant liver mass (13, 16, 17). Besides its effects on cell growth, activin A also stimulates the production of extracellular matrix proteins and is thought to be involved in the pathogenesis of liver fibrosis (7).

In addition to the $\beta_A$- and $\beta_B$-subunits of activin, two other types of activin subunit, namely $\beta_C$ and $\beta_E$, are expressed abundantly in the liver (11, 33). Interestingly, the expression pattern of $\beta_C$ and $\beta_E$ is quite different from that of the $\beta_A$-subunit in the liver. Under a normal condition, the expression level of the $\beta_A$-subunit is relatively low, whereas it is markedly increased 24–48 h after partial hepatectomy (28). In contrast, the expression level of the $\beta_C$- and $\beta_E$-subunits is quite high in normal liver but decreases markedly after partial hepatectomy (9, 11, 19). The expression pattern is reciprocal to that of the $\beta_A$-subunit. This raises the possibility that the role of the $\beta_C$- and $\beta_E$-subunits of activin, if any, may be different from that of the $\beta_A$-subunit. However, the function of the $\beta_C$- or $\beta_E$-subunit of activin has not been elucidated to date. Recently, Lau et al. (18) generated mutant mice lacking the $\beta_C$ and/or $\beta_E$ gene by a homologous recombination technique. They showed that the liver of the mice lackng the $\beta_C$ and/or $\beta_E$ gene was morphologically normal and that liver regeneration occurred normally after partial hepatectomy. They concluded that the $\beta_C$- and $\beta_E$-subunits are dispensable during development and regeneration of the liver (18). It is not totally certain at present whether or not the $\beta_C$- and $\beta_E$-subunits have any specific function in the liver. The present study was conducted to address the function of the $\beta_C$-subunit of activin in hepatocytes. The results suggest that the $\beta_C$-subunit has unique functions that are different from those of the $\beta_A$-subunit.

MATERIALS AND METHODS

Materials. AML12 (25), a hepatocyte cell line expressing TGF-$\alpha$ was provided by Dr. Hitoshi Takagi of Gunma University, who received the cells from prof. Nelson Fausto of Brown University. Recombinant human activin A was provided by Dr. Y. Eto of the Central Research Laboratory of Ajinomoto (Kawasaki, Japan). Dulbecco’s modified Eagle’s medium (DMEM)-Ham’s F-12 (1:1, vol/vol), Ham’s F-12, penicillin, streptomycin sulfate, and ITS solution (5 $\mu$g/ml insulin, 5 $\mu$g/ml transferrin, and 5 ng/ml selenious acid) were purchased from GIBCO (Grand Island, NY). Sulfated cellulofine was purchased from Seikagaku (Tokyo, Japan). Anti-FLAG M2 monoclo-

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nal antibody and anti-FLAG M2 affinity gel were purchased from Sigma (St. Louis, MO). Monoclonal anti-β-subunit antibody was described previously (23). [3H]thymidine and [3P]deoxyctydine triphosphates were purchased from NEN Life Science Products (Boston, MA). Recombinant human activin C was generously provided by Biopharm (Heidelberg, Germany).

Cell culture. AML12 cells were cultured in DMEM-Ham’s F-12 supplemented with 10% fetal bovine serum (FBS, Gibco), ITS solution, 0.1 mM dexamethasone, penicillin, and streptomycin under a humidified atmosphere of 95% air-5% CO2 at 37°C. They were routinely subcultured using trypsin-EDTA every 5–7 days. Mouse erythroleukemia (F5–5) cells and Chinese hamster ovary (CHO) cells were provided by the Riken Cell Bank (Tsukuba, Japan). These cells were cultured in Ham’s F-12 supplemented with 10% FBS, 0.1 mM dexamethasone, penicillin, and streptomycin under a humidified atmosphere of 95% air-5% CO2 at 37°C.

RNA extraction and Northern blot analysis. Total RNA was extracted from liver homogenates with the TRIZol reagent (Invitrogen). mRNA was extracted using Oligotex-dT30 (Super Roche) according to the manufacturer’s instructions. mRNA was separated by electrophoresis on a 1.0% agarose gel containing 2.2 M formaldehyde and 40 mM MOPS (pH 7.0) and transferred to a nylon membrane (Hybond, Amersham Pharmacia Biotech) by capillary blotting technique after hybridization with the cDNA for the mouse βC-subunit of activin and cDNA for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Clontech, Palo Alto, CA). For quantification of the relative βC mRNA content, the intensities of the autoradiographic signals for βC and GAPDH were quantified and expressed in arbitrary density units. The βC-to-GAPDH ratio (based on integrated signals) was determined for each sample.

Reverse Transcription-PCR. mRNA was isolated from the liver with the TRIZol reagent and Oligotex-dT30 (Super). First-strand cDNA was made from mRNA by using the Superscript Preamplification System (Invitrogen) according to the manufacturer’s instructions. Contaminating genomic DNA was removed with RNase-free DNase. Five micrograms of DNA-treated RNA were incubated with 1 μl of oligo(dT) at 70°C for 10 min. Two microliters of 10× PCR buffer, 1 μl of DTT (0.1 M), 2 μl of dNTP mixture (10 mM), and 2 μl of MgCl2 (25 mM) were added to each reaction. After incubation for 5 min at 1°C, 1 μl of reverse transcriptase was added. Samples were incubated at 42°C for 50 min and then at 70°C for 15 min. RNase H (1 μl) was added to each reaction, and samples were incubated at 37°C for 20 min. PCR was performed as indicated by the manufacturer (Perkin-Elmer, Norwalk, CT) with the following primers: mouse βC: a 5’-end-specific forward primer (5’-AAGATGGCCTCCTCT- TGCTCTGGCT-3’) and a 3’-end-specific reverse primer (5’-AGCGTAACTACCCCGAGGCTCGAAC-3’). Reactions included 5 μl of 10× PCR buffer, 1 μl of MgCl2 (50 mM), 1.5 μl of dNTP mixture, 1 μl of 5’ primer, 1 μl of 3’ primer, 0.5 μl of PLATINUM Pfx DNA polymerase (Invitrogen), and 1 μl of cDNA. Samples were incubated at 94°C for 5 min, followed by the indicated number of cycles of 33 s at 94°C, 33 s at 60°C, and 33 s at 72°C, with a final extension at 72°C for 10 min in a Perkin-Elmer DNA thermal cycler.

Recombination procedures and transfection to CHO cells. The mouse cDNA clone was subcloned into the EcoRI site of the vector pcDNA3 (Invitrogen). To introduce the FLAG epitope tag into the carboxyl terminus of the βC-subunit of activin, we amplified a fragment of the βC by PCR using the sense (5’-GGGAATTCCTACTTGTCGTCATCGTCTTTGTAGTCCATG-3’) and the antisense (5’-GGGAATTCCTACTTGTGCTATCGTCTTTGTAGTCCATG- GTACTACCCCGAGGCTCGAAC-3’) primers; the PCR products were then used for native βC cDNA. The PCR-purified product was cloned into the pcDNA3 vector for transfection into CHO cells. The βC expression vectors were purified using the Concert High Purify Plasmid Maxiprep System (Invitrogen) according to the manufacturer’s instructions. CHO cells were transfected using Lipo-
RESULTS

Effect of conditioned medium of βC-transfected cells on the growth of AML12 cells. To examine the function of the βC-subunit of activin, we established a permanent cell line expressing a FLAG-tagged βC-subunit (CHO-βC cells). The expression of the endogenous βA or βC-subunit of activin was undetectable in these cells. Morphology and growth rate of CHO-βC cells were similar to those in mock-transfected CHO cells (data not shown). The conditioned medium of CHO-βC cells contained activin C, a dimer of βC, as well as the precursor (Fig. 1A). We incubated AML12 cells, a hepatocyte cell line (27), with the conditioned medium of mock-transfected and βC-transfected cells. As shown in Fig. 1B, conditioned medium obtained from the CHO-βC cells significantly increased the cell number, whereas that obtained from mock-transfected cells had no effect. The conditioned medium obtained from CHO-βC cells but not from mock-transfected cells also stimulated [3H]thymidine incorporation in AML12 cells (Fig. 1C). The effect of the CHO-βC cell conditioned medium was dependent on its concentration. When activin C was depleted from the conditioned medium by anti-FLAG Affinity gel, the growth-stimulatory effect was markedly inhibited, and the value was not statistically different from the control value (Fig. 1D). As reported previously, activin A inhibits growth of AML12 cells (32). The effect of the conditioned medium obtained from CHO-βC cells was the opposite of that of activin A. To confirm that the effect of the conditioned medium was independent of the activin A signaling system, we examined the effect of the conditioned medium in AML12 cells expressing the dominant-negative mutant type II activin receptor (AMLtAR cells). In these cells, the effect of activin A was blocked completely, as activin A was trapped by the mutant receptor lacking an intracellular kinase domain (32). As shown in Fig. 2A, activin A inhibited epidermal growth factor-induced [3H]thymidine incorporation into AML12 cells, whereas it did not elicit any effect in AMLtAR cells, confirming that the activin signaling system was blocked in these cells. In AMLtAR cells, the conditioned medium of CHO-βC cells stimulated [3H]thymidine incorporation (Fig. 2B). It is of note that the conditioned medium did not affect DNA synthesis in Balb/c 3T3 fibroblasts (data not shown).

We then examined whether or not the conditioned medium of CHO-βC cells could support the survival of AML12 cells. AML12 cells were seeded sparsely and cultured with serum-free conditioned medium of either mock-transfected or CHO-βC cells. Some of the AML cells cultured in the conditioned medium from mock-transfected CHO cells died, and the
number of AML12 cells was ∼75% after 48 h (Fig. 3A). In contrast, most of the AML12 cells cultured in the conditioned medium of CHO-βC cells remained alive. Morphologically, cells cultured in the conditioned medium from βC-transfected CHO cells were different from those cultured in the mock-transfected CHO cell conditioned medium. Cells cultured in conditioned medium obtained from mock-transfected CHO cells shrank after 48 h of culture, and some of them were crenated (Fig. 3Ba). Note that a similar morphological change was observed in AML12 cells cultured in serum-free medium (data not shown). In contrast, cells cultured in conditioned medium obtained from βC-transfected cells were flat, and some of them displayed extended processes (Fig. 3Bb).

Effect of conditioned medium of βC-transfected cells on differentiation of erythroleukemia cells. We then examined whether or not the conditioned medium induced erythroid differentiation (10). Activin A induces differentiation of erythroleukemia cells to erythrocytes, and the bioactivity of activin A has been assessed using this cell system (10). Activin A induces differentiation of erythroleukemia cells. Note that the conditioned medium of mock-transfected cells slightly inhibited the action of activin A on differentiation. This inhibition could have been due to follistatin produced in CHO cells. We therefore treated the conditioned medium of both mock-transfected CHO cells and CHO-βC cells with sulfated cellulose to remove follistatin (13). As expected, the conditioned medium of mock-transfected cells treated with sulfated cellulose did not affect activin A-evoked erythroid differentiation (Fig. 4B). Nonetheless, the conditioned medium of CHO-βC cells inhibited the activin A action even after the treatment with sulfated cellulose, suggesting that the inhibition by the CHO-βC conditioned medium was not due to follistatin produced in CHO cells.

Transfection of βC-subunit in AML12 cells with adenovirus vector. To further assess the function of the βC-subunit, we transfected AML12 cells with CDNA for this subunit by using adenovirus vector. Transfected cells were incubated in serum-free medium, and [3H]thymidine incorporation was measured differentiation by itself (data not shown). However, as shown in Fig. 4A, it markedly inhibited activin A-induced differentiation of erythroleukemia cells.
We therefore examined whether or not infected with the FLAG-tagged adenovirus vector, and then incubated in serum-free medium for 48 or 72 h, and [3H]thymidine incorporation was then measured. Values are means ± SE for 4 experiments. *P < 0.05 vs. mock transfection. A: AML12 cells were transfected with the βc-subunit (filled bars) or vector alone (open bars), using adenovirus vector and then incubated in serum-free medium for 48 or 72 h, and [3H]thymidine incorporation was then measured. Values are means ± SE for 4 experiments. *P < 0.05 vs. mock transfection. B: expression of mRNA for endogenous βA- and βc-subunits. AML12 cells were incubated for 24 h in medium with (growing) or without (quiescent) 10% FBS, and RNA was extracted. Northern blotting was done to detect mRNA for the βA- and βc-subunits. C: AML12 cells were transfected with FLAG-tagged βc-subunit by using adenovirus vector with different multiplicity of infection (MOI). Cells were then incubated for 48 h in the presence or absence of 10% FBS. The medium was obtained and immunoprecipitated with anti-FLAG antibody, and then immunoblotting (IB) was done by using anti-βA or anti-FLAG antibody. D: AML12 cells were transfected with FLAG-tagged βc-subunit by using adenovirus vector at titers of 10 and 50 MOI. Cells were then incubated for 48 h in the presence of 10% FBS. Immunoprecipitation and immunoblotting were done as described.

Fig. 5. Transfection of βc-subunit with adenovirus vector. A: AML12 cells were transfected with the βc-subunit (filled bars) or vector alone (open bars), using adenovirus vector and then incubated in serum-free medium for 48 or 72 h, and [3H]thymidine incorporation was then measured. Values are means ± SE for 4 experiments. *P < 0.05 vs. mock transfection. B: expression of mRNA for endogenous βA- and βc-subunits. AML12 cells were incubated for 24 h in medium with (growing) or without (quiescent) 10% FBS, and RNA was extracted. Northern blotting was done to detect mRNA for the βA- and βc-subunits. C: AML12 cells were transfected with FLAG-tagged βc-subunit by using adenovirus vector with different multiplicity of infection (MOI). Cells were then incubated for 48 h in the presence or absence of 10% FBS. The medium was obtained and immunoprecipitated with anti-FLAG antibody, and then immunoblotting (IB) was done by using anti-βA or anti-FLAG antibody. D: AML12 cells were transfected with FLAG-tagged βc-subunit by using adenovirus vector at titers of 10 and 50 MOI. Cells were then incubated for 48 h in the presence of 10% FBS. Immunoprecipitation and immunoblotting were done as described.

Fig. 6. Effect of recombinant activin C on AML12 cell growth. AML12 cells were incubated with serum-free medium containing various doses of recombinant activin C for 48 h, and the number of cells (A) or [3H]thymidine incorporation (B) was measured. Values are means ± SE for 4 experiments. *P < 0.05 vs. none.
obtained from cells stably transfected with the vector and by testing the effect of the conditioned medium on DNA synthesis in rat hepatocytes. By incubating with various concentrations of activin A (A) and activin C (B), and 125I-activin A binding to the cellsurface receptors (A) and follistatin (B) was measured. Values are means ± SE of 3 experiments.

**Effect of conditioned medium of βC-transfected cells on DNA synthesis in rat hepatocytes.** Finally, we examined the effect of conditioned medium on DNA synthesis in rat hepatocytes in primary culture. DNA synthesis in hepatocytes incubated in conditioned medium obtained from βC-transfected cells was slightly but significantly higher than that in cells incubated in the conditioned medium from mock-transfected cells (Fig. 8).

**DISCUSSION**

In the present study, we addressed whether or not activin C has any function in hepatocytes. Because of the limited amount of recombinant activin C available, we employed two alternate approaches to assess the effect of activin C: by testing the effect of transfection of the vector and by testing the effect of conditioned medium obtained from cells expressing the vector and follistatin. 125I-activin A was incubated with various concentrations of activin C. These different approaches provided essentially similar results.

The conditioned medium obtained from cells expressing the βC-subunit contained a considerable amount of activin C, a βC homodimer. Surprisingly, when added to AML12 cells, it stimulated DNA synthesis and increased the number of cells in serum-free medium. Furthermore, the conditioned medium supported the survival of AML12 cells cultured in a serum-free condition. These effects were the opposite of that of activin A, a βA homodimer. Indeed, the effect was observed in AML12 cells expressing the dominant-negative activin receptor, indicating that activin C can exert its action independently of the activin receptor signaling. A similar stimulatory effect was observed by addition of recombinant activin C. A small stimulatory effect on DNA synthesis was also observed in rat hepatocytes in primary culture. Collectively, unlike activin A, activin C functions as a growth-promoting factor as well as a survival factor in hepatocytes. Perhaps activin C binds to its unique receptor and transmits growth-promoting signals. Elucidation of the putative receptor and the mechanism of action of activin C await further studies.

Besides the action on hepatocytes as a ligand, the βC-subunit also elicits its function by another mechanism, namely formation of a heterodimer with the βA-subunit. Mellor et al. (23) showed that βA- and βC-subunits form a heterodimer in prostate cells. They postulated that the βC-subunit antagonizes activin A by forming a nonfunctioning βA-βC subunit (22, 23). Our results are consistent with their proposal. If the expression of the βC-subunit increases, it would prevent the formation of activin A, a βA homodimer. In normal liver, the expression of the βA-subunit is low, whereas that of βC is quite high. Hence, formation of the growth-inhibitory βA homodimer may be prevented by abundant expression of the βC-subunit in intact liver. Although the function of the βA-βC heterodimer is not certain at present, it is perhaps less potent than the βA-βA homodimer in inhibiting hepatocyte growth. Again, the βC-subunit functions to attenuate the growth-inhibitory action of the βA-subunit.

Another aspect of βC function was obtained in experiments done in erythroleukemia cells. In these cells, activin A induces erythroid differentiation, and this effect has been used for the bioassay of activin A (10). In these cells, the conditioned medium from βC-transfected cells, but not from mock-transfected cells, blocked the effect of activin A. Because activin C does not affect the binding of activin A to its receptor, activin C in the conditioned medium may inhibit the activin A signaling at a step distal to the receptor binding. At present, the precise mechanism of the action of activin C is not clear. Identification of the putative receptor for activin C would help to answer this question.

Collectively, these results demonstrate three aspects of the function of the βC-subunit of activin. Intracellularly, it prevents the formation of the βA dimer activin A. Once secreted as a homodimer, activin C stimulates hepatocyte growth by itself. Activin C also attenuates the action of activin A by acting on the postreceptor step. Therefore, the function of the βC-subunit is to antagonize the action of the βA-subunit. In this regard, Lau et al. (18) generated βC-null mice and found no abnor-

**Fig. 7.** Effect of recombinant activin C on binding of 125I-labeled activin A to receptors and follistatin. 125I-activin A was incubated with various concentrations of activin A (A) and activin C (B), and 125I-activin A binding to the cell-surface receptors (A) and follistatin (B) was measured. Values are means ± SE of 3 experiments.

**Fig. 8.** Effect of conditioned medium (CM) on DNA synthesis in rat hepatocytes. Rat hepatocytes were incubated for 48 h with conditioned medium obtained from βC-transfected or mock-transfected CHO cells, and [3H]thymidine incorporation was measured. As a positive control, hepatocytes were incubated with 1 nM EGF and conditioned medium from mock-transfected cells. Values are means ± SE for 4 experiments and expressed as a percentage of the value obtained in cells treated with mock-transfected conditioned medium (1,035 ± 62 cpm). **P < 0.05 and ***P < 0.005 vs. mock transfected.
mality in the liver. Because the activin-follistatin system is very complex, comprising many types of ligands, binding proteins, and receptors, deletion of one or two ligands may have been compensated for by other ligands, including other members of the TGF-β superfamily and possibly by other types of growth factors. For example, although activin A exerts numerous effects and regulates cellular functions in various organs (1, 2, 12, 15, 19, 21, 29), βA-null mice have abnormalities in only limited numbers of organs (6). Because hepatocyte growth and differentiation are regulated by many growth factors, it is not surprising that the βc-null mice have minimal abnormalities in the liver. Recently, Chabiovsky et al. (5) reported that transfecion of the mouse liver with the βc-subunit reduced DNA synthesis in mice. Their results are apparently opposite to ours. They introduced the βc gene by using naked plasmid DNA and measured the regeneration after the damage induced by the gene transfer. They concluded that the βc gene inhibited regenerative DNA synthesis in hepatocytes. The transfection was achieved in only 6% of hepatocytes of the H9252 line. The production of activin A is shut off by both reduction of the βA-transfected mice was comparable to that in LacZ-transfected mice. Furthermore, DNA content and the liver weight in βc-transfected mice were not different from those in LacZ-transfected mice. The reduced labeling of BrdU on day 2, therefore, could be explained simply by the phase shift of the DNA synthesis. More importantly, the DNA content and the liver weight in the βc-transfected liver were unchanged, whereas those in the βA-transfected liver were significantly reduced. Consequently, their results rather indicate that the function of the βc is quite different from that of the βA. Vejda et al. (26) recently reported that transfection of the βc-subunit induced apoptosis in Hep G2 cells. As we reported previously (20), the activin-follistatin system is deranged in many respects in hepatoma cells including Hep G2 cells. Interpretation of data obtained in hepatoma cells should be done carefully.

The present results suggest that the activin-follistatin system in the liver is much more complex than thought previously. The βA-subunit is expressed at relatively low levels in hepatocytes in normal liver. Considering that the βA- and βc-subunits form a heterodimer, activin A, a homodimer of the βA, may not be formed in normal liver, since the expression of the βC-subunit is high. Instead, activin C, a homodimer of the βC-subunit, may be formed abundantly in this condition. After partial hepatectomy, the expression of the βC decreases whereas that of the βA is upregulated (9, 33). This allows the formation of the βA dimer activin A to occur, which participates in termination of the regeneration processes (12, 17). Then the production of activin A is shut off by both reduction of the βA expression and restoration of the βc expression. Regarding the termination of the activin A action, we need to mention that follistatin, an antagonist of activin A, is also produced in hepatocytes (31). Indeed, the expression of follistatin is augmented by activin A (6). It is quite likely that follistatin also modifies the effect of activin A during liver regeneration.

In conclusion, the action of the βA dimer activin A is antagonized not only by follistatin but also by a related ligand activin C, a homodimer of the βc-subunit. In the liver, the βc-subunit is also expressed at high levels, and the expression pattern is similar to that of the βc-subunit. Although we do not know the function of the βc-subunit at present, the βc-subunit may have its own function. Collectively, the activin-follistatin system may regulate the cells in the liver in a much more complex fashion.

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