βA- and βC-activin, follistatin, activin receptor mRNA and βC-activin peptide expression during rat liver regeneration

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Abstract

The mRNA expression of two activin growth factor subunits (βA- and βC-activin), activin receptor subunits (ActRIIA, ActRIIB) and the activin-binding protein follistatin, and peptide expression of βA-activin and βC-activin subunits, were examined in regenerating rat liver after partial hepatectomy (PHx). Liver samples were collected from adult, male Sprague–Dawley rats, 12–240 h (n=3–5 rats per time point) after PHx or from sham-operated controls at the same time points. Hepatocyte mitosis and apoptosis were assessed histologically and by in situ cell death detection. RT and PCR were used to assess relative gene expression. βA- and βC-activin peptide immunoreactivity was assessed in liver and serum samples by western blotting, whereas cellular expression was investigated by immunohistochemistry, using specific monoclonal antibodies. βA- and βC-activin mRNA dropped to <50% of sham control values 12 h after PHx and remained at this level until 168 h post-PHx, when βA-activin expression increased to three times sham control values and βC-activin mRNA returned to pre-PHx levels. A peak in follistatin expression was observed 24–48 h post-PHx, coincident with an increase in hepatocyte mitosis. No changes were observed in ActRIIA mRNA, whereas ActRIIB expression paralleled that of βA-activin mRNA. βC-activin immunoreactive homo- and heterodimers were observed in regenerating liver and serum. Mitotic hepatocytes frequently contained βC-activin immunoreactivity, whereas apoptotic hepatocytes were often immunoreactive for βA-activin. We conclude that βA- and βC-activin subunit proteins are autocrine growth regulators in regenerating liver and when expressed independently lead to hepatocyte apoptosis or mitosis in a subset of hepatocytes.

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Introduction

Cell proliferation and cell death are subject to control by stimulatory and inhibitory factors. Simplistically, trophic factors will stimulate mitosis and at the same time inhibit cell death, while negative growth signals will inhibit mitosis and cause apoptosis. In contrast to the many heterotropic factors that have been identified, very few growth inhibitors or apoptosis inducers have been characterised in the liver (Fausto et al. 1995). The activins belong to the transforming growth factor-β (TGF-β) gene family. Members of this family share structural similarities, but are diverse in their biological activities (Kingsley 1994). Activins are hetero- or homodimers of inhibin β-subunits held together by disulphide bonds (Ying 1988). Activins control cell proliferation and programmed cell death in a variety of tissues including the liver (Schwall et al. 1993, Yasuda et al. 1993, Hully et al. 1994). Activin A is a homodimer of the inhibin βA-activin subunit. It is expressed in hepatocytes and has been shown to be a negative regulator of hepatocyte cell growth (Schwall et al. 1993, Yasuda et al. 1993, Hully et al. 1994). In the normal liver the expression of activin A is relatively low (Yasuda et al. 1993). Activin A is involved in the maintenance of constant liver mass, since blocking activin A action by administrating the activin-binding protein follistatin leads to hepatocyte proliferation (Kogure et al. 1995, 1996, 1998, 2000, Koizan et al. 1997, Zhang et al. 1997a). Activin A is also up-regulated in the development of liver cirrhosis concomitant with an increase in hepatocyte apoptosis (Gold et al. 2003).

βC-activin mRNA appears to be predominately expressed in the liver (Hotten et al. 1995, Lau et al. 1996, 2000, Schmitt et al. 1996, Gold et al. 2004) and is down-regulated 12 h after partial hepatectomy (PHx) in the rat, suggesting that it too may be a negative regulator of liver cell growth (Esquela et al. 1997, Zhang et al. 1997b). Two recent studies show that βC-activin
inhibits DNA synthesis of hepatic cells in vivo (Chabicovsky et al. 2003) and induces apoptosis in hepatoma cells in vitro (Vejda et al. 2003). Stimulation of hepatocyte proliferation by administration of di-n-butyl phthalate was accompanied by a rise in βB- and βC-activin mRNA and a decrease in βα-activin mRNA (Kobayashi et al. 2002). The βC-activin subunit can form heterodimers with the βα-, βB- and βE-activin subunits in vitro (Mellor et al. 2000, 2003, Vejda et al. 2002) and acts at the intra-cellular level to modulate the synthesis of activin A or activin B via the formation of activin AC or BC heterodimers (Mellor et al. 2000, 2003, Kobayashi et al. 2002). Recent research demonstrates that βC-activin homodimer can be growth promoting and confirms that intracellular AC dimerisation prevents the formation of activin A (Wada et al. 2004). Thus βC-activin has been shown to be either growth inhibitory (Esquela et al. 1997, Zhang et al. 1997b) or growth promoting (Kobayashi et al. 2002, Wada et al. 2004) in liver. A balance between cell proliferation and apoptosis is crucial for regulating normal liver function (Chen et al. 2000), since abnormalities in liver regeneration may contribute to chronic hepatitis, cirrhosis and cancer (Leevy 1998). Cellular control of activin expression appears to occur at many levels, including the amount of growth factor, dimer combinations formed, the presence of binding proteins, receptor recruitment and presence of other peptides (Phillips 2000). The presence of β-activin subunits, follistatin and activin receptor mRNAs, and the ability of these proteins to affect hepatic cell division, implies that these growth factors may be involved in the liver’s regenerative response. In order to examine the ability of the βC-activin subunit to antagonise activin A action in vivo, we examined hepatocyte cell death and division after PHx in the liver of male rats and assessed mRNA expression of βα-activin, βC-activin, follistatin, and activin receptor subunits ActRIIA and ActRIIB; and also βα- and βC-activin subunit dimer formation. We hypothesised that βC-activin expression is associated both cellurally and temporally with βα-activin expression, at times of liver growth. If this hypothesis is correct we would predict co-localisation of the two activin subunits in mitotic or resting hepatocytes. Furthermore we predict βα-activin expression to be high, and the expression of antagonists of activin A (βC-activin and follistatin) to be decreased at times of maximal hepatocyte apoptosis.

Methods

Ethical approval

All experiments using animals were performed in accordance with the 1999 Animal Welfare Act regulations of New Zealand after approval was granted by the University of Otago Medical School Animal Ethics Committee.

Surgery

Male (220–250 g) Sprague–Dawley rats were randomly allocated to a PHx or control group. PHx was performed under ether anaesthesia according to a previously described method (Higgins & Anderson 1932). Control sham operations consisted of opening the abdominal cavity and briefly handling the liver. Two sham-operated control animals were killed at 12, 24, 48, 72, 96, 168 and 240 h post-sham PHx. Five PHx rats were killed at 12, 48 and 168 h; while three PHx rats were killed at 24, 72, 96 and 240 h post-PHx. Samples of caudate lobe from regenerating or sham control liver samples were removed and either immediately stored at −80 °C or prepared for histology. The caudate lobe is not affected by the surgery, but does participate in the restoration of liver mass (Higgins & Anderson 1932).

Histology

Samples were fixed in 4% paraformaldehyde and processed to 4 µm thick sections. Serial sections were used for immunohistochemistry, cell death detection, and haematoxylin and eosin staining for histology. Hepatocyte cell death was assessed with an in situ cell death detection kit (TUNEL, Roche) and was confirmed by counting apoptotic bodies in serial sections, as previously described (Gold et al. 2003). Mitotic hepatocytes were identified based on histology (prophase, metaphase, anaphase or telophase). Within sections, the percentage of mitotic hepatocytes or TUNEL-positive hepatocytes were counted. Five randomly selected fields were assessed in duplicate sections, in three to five animals per time point and the results are presented as means ± s.d.

Molecular biology

Total RNA was extracted from liver using a RNeasy Mini Kit (Qiagen) according to manufacturer directions. RNA concentration was determined by spectrophotometry at 260 nm. An equal amount of RNA from each tissue was used for generation of first-strand cDNA. Primers for PCR were designed based on published rat sequences, or from consensus sequence of human and mouse βC-activin cDNA (Gold et al. 2003). All primers were designed so that the amplicon was derived from two or more exons. Preliminary PCR assays were performed to determine the optimal annealing temperature, MgCl₂ concentration and range of cycles over which the amplified cDNA samples remained in the exponential phase of amplification (Gold et al. 2003). Reactions without template, genomic DNA and RNA
controls, were run alongside all experimental samples. PCR products were separated on agarose gel, photographed and semi-quantified by densitometry as previously described (Gold et al. 2003). The density of amplified product for each sample was normalised with respect to the density of β-actin mRNA. To confirm amplicon identity, PCR products from two separate reactions were sequenced in both directions using the direct-cycle method, by the University of Otago Centre for Gene Research sequencing facility.

**Immunoblotting**

The identity of activin dimers present in tissue extracts and serum was determined by western immunoblotting. Protein samples were prepared for electrophoresis by homogenising 50 mg tissue in 500 µl non-reducing sample buffer (4% (w/v) SDS, 2 mM EDTA, 50 mM Tris–HCl), heating to 100 °C for 5 min and centrifuging at 10 000 g for 10 min. Total protein concentration was determined with a Sigma BCA kit (Sigma–Aldrich). Equal amounts of protein (2 mg) were loaded onto 15% polyacrylamide gels and separated by electrophoresis under non-reducing conditions. The proteins were transferred to polyvinylidene difluoride membrane with a reducing transfer buffer (700 mM glycine, 300 mM Tris, 15·6% ethanol, 15 mM dithiothreitol). Antibodies were diluted [βA-activin 1:2000 (R&D Systems, Inc., Minneapolis, MN, USA) and βC-activin 1:3000 (Mellor et al. 2000)], incubated overnight at 4 °C in Tris-buffered saline (TBS)–5% milk powder. Biotinylated goat anti-mouse secondary antibody (Amersham) was diluted 1:3000 in TBS containing 1% BSA (Sigma) and sections were incubated with TBS containing 1% BSA (Sigma) and sections washed with TBS containing 0·1% Tween-20. Negative controls were included in every experiment: sections were incubated with primary antibody only, secondary antibody only or mouse monoclonal IgG substituted for primary antibody (Sigma–Aldrich). Specificity of staining was also assessed by pre-absorption of the βA- or βC-activin primary antibodies with 500 µg/ml antigenic peptide as described previously (Mellor et al. 2000). The percentages of intensely stained βA- and βC-activin hepatocytes undergoing mitosis or apoptosis were assessed in liver sections. Slides from regenerating liver were analysed for positive β-activin subunit staining in apoptotic (12 h, 7 day and 10 day sections) or mitotic hepatocytes (24 h, 48 h and 72 h sections). Apoptotic hepatocytes in βA- or βC-activin-stained sections were identified by their histological appearance (Gold et al. 2003) and by positive TUNEL staining in the adjacent section. Mitotic hepatocytes in prophase, metaphase, anaphase or telophase were identified by histological appearance. These time points were chosen as they contained increased evidence of apoptosis or mitosis compared with the other time points (see Fig. 1). The frequencies of apoptotic or mitotic hepatocytes that exhibited intense activin subunit immunoreactivity were presented as a staining index (stained hepatocytes/total hepatocytes in mitosis or apoptosis × 100).

**Statistics**

The normalised densities of PCR products (n = 41) were log transformed and variation with time assessed by ANOVA (SPSS for Windows 6·1). In figures, normalised
densities are expressed as a percentage of sham-operated controls at each time point. Gene expression results are presented as means ± S.D. of 3 independent PCR reactions with different cDNAs, n = 3–5 PHx and 2 sham controls per time point.

Mitotic/apoptotic staining percentages were analysed by Mann–Whitney U-test (SPSS for Windows 6.1).

Results

Body weight

Rats lost 4% of their body weight post-PHx, returning to pre-operation weight after 5 days (120 h). At 7 days (168 h) post-PHx, by which time liver mass had been restored, rat body weight was steadily increasing (14% increase compared with pre-operation weight). Control rats did not lose weight after the sham operation and their body weight increased throughout the study (25% increase by day 7).

Histology

The percentages of mitotic and apoptotic hepatocytes in regenerating male rat liver are presented in Fig. 1. Mitosis was observed rarely in sham-operated control liver. An increase in the percentage of mitotic hepatocytes was evident in the caudate lobe 48 h (P < 0.001) and 72 h (P < 0.02) post-PHx. Cell death was low in sham-operated control liver, but an increased rate of TUNEL staining was evident at 12 h and from 96 h on in regenerating male rat liver.

Gene expression

Concentrations of β-actin mRNA did not vary significantly after control sham operations or during the course of liver regeneration (P = 0.537, ANOVA). Control sham operations did not lead to significant changes in βA- or βC-activin, ActRIIA, ActRIIB or follistatin mRNA (Fig. 2). Results are mean ± range in n = 2 control rats at each time point. Follistatin mRNA expression was very low in control livers. Consistently more βC-activin than βA-activin mRNA and equivalent amounts of ActRIIA and ActRIIB mRNA were detectable.

Results from the caudate lobe of regenerating rat liver are presented graphically as a percentage of sham controls. Significantly lower amounts of βA-activin mRNA were measured in the caudate lobe of regenerating liver at 12 and 24 h following PHx, compared with sham-operated controls (P < 0.05, Fig. 3A). βA-activin mRNA increased at 168 h, when liver mass appeared fully restored (P < 0.001). Amounts of βC-activin mRNA decreased significantly compared with controls levels at 12, 24 (P < 0.05) and 72 h (P < 0.001) post-PHx (Fig. 3A). Follistatin mRNA increased from very low values to a peak at 24 and 48 h in regenerating liver (P < 0.001), decreasing to barely measurable values at 72, 168 and 240 h compared with controls (P < 0.001, Fig. 3B). The expression of ActRIIA mRNA showed no significant changes with restoration of liver mass, whereas ActRIIB receptor mRNA increased markedly at 168 h (P < 0.001, Fig. 3C), compared with controls.
**Activin peptides in liver**

Activin subunit immunoreactivity in control and restoring rat liver are shown in Fig. 4. Immunoblotting revealed that the caudate lobe of sham-operated male rat liver contained moderate amounts of activin pro-proteins (above 32 kDa), small amounts of a βA-activin homodimer, no evidence of the βAβC (23 kDa) band and moderate levels of the βC-activin homodimer at 21 kDa. βA-activin immunoreactive bands corresponding to pro-peptides (above 36 kDa), βAβA-activin (26 kDa) and βAβC-activin (23 kDa) were evident in the regenerating caudate lobe (Fig. 4A). There were βC-activin immunoreactive bands above 36 kDa (likely to be βC-activin subunit pro-peptides), at 23 kDa (βAβC-activin) and at 21 kDa (βCβC-activin) in regenerating liver (Fig. 4B).

Activin dimer combinations appeared to alter in restoring rat liver (Fig. 4), but no attempt was made to quantify amounts of immunoreactivity on the blots. The 12-h time point showed little evidence of dimers of either subunit. The proportion of βAβA (26 kDa) and βAβC (23 kDa) activin dimers appeared to increase at 24 h. There was proportionally more βAβC activin (23 kDa) at 48 h than either βAβA (26 kDa) or βCβC (21 kDa) and at 96 h there appeared to be more βC-activin than βA-activin immunoreactivity compared with other time points. At 168 and 240 h the male rat restoring caudate lobe showed evidence of more βAβA activin (26 kDa) and βAβC activin (23 kDa), than βCβC activin (21 kDa).

**Activin peptides in serum**

In the serum of PHx rats the anti-βA-activin antibody detected a double band at 23–26 kDa; this is likely to be activin AC (βAβC dimer), activin AB (βAβB dimer) and/or activin A (βAβA, Fig. 5A). The βC-activin antibody also detected two circulating peptides in regenerating and control rat serum (Fig. 5B). These bands may be activin AC (23 kDa), activin BC and/or activin C (βCβC dimer, 21 kDa). The unidentified intensely immunoreactive band evident at around 30 kDa for both β-activin antibodies is likely to be an artefact caused by excess loading of serum onto the gel.
Activin subunit immunohistochemistry

Representative views of activin subunit immunoreactivity are displayed in Fig. 6. Mild βA-activin subunit immunoreactivity was present in hepatocyte cytoplasm in control and regenerating male rat liver. At 48 h post-PHx, staining was mainly associated with granular or vesicular structures, rather than hepatocyte cytoplasm (Fig. 6A). In the later stages of restoration of liver mass, there was increased βA-activin subunit immunoreactivity associated with apoptotic hepatocytes (Fig. 6B).

Moderate βC-activin subunit immunoreactivity was observed in the majority of hepatocytes in control male liver. At 48 h after PHx, there was intense βC-activin subunit immunoreactivity associated with mitotic cells (Fig. 6C). βC-activin immunoreactivity was evident in approximately 65% of hepatocyte nuclei, as well as in the cytoplasm, at 168 and 240 h post-PHx (Fig. 6D).

βA-activin subunit staining was assessed in 355 mitotic hepatocytes and 29 were strongly positive. βC-activin subunit immunoreactivity was assessed in 243 mitotic hepatocytes and 218 were intensely immunoreactive. Of 195 apparently apoptotic hepatocytes assessed for βA-activin subunit staining, 144 were markedly positive, whereas 32 of 191 apoptotic hepatocytes assessed for βC-activin subunit immunoreactivity were positive. These data are shown in Fig. 7. Thus an increase in βA-activin subunit peptide was associated with hepatocyte cell death (P<0·01), whereas an increase in βC-activin subunit immunoreactivity was significantly associated with hepatocyte cell division (P<0·001).

Discussion

Temporal changes in mRNA expression of βA- and βC-activin subunits, follistatin and activin receptor RIIB were observed in the regenerating caudate lobe of male rat liver.

The ratio of βA- and βC-activin subunit dimers appeared to alter in restoring rat liver. An increase in βA-activin subunit protein was associated with
hepatocyte apoptosis, while increased βC-activin subunit protein was associated with hepatocyte mitosis.

Activin A and activin receptor subunit ActRIIA have previously been shown to be associated with apoptosis (Schwall et al. 1993, Yasuda et al. 1993, Hully et al. 1994, Coerver et al. 1996, Draper et al. 1997). Our results indicate that locally produced activin A acts as an autocrine factor to regulate hepatocyte cell proliferation, however we failed to demonstrate changes in ActRIIA mRNA. Activin-induced apoptosis of hepatoma cells is blocked by dominant negative forms of ActRIIB or Smad 2, whereas over-expression of either ActRIB, ActRIIB, Smad 2 or Smad 4 is sufficient to stimulate apoptosis in the absence of activin A (Chen et al. 2000, 2002). This suggests that activin A signals through ActRIB/ActRIIB receptors and the receptor Smad pathway plays a central role in determining cellular fate. Hubner et al. (1999) and Rosendahl et al. (2001) have examined activin receptor type II mRNA in wound healing and lung inflammation. Neither study demonstrates changes in these mRNAs in their in vivo models. To the best of our knowledge the data presented here represent the first report of dynamic regulation of activin type IIB receptor subunit mRNA.

The mechanisms by which activin A regulates hepatocyte growth arrest are largely unknown. In HepG2 cells, retinoblastoma hypophosphorylation – via modulation of p33, p21 and the cyclin-dependent kinase Cdk4 – is involved in activin-mediated cell growth inhibition (Zauberman et al. 1997). Activin has also been shown to induce hepatocyte growth arrest through induction of the cyclin-dependent kinase inhibitor p15 and transcription factor Sp1 (Ho et al. 2004). Examining the expression of genes shown to be associated with activin-mediated cell cycle arrest in vitro in an in vivo model of liver regrowth may provide further evidence as to the mechanisms by which activins control hepatocyte cell growth. Follistatin has previously been shown to increase DNA synthesis and to antagonise the inhibitory effects of activin A in regenerating liver (Kogure et al. 1993, 1996, 1998, 2000, Zhang et al. 1997a). The increase in follistatin demonstrated in our study may therefore serve to protect proliferating hepatocytes from the apoptotic effects of activin A. Mitosis was evident at 48–72 h when follistatin mRNA expression was high. This may indicate that increased follistatin expression promotes hepatocyte cell division, either independently or by blocking the negative growth effects of activin A.

βC-activin has been shown to be growth inhibitory in liver (Esquela et al. 1997, Zhang et al. 1997b) or growth promoting (Kobayashi et al. 2002, Wada et al. 2004). Lau et al. (2000) demonstrated, by RNase protection assay with a probe from the 3′ untranslated region of C-activin cDNA, an increase in βC-activin mRNA 12–24 h after PHx; this differs both from our results and from those of Esquela et al. (1997) and Zhang et al. (1997b). Discrepancies may be related to the region of βC-activin cDNA examined or the part of the liver used in this study. In our study, βC-activin mRNA was low in extracts of regenerating liver, but significantly associated histologically with mitotic hepatocytes. Thus the gene expression data presented support the theory that
βC-activin may act as a liver chalone, whereas the immunohistochemical data suggest a local role for βC-activin in hepatocyte proliferation. In addition, our data suggest that activin mRNA may be labile and translated peptide stored in hepatocytes, as mRNA levels did not accurately reflect the amount of activin subunit proteins evident by immunohistochemistry or immunoblotting. These discrepancies highlight the importance of examining activin at both the mRNA and protein level.

It has been proposed that high levels of βC-activin subunit peptide may protect dividing cells from the apoptotic effects of activin A by forming intracellular heterodimers (Mellor et al. 2000). There was, however, very little evidence of βA-activin subunit immunoreactivity in mitotic hepatocytes, indicating that the heterotrophic effects of βC-activin subunit protein were independent of βA-activin subunit protein within mitotic hepatocytes. Mellor et al. (2000, 2003) and

Figure 6 Selected views of βA-activin and βC-activin subunit peptide immunoreactivity in paraffin-embedded, paraformaldehyde-fixed sections of the caudate lobe of male rat regenerating liver. (A) Granular βA-activin subunit immunoreactivity in hepatocytes. Adjacent hepatocytes undergoing mitosis show no positive staining with the βA-activin antibody. (B) βA-activin subunit immunoreactivity in a cell undergoing apoptosis. (C) Cytoplasmic βC-activin subunit immunoreactivity in association with mitosis. (D) βC-activin subunit immunoreactivity within hepatocyte nuclei, 10 days after PHx. (E and F) βA- and βC-activin control sections incubated with mouse IgG. Final magnifications: A, B and C, ×400; D, ×40; E and F, ×200. No staining was observed in control sections incubated with primary antibody pre-absorbed with 100 times excess βA-or βC-activin subunit synthetic peptide (not shown, see Gold et al. 2003).
Vejda et al. (2002) reported the formation of βC-activin subunit peptide homodimers and heterodimers in vitro. Our study confirms that βC-activin homo- and heterodimers also form in vivo. When non-reduced samples were assessed, the β-activin subunit antibodies detected a range of activin dimers. The presence of a βAβC activin dimer was confirmed with both the βA- and βC-activin subunit antibodies. The exact identity of other in vivo βC-activin peptides needs to be confirmed by immunoprecipitation studies. To the best of our knowledge our study is the first evidence of in vivo βC-activin subunit peptide dimers in regenerating rat liver. Wada et al. (2004) propose that activin C homodimer, rather than activin A, is formed in normal liver and PHx may lead to increased activin A, terminating liver regeneration (Wada et al. 2004). We show activin homodimers are present at low to moderate levels in control liver while increased activin AC was evident in regenerating liver. Therefore the formation of activin heterodimers may antagonise the formation of homodimers and allow restoration of liver mass. βE-activin is also expressed in the liver and can dimerise with βA- and βC-activin subunits, and is therefore likely to be an additional player in the restoration of liver mass (Lau et al. 2000, Vejda et al. 2002).

Significant amounts of βC-activin subunit peptide were detected in the serum of rats by western blotting. During regrowth the liver is able to carry out most normal physiological functions; however, functional reserve is very low. Therefore some peptides, rather than being processed in the liver, are secreted (Fausto 1990). The presence of circulating βC-activin subunit dimers in control rat serum suggests these dimers are not merely a by-product of a liver with low functional reserve. However the local effects of liver activins are difficult to reconcile with evidence of secreted activins. These results may indicate that βC-activin subunit proteins, like activin A, have endocrine, autocrine and paracrine roles in vivo. These findings also raise the possibility that serum levels of βC-activin subunit peptide may be able to be used as a diagnostic marker of liver health.

Figure 7 Mitotic (filled columns) and apoptotic (open columns) labelling indices for βA-activin and βC-activin subunit immunoreactivity. Slides from regenerating liver were assessed for markedly positive β-activin subunit staining in apoptotic (12 h, 7 days and 10 days PHx) or mitotic hepatocytes (24 h, 48 h and 72 h PHx). Hepatocytes undergoing cell death were identified based on histological appearance and apoptosis confirmed on an adjacent TUNEL-stained section (not shown). Mitotic hepatocytes were identified based on histological appearance. Apoptotic or mitotic cells that were markedly positive for activin subunit immunoreactivity were counted and divided by the total number of cells assessed; results are presented as a staining index (%).

Lau et al. (2000) proposed that βC-activin subunit antibody in the later stages of regeneration of male rat liver mass (168 and 240 h) was unexpected. Nuclear localisation of βA-activin subunit in rat seminiferous epithelium was presented by Blauer and colleagues in 1999. This group showed the βA-activin subunit precursor contained a functional nuclear localisation sequence (Blauer et al. 1999). A search of protein databases (Psort database) indicates that human βC-activin contains a leucine zipper domain at amino acids 148–169 (http://psort.nibb.ac.jp). Despite the specific times post-hepatectomy at which nuclear staining was observed and our observation of nuclear βC-activin staining with a polyclonal antibody, in mouse ovarian cysts (Richardson et al. 2005), we cannot entirely discount the possibility that nuclear staining may be a tissue fixation artefact. In order to determine if nuclear localisation of βC-activin subunit in the later stages of regeneration of male rat liver mass indicates a novel signalling pathway, future studies will perform western blotting on nuclear extracts of regenerating rat liver.

We have demonstrated concurrent changes in the expression of βA-activin, βC-activin, follistatin and activin receptor subunit mRNA in the caudate lobe of regenerating rat liver, over 240 h post-PHx. βC-activin appears to be regulated in parallel to βA-activin in vivo, however the two subunits are not always localised in the same cell types.

We conclude that βA- and βC-activin subunit proteins are autocrine growth regulators in regenerating liver and when expressed independently lead to hepatocyte apoptosis (βA-activin) or mitosis (βC-activin) in a subset of hepatocytes. In the liver, apoptosis is an important part of the tightly controlled homeostatic
mechanisms regulating liver function (Chen et al. 2000), perhaps indicating the importance of locally produced, autocrine growth regulators such as the activins in the liver. The mechanism by which βC-activin promotes hepatocyte mitosis and the role of the closely related βE-activin subunit in liver regeneration will form the basis of future studies.

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