Insulin-Like Growth Factor Binding Protein 5 Is Associated With Involution of the Ventral Prostate in Castrated and Finasteride-Treated Rats

Lynn N. Thomas,[1] Pinchas Cohen,[2] Robert C. Douglas,[1] Catherine Lazier,[3] and Roger S. Rittmaster[1]*

[1]*Department of Medicine and Physiology/Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada*
[2]*Department of Pediatrics, University of Pennsylvania, Philadelphia, Pennsylvania*
[3]*Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada*

BACKGROUND. Insulin-like growth factor binding protein (IGFBP)-5 has been proposed as a signal for apoptosis in the ovary. To determine the relationship between IGFBP-5 and apoptosis during regression of the androgen-deprived prostate, rats were castrated or treated with the 5α-reductase inhibitor finasteride for 4, 9, 14, 21, and 28 days.
METHODS. Ventral prostate tissue was immunostained for IGFBP-5, and apoptotic cells were identified by in situ end-labeling of fragmented DNA (TUNEL). To compare the distribution of IGFBP-5 with the distribution of apoptotic cells, mirror-image serial sections of prostate tissues from normal and day 4 finasteride-treated rats were examined.
RESULTS. In normal rats, 4 ± 1% of prostate epithelial cells stained positively for IGFBP-5, and 0.1 ± 0.03% demonstrated DNA fragmentation. IGFBP-5 staining peaked at day 9 with 93 ± 2% and 64 ± 13% of epithelial cells staining positively in castrated and finasteride-treated rats, respectively. In contrast, DNA fragmentation peaked at day 4 in tissues from both castrated and finasteride-treated rats with 7 ± 1% and 0.7 ± 0.3% of epithelial cells, respectively, staining. In the serial sections, TUNEL and IGFBP-5 staining were not usually expressed in the same cells.
CONCLUSIONS. Prostatic involution involves both programmed cell death and inhibition of cell growth. Because of the distribution of staining and the delayed expression of IGFBP-5 relative to initiation of apoptosis, we postulate that IGFBP-5 functions as an inhibitor of cell proliferation rather than as a signal for apoptosis. *Prostate 35:273–278, 1998.*

KEY WORDS: **apoptosis; atrophy; androgen withdrawal; IGF; IGFBP-5**

INTRODUCTION

Testosterone, the major circulating androgen, must be converted in the prostate to dihydrotestosterone (DHT) by the enzyme 5α-reductase for normal prostatic growth and secretory activity [1]. In the absence of androgens, regression of the prostate occurs through a combination of atrophy and programmed cell death [2–6]. In the presence of testosterone and finasteride, which cause 5α-reductase inhibition, partial regression is observed [4].

The stimulatory effect of androgens on prostate growth and secretory function is likely due, in part, to modulation of intraprostatic growth factors such as insulin-like growth factor-I and -II (IGF-I and -II). IGFs promote cell growth and differentiation in many in vitro and in vivo systems [7]. However, recent studies have suggested that modulation of IGF activity does not occur through alterations in IGF gene expression

Contract grant sponsor: Medical Research Council of Canada; Contract grant sponsor: Merck Frosst Canada.

*Correspondence to: Roger S. Rittmaster, M.D., Victoria Building, 1278 Tower Road, Room 2035D, Halifax, Nova Scotia B3H 2Y9, Canada. E-mail: ritt2@is.dal.ca

Received 22 July 1997; Accepted 21 November 1997

or protein concentration, but rather through alterations in IGF-binding protein (IGFBP) concentrations [8–12]. IGFBPs modify IGF activity by competing with the IGF receptor for the growth factors [7]. In the ovary, IGFBP-5 has been proposed as a signal for apoptosis. IGFBP-5 mRNA was shown by in situ hybridization to be expressed in the granulosa cells of atretic but not in dominant rat ovarian follicles [13].

The purpose of this study was to explore the possibility that IGFBP-5 plays a similar role in the androgen-deprived prostate. To investigate the relationship between IGFBP-5 and apoptosis in the prostate, immunocytochemical techniques were used to examine the prostates of rats which had been left intact, castrated, or treated with the 5α-reductase inhibitor finasteride for up to 28 days. Tissues were immunostained for IGFBP-5 or in situ end-labeled for DNA fragmentation, an early marker of apoptosis [14], and the pattern and timing of IGFBP-5 staining and DNA fragmentation were compared.

MATERIALS AND METHODS

Animal Protocol

Male Sprague-Dawley rats (55 days old at the start of the experiment) were purchased from Charles River Laboratories (Montreal, Quebec, Canada) and were fed water and Purina Rat Chow ad libitum. 5α-reductase inhibition was achieved with finasteride (Proscar; Merck, Sharp and Dohme Research Laboratories, Rahway, NJ). Rats were divided into three groups: normal, castrated, and finasteride-treated. Castration was performed via the scrotal route with ketamine/xylazine anesthesia. Finasteride-treated animals were given 40 mg/kg daily by sc injection in 1 ml sesame oil containing 10% ethanol [4]. The rats were killed after 4, 9, 14, 21, and 28 days of treatment. The prostates were immediately removed, weighed, and prepared for histological examination.

Immunostaining for IGFBP-5

Ventral prostate tissue was fixed in 10% phosphate-buffered formalin, and embedded in paraffin. Five-micrometer sections were deparaffinized, rehydrated, and covered with 5% horse serum for 60 min at room temperature to block nonspecific binding activity. The primary antibody used for this procedure was rabbit anti-human IGFBP-5 (supplied by Dr. Michael Kiefer, Chiron Corp., Emeryville, CA). This antibody has been shown, by Western blotting techniques, not to cross-react with IGFs or any other IGFBPs present in prostate tissue [15]. The tissues were incubated with the primary antibody overnight at 4°C in 100% humidity, washed in phosphate buffered saline (PBS), incubated with biotinylated goat anti-rabbit IgG (Dimension Laboratories, Mississauga, Ontario, Canada) for 60 min at room temperature, washed in PBS, and then incubated for 60 min at room temperature with streptavidin-alkaline phosphatase (Dimension Laboratories). Alkaline phosphatase activity was detected with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. The tissue was counterstained with methyl green. Ducts were randomly selected, and all epithelial cells in those ducts were counted at a magnification of ×400. The number of positively stained cells was expressed as a percentage of total epithelial cells. Negative controls consisted of the omission of the primary antibody from the staining procedure and the substitution of normal rabbit serum for the primary antibody. No specific staining was seen in either case.

Staining for DNA Breaks

A modification of the TUNEL technique for in situ end-labeling of DNA breaks, an early apoptotic event, was used to identify apoptotic cells [4,14]. The staining was carried out using terminal transferase to add biotinylated dAMP to the 3′ ends of fragmented DNA strands. Streptavidin-horseradish peroxidase was used to amplify the reaction, and peroxidase activity was visualized with 3-amino-9-ethyl carbazole. Ducts were randomly chosen and all epithelial cells in those ducts were counted at a magnification of ×400. The number of positively stained cells was expressed as a percentage of total epithelial cells. In the negative control, TdT was omitted and no staining was observed. We have previously reported results of TUNEL staining in these animals up to 21 days of treatment [4].

Technique for Direct Photographic Comparison of IGFBP-5 Staining and TUNEL Staining

Two-micrometer mirror-image serial sections were stained for IGFBP-5 or in situ end-labeled for DNA breaks. Identical fields of view from each were photographed and superimposed using Adobe Photoshop (Adobe Systems, Inc., Mountain View, CA) on a Macintosh computer. Cells immunostained for IGFBP-5 were purple with green counterstaining, and TUNEL-stained cells were red with blue counterstaining. When the TUNEL photographs were scanned into Adobe Photoshop, the blue counterstain was recolored green to avoid confusing purple IGFBP-5 staining with the overlaid blue counterstain of the TUNEL sections. Background colors were also adjusted to attain as close a match as possible between the two photographs. The color adjustments resulted in blue IGFBP-5 staining, green counterstaining, and red TUNEL staining. The photographs were overlaid us-

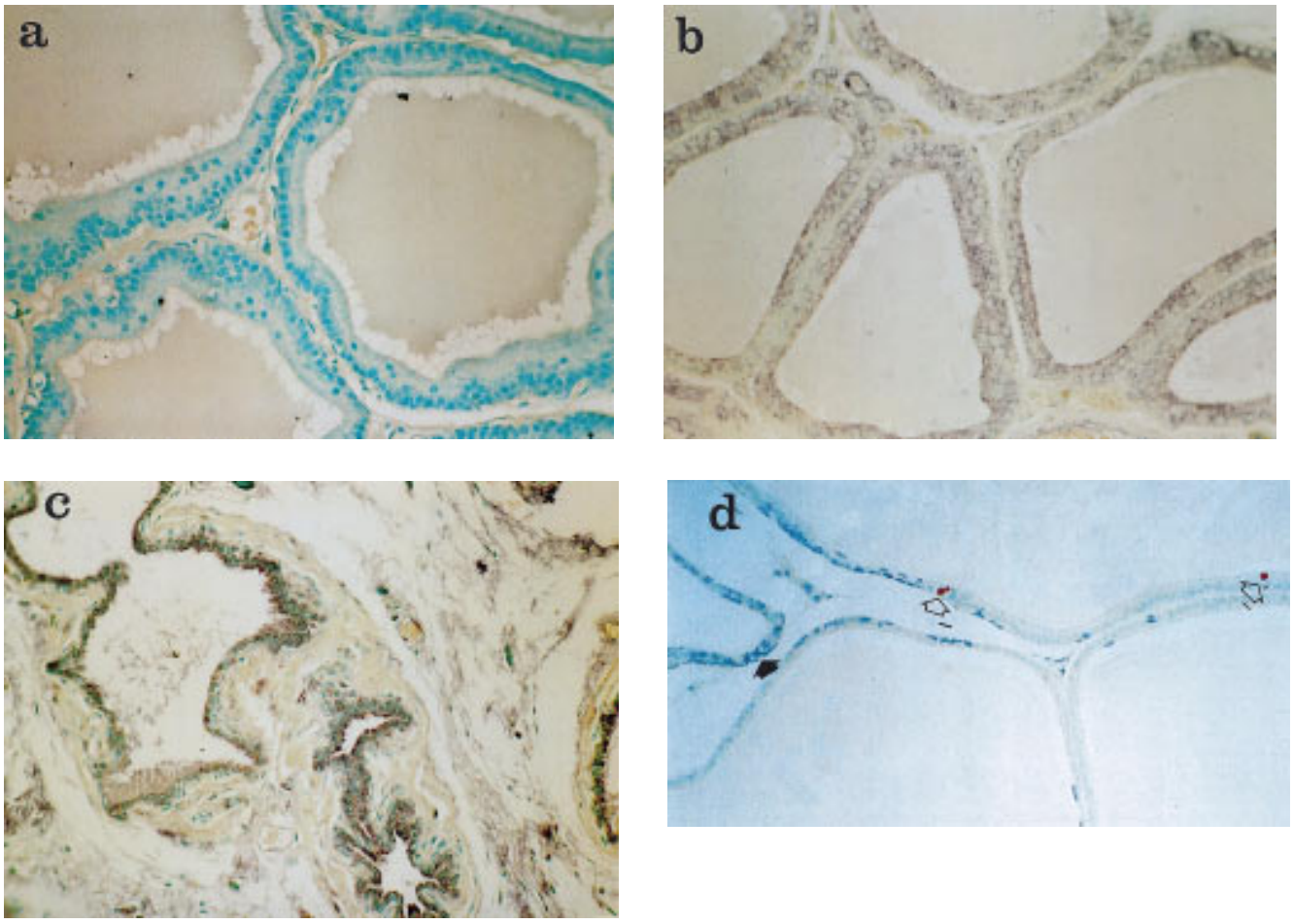

Fig. 1. IGFBP-5 staining in ventral prostate tissue from a normal **(a),** a 9-day finasteride-treated **(b),** and a 9-day castrated **(c)** rat. IGFBP-5 staining is purple, and the nuclear counterstain is green. There is negligible staining in the prostate of the normal rat because staining in these prostates was mainly confined to atrophic ducts, and normal ducts are pictured here. **d:** An overlaid photograph showing IGFBP-5 staining (dark blue, indicated by solid arrow) and TUNEL staining (red, indicated by open arrow) in ventral prostate tissue from a 4-day finasteride-treated rat. The apoptotic cells in the photograph did not stain for IGFBP-5 (×200).

ing Adobe Photoshop's hard light overlay technique, which allowed TUNEL staining to show up best.

Statistics

Results are expressed as mean ± SEM unless otherwise noted. Statistics were performed using StatView (Abacus Concepts, Berkeley, CA) on a Macintosh computer. Group means were compared using analysis of variance followed by Fisher's protected least significant difference test. $P < 0.05$ was considered statistically significant.

RESULTS

Immunostaining for IGFBP-5

Both castration and finasteride treatment resulted in a significant increase in IGFBP-5 staining (Fig. 1a–c), with castration having the greater effect (Fig. 2). In normal rats, a mean of 4 ± 1% of epithelial cells showed positive staining and there was no significant difference in staining levels throughout the duration of the experiment. The majority of positive cells in normal rats were in atrophic-looking ducts. In castrated rats, the percentage of epithelial cells staining positively increased significantly to 41 ± 9% by day 4 and reached a peak value of 93 ± 2% by day 9. At 14 days, staining levels decreased to 35 ± 7%, but they remained significantly higher than baseline values, and no further decline was seen thereafter ($P < 0.0001$ for 4, 9, 14, and 28 days; $P < 0.05$ for 21 days). A similar time course of staining was observed in the finasteride-treated rats. By day 4 of treatment, the percentage of epithelial cells showing positive staining increased significantly, to 22 ± 14% ($P < 0.05$). Staining levels

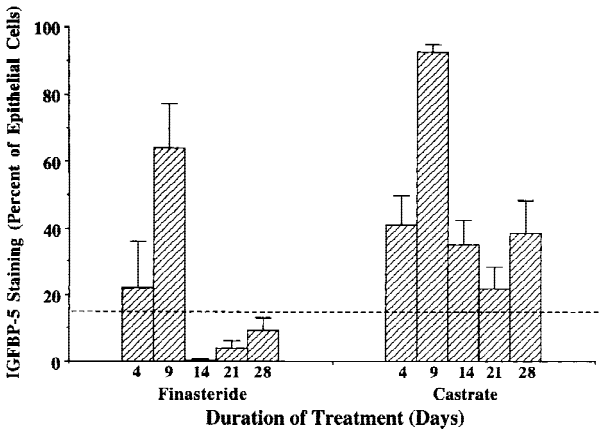

Fig. 2. Percentage of epithelial cells staining positively for IGFBP-5 in prostate tissues from 4-, 9-, 14-, 21-, and 28-day finasteride-treated and castrated rats. Dotted line indicates upper limit of normal (mean + 2 SD) for tissues from intact rats.

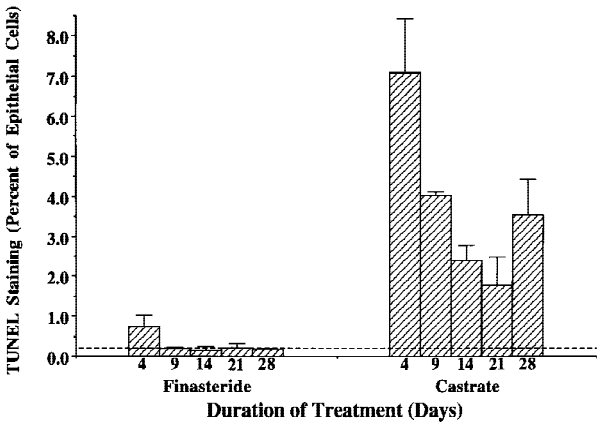

Fig. 3. Percentage of epithelial cells staining positively for TUNEL in prostate tissues from 4-, 9-, 14-, 21-, and 28-day finasteride-treated and castrated rats. Dotted line indicates upper limit of normal (mean + 2 SD) for tissues from intact rats.

peaked by day 9 at 64 ± 13% ($P < 0.0001$) and thereafter returned to baseline.

Staining for Apoptotic Cells

A mean of 0.1 ± 0.03% of epithelial cells demonstrated apoptosis in normal rats. There was no significant variation over the duration of the experiment. Both castration and finasteride treatment resulted in increased numbers of apoptotic cells. However, a much greater increase was seen in prostates from castrated than from finasteride-treated rats (Fig. 3). In prostates from castrated rats, the percentage of apoptotic cells peaked by day 4, with 7 ± 1% of cells staining positively. Thereafter a gradual decrease was seen, although staining levels remained significantly higher than baseline values ($P < 0.0001$ for 4, 9, and 28 days; $P < 0.01$ for 14 and 21 days). In prostates from finasteride-treated rats, the percentage of positively stained cells increased significantly by day 4 to 0.7 ± 0.3% ($P < 0.001$), and then staining levels returned to baseline for the duration of the experiment.

Photographic Comparison of IGFBP-5 Staining and TUNEL Staining

In order to further examine the relationship between IGFBP-5 distribution and apoptosis in the prostate, serial sections were stained for IGFBP-5 or for DNA fragmentation and compared. The majority of TUNEL-stained cells were not positive for IGFBP-5 staining in tissues from normal and day 4 finasteride-treated rats. IGFBP-5 staining was too pervasive to differentiate from TUNEL staining in the prostates from day 9 finasteride-treated and all castrated rats. However, in sections where IGFBP-5 staining was more discrete, apoptosis and IGFBP-5 staining usually occurred in different areas. Atrophic ducts (those with flattened epithelial cells) were always heavily stained for IGFBP-5, sometimes with as many as 90–100% of epithelial cells showing positive staining, but the ducts often had no apoptotic cells. Conversely, many normal ducts had one or two apoptotic cells but no IGFBP-5 staining. Most often, however, ducts were seen with IGFBP-5 staining in one area of the epithelium and one or two apoptotic cells in another area of the epithelium, with occasional overlap (Fig. 1d).

DISCUSSION

Regulation of IGF activity can be accomplished by hormonal control of IGFBP gene expression and protein stability. IGFBPs have been shown to inhibit IGF-induced cell proliferation in many cell systems. For example, high levels of endogenous IGFBP-5 render U-2 human osteosarcoma cells unresponsive to stimulation by exogenous IGF-I [16]. An IGFBP from PC3 human prostatic carcinoma cells inhibits both basal mouse osteoblast proliferation and IGF-I-induced osteoblast mitogenesis, and an IGFBP from human decidua inhibits IGF-I activity in choriocarcinoma cells [17,18]. In many other in vitro systems, IGF activity is attenuated by the addition of IGFBPs [19–24]. It is likely that IGFBPs negate the mitogenic effects of IGFs by preventing them from binding to the IGF-I receptor. IGFBPs have also been shown to enhance IGF activity. Preincubation of IGFBPs with cultured cells is required to achieve this effect [21,25–28]. The mechanism of this potentiating activity is unknown, but it has been suggested that membrane-bound IGFBPs may modulate the interaction of IGFs with IGF-I re-

ceptor, or they may act as a reservoir for IGFs [25,27]. Hormonal control of IGFBP gene expression has been demonstrated in several studies, including one which described estrogen regulation of PEO4, an estrogen receptor-positive ovarian cancer cell line. Estrogen was shown to have no effect on IGF-I expression; it stimulated PEO4 growth by downregulating IGFBP expression [9].

Much attention has been focused on the ovary and the relationship between IGFBPs and follicular atresia, a process which involves apoptosis of granulosa cells [29,30]. Several studies have identified the presence of increased concentrations of IGFBP-4 and -5 in atretic follicles but not in dominant follicles in rat, sheep, and porcine ovaries [8,13,31–35]. The accumulated evidence has led to the proposal that follicular atresia is caused by a process involving GnRH, which mediates enhanced IGFBP-4 and -5 production.

In the present study, a significant increase in IGFBP-5 staining occurred during prostatic regression. This effect was observed whether regression was caused by castration or by finasteride treatment, although castration had a greater effect. IGFBP-5 was significantly increased by day 4 of finasteride treatment or castration, with peak values being achieved by day 9. The number of apoptotic cells, as measured by the TUNEL technique, peaked in tissues from both castrated and finasteride-treated rats by day 4 of treatment. Although a relationship between prostatic involution and IGFBP-5 concentrations is apparent in the rat ventral prostate, these results argue against IGFBP-5 as a signal for apoptosis. If IGFBP-5 were a signal for apoptosis in the androgen-deprived prostate, IGFBP-5 staining would be expected to peak earlier than or at the same time as TUNEL staining. It might also be expected that cells which stain positively for IGFBP-5 would also stain positively for DNA breaks; however, such costaining was uncommon in our study.

In an effort to determine whether the increased IGFBP-5 protein distribution seen during prostatic regression is due to increased gene expression, polyA RNA was isolated from the prostate tissues of day 4 normal, finasteride-treated, and castrated rats. Three micrograms of polyA RNA from each sample were used for Northern blot analysis. A 30-mer oligonucleotide complementary to rat IGFBP-5 mRNA near the 5′ end and an 863-bp probe obtained by RT-PCR from the middle of the coding region were used as probes for the Northern analyses. The appropriate bands, as defined by Shimasaki et al. [36], who cloned and sequenced the rat IGFBP-5 gene, could not be consistently identified because they were extremely faint. Because of this inconsistency, mRNA concentrations could not be quantitated and compared among the groups. Shimasaki et al. [36], also using 3 μg of polyA RNA for Northern blot analysis, showed only very faint bands in some of the rat tissues they tested as well. They did not demonstrate IGFBP-5 mRNA in rat prostate tissue. One possible explanation for our inability to localize IGFBP-5 mRNA in the rat prostate is that it is unstable and transiently expressed, while the protein is more stable. Alternatively, it is possible that rat IGFBP-5 mRNA in the prostate is expressed at a low but steady rate and that regulation of IGFBP-5 occurs through changes in IGFBP protease activity.

Finasteride treatment provided a useful method of substantiating the results obtained from castrated animals. Previous studies have demonstrated that 5α-reductase inhibition by finasteride causes a lesser degree of prostatic involution than does castration [3–6]. In the present study, castration caused a maximum 93% reduction in prostate weight by day 14 with no further reduction seen thereafter, whereas finasteride treatment caused a maximum 65% reduction in prostate weight by day 21 with no further decrease seen thereafter. As would be expected, castration also caused a larger increase in IGFBP-5 staining compared to 5α-reductase inhibition. These results reinforce the previous results of other investigators and ourselves that finasteride does not cause the level of androgen ablation achieved by castration. The elevated intraprostatic testosterone concentrations present in finasteride-treated rats attenuate the effects of DHT withdrawal.

CONCLUSIONS

In summary, we have found that androgen deprivation is associated with increased levels of IGFBP-5 in the rat ventral prostate. The delayed expression of IGFBP-5, relative to the onset of apoptosis, argues against a role for IGFBP-5 as a signal for apoptosis. Its prominent expression in atrophic ducts, but not in cells in the early stages of apoptosis, suggests that this binding protein may serve to prevent initiation of new cell growth in the androgen-deprived prostate.

REFERENCES

1. Mee S, Bigsby RM, Higgins SJ, Yoshiki S: The endocrinology and developmental biology of the prostate. Endocr Rev 1987;8: 338–362.
2. Kyprianou N , Isaacs JT: Activation of programmed cell death in the rat ventral prostate after castration. Endocrinology 1988;122: 552–562.
3. Rittmaster RS, Magor KE, Manning AP, Norman RW, Lazier CB: Differential effect of 5 alpha-reductase inhibition and castration on androgen-regulated gene expression in rat prostate. Mol Endocrinol 1991;5:1023–1029.
4. Rittmaster RS, Manning AP, Wright AS, Thomas LN, Whitefield

S, Norman RW, Lazier CB, Rowden G: Evidence for atrophy and apoptosis in the ventral prostate of rats given the 5 alpha-reductase inhibitor finasteride. Endocrinology 1995;136:741–748.
5. Shao TC, Kong A, Marafelia P, Cunningham GR: Effects of finasteride on the rat ventral prostate. J Androl 1993;14:79–86.
6. Lamb JC, English H, Levandoski PL, Rhodes GR, Johnson RK, Isaacs JT: Prostatic involution in rats induced by a novel 5 alpha-reductase inhibitor, SK&F 105657: Role for testosterone in the androgenic response. Endocrinology 1992;130:685–694.
7. Cohick WS, Clemmons DR: The insulin-like growth factors. Annu Rev Physiol 1993;55:131–153.
8. Monget P, Monniaux D, Pisselet C, Durand P: Changes in insulin-like growth factor-I (IGF-I), IGF-II, and their binding proteins during growth and atresia of ovine ovarian follicles. Endocrinology 1993;132:1438–1446.
9. Krywicki RF, Figueroa JA, Jackson JG, Kozelsky TW, Shimasaki S, Von Hoff DD, Yee D: Regulation of insulin-like growth factor binding proteins in ovarian cancer cells by oestrogen. Eur J Cancer 1993;29A:2015–2019.
10. Kanety H, Madjar Y, Dagan Y, Levi J, Papa MZ, Pariente C, Goldwasser B, Karasik A: Serum insulin-like growth factor-binding protein-2 (IGFBP-2) is increased and IGFBP-3 is decreased in patients with prostate cancer: Correlation with serum prostate-specific antigen. J Clin Endocrinol Metab 1993;77:229–233.
11. Cohen P, Peehl DM, Graves HC, Rosenfeld RG: Biological effects of prostate specific antigen as an insulin-like growth factor binding protein-3 protease. J Endocrinol 1994;142:407–415.
12. Conover CA, Perry JE, Tindall DJ: Endogenous cathepsin D-mediated hydrolysis of insulin-like growth factor-binding proteins in cultured human prostatic carcinoma cells. J Clin Endocrinol Metab 1995;80:987–993.
13. Erickson GF, Nakatani A, Ling N, Shimasaki S: Localization of insulin-like growth factor-binding protein-5 messenger ribonucleic acid in rat ovaries during the estrous cycle. Endocrinology 1992;130:1867–1878.
14. Gavrieli Y, Sherman Y, Ben Sasson SA: Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493–501.
15. Kiefer MC, Schmid C, Waldvogel M, Schlapfer I, Futo E, Masiarz FR, Green K, Barr PJ, Zapf J: Recombinant human insulin-like growth factor binding proteins 4, 5, and 6: Biological and physiochemical characterization. Growth Regul 1993;3:56–59.
16. Conover CA, Kiefer MC: Regulation and biological effect of endogenous insulin-like growth factor binding protein-5 in human osteoblastic cells. J Clin Endocrinol Metab 1993;76:1153–1159.
17. Perkel VS, Mohan S, Baylink DJ, Linkhart TA: An inhibitory insulin-like growth factor binding protein (In-IGFBP) from human prostatic cell conditioned medium reveals N-terminal sequence identity with bone derived In-IGFBP. J Clin Endocrinol Metab 1990;71:533–535.
18. Ritvos O, Ranta T, Jalkanen J, Suikkari AM, Voultilainen R, Bohn H, Rutanen EM: Insulin-like growth factor (IGF) binding protein from human decidua inhibits the binding and biological action of IGF-I in cultured choriocarcinoma cells. Endocrinology 1988;122:2150–2157.
19. Burch WM, Correa J, Shively JE, Powell DR: The 25-kilodalton insulin-like growth factor (IGF)-binding protein inhibits both basal and IGF-I-mediated growth of chick embryo pelvic cartilage in vitro. J Clin Endocrinol Metab 1990;70:173–180.
20. Cheung PT, Smith EP, Shimasaki S, Ling N, Chernausek SD: Characterization of an insulin-like growth factor binding protein (IGFBP-4) produced by the B104 rat neuronal cell line: Chemical and biological properties and differential synthesis by sublines. Endocrinology 1991;129:1006–1015.
21. Conover CA, Ronk M, Lombana F, Powell DR: Structural and biological characterization of bovine insulin-like growth factor binding protein-3. Endocrinology 1990;127:2795–2803.
22. Frauman SG, Tsuzaki S, Moses AC: The binding characteristics and biological effects in FRTL5 cells of placental protein-12, an insulin-like growth factor-binding protein purified from human amniotic fluid. Endocrinology 1989;124:2289–2296.
23. Blat C, Delbe J, Villaudy J, Chatelain G, Golde A, Harel L: Inhibitory diffusible factor 45 bifunctional activity. As a cell growth inhibitor and as an insulin-like growth factor I-binding protein. J Biol Chem 1989;264:12449–12454.
24. Mohan S, Bautista CM, Wergedal J, Baylink DJ: Isolation of an inhibitory insulin-like growth factor (IGF) binding protein from bone cell-conditioned medium: A potential local regulator of IGF action. Proc Natl Acad Sci USA 1989;86:8338–8342.
25. Conover CA: Potentiation of insulin-like growth factor (IGF) action by IGF-binding protein-3: Studies of underlying mechanism. Endocrinology 1992;130:3191–3199.
26. Clemmons DR, Gardner LI: A factor contained in plasma is required for IGF binding protein-1 to potentiate the effect of IGF-I on smooth muscle cell DNA synthesis. J Cell Physiol 1990; 145:129–135.
27. Jones JI, Gockerman A, Busby WH Jr, Camacho Hubner C, Clemmons DR: Extracellular matrix contains insulin-like growth factor binding protein-5: Potentiation of the effects of IGF-I. J Cell Biol 1993;121:679–687.
28. Elgin RG, Busby WH, Clemmons DR: An insulin-like growth factor binding protein enhances the biological response to IGF-I. Proc Natl Acad Sci USA 1987;84:3254–3258.
29. Tilly JL, Kowalski KI, Johnson AL, Hsueh AJ: Involvement of apoptosis in ovarian follicular atresia and postovulatory regression. Endocrinology 1991;129:2799–2801.
30. Tilly JL: Ovarian follicular atresia: A model to study the mechanisms of physiological cell death. Endocr J 1993;1:67–72.
31. Mondschein JS, Etherton TD, Hammond JM: Characterization of insulin-like growth factor-binding proteins of porcine ovarian follicular fluid. Biol Reprod 1991;44:315–320.
32. Howard HJ, Ford JJ: Insulin-like growth factor binding proteins in fluid of developing follicles: Potential intra-ovarian regulators of steroidogenesis. Biol Reprod [Suppl] 1991;44:152.
33. Adashi EY, Resnick CE, Hernandez ER, Hurwitz A, Rosenfeld RG: Follicle-stimulating hormone inhibits the constitutive release of insulin-like growth factor binding proteins by cultured rat ovarian granulosa cells. Endocrinology 1990;126:1305–1307.
34. Erickson GF, Li D, Sadrkhanloo R, Liu XJ, Shimasaki S, Ling N: Extrapituitary actions of gonadotropin-releasing hormone: Stimulation of insulin-like growth factor-binding protein-4 and atresia. Endocrinology 1994;134:1365–1372.
35. Onoda N, Li D, Mickey G, Erickson G, Shimasaki S: Gonadotropin-releasing hormone overcomes follicle-stimulating hormone's inhibition of insulin-like growth factor-5 synthesis and promotion of its degradation in rat granulosa cells. Mol Cell Endocrinol 1995;110:17–25.
36. Shimasaki S, Shimonaka M, Zhang H, Ling N: Identification of five different insulin-like growth factor binding proteins (IGFBPs) from adult rat serum and molecular cloning of a novel IGFBP-5 in rat and human. J Biol Chem 1991;266:10646–10653.