Regional Loss of Imprinting of the Insulin-like Growth Factor II Gene Occurs in Human Prostate Tissues

David F. Jarrard, Marion J. G. Bussemakers, G. Steven Bova, and William B. Isaacs

Brady Urological Institute, Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, Maryland 21287 [D. F. J., G. S. B., W. B. I.]; and Urology Research, University Hospital, 6500 HB Nijmegen, the Netherlands [M. J. G. B.]

Abstract

In most tissues, the insulin-like growth factor II gene (IGF-II) demonstrates imprinting, being expressed exclusively from the paternal allele. Recently, a loss of IGF-II imprinting (i.e., biallelic expression) has been found in sporadic Wilm's tumors and lung carcinomas, and this molecular event may contribute to the pathogenesis of these tumors. Here, we report that in prostates removed at radical surgery for localized adenocarcinoma, both the cancer and the associated normal peripheral zone tissue have a pronounced biallelic expression of the IGF-II gene. However, this pattern of gene expression is uncommon in periurethral samples of benign prostatic hyperplasia (BPH) from the same specimens. We analyzed the status of genomic imprinting at the IGF-II locus in prostate specimens removed for carcinoma using an ApaI polymorphism in the 3′ untranslated exon of the IGF-II gene. First-strand cDNA synthesis and subsequent PCR amplification were performed on 13 of 35 radical prostatectomy specimens found to be informative for analysis of allele-specific expression. Biallelic expression for IGF-II RNA was demonstrated in 10 (83%) of 12 tumor samples and 8 (73%) of 11 matched peripheral zone prostate samples but in only 2 (18%) of 11 BPH samples. RNA transcripts were readily demonstrated by Northern blot analysis, and differences in expression were not noted among normal, BPH, and tumor prostate tissues. In situ hybridization revealed production of IGF-II by both the epithelium and stroma. The finding of a frequent biallelic expression of IGF-II in peripheral prostate specimens suggests a regional pattern of IGF-II gene regulation exists in prostate tissue. We hypothesize that this tissue-specific pattern of gene expression may participate in the marked predilection of peripheral prostatic tissue for the development of carcinogenesis.

Introduction

Prostate cancer is now the most commonly diagnosed cancer in U.S. males, even exceeding lung cancer, and is also believed to exist in a latent, clinically undetectable form in an additional 10–30% of men over the age of 50 years (1). More than 75% of prostate cancers arise in the peripheral region of the prostate. This region is believed to have embryological origins distinct from the central (periurethral) prostate (2), the site of development of BPH. The fundamental molecular events underlying this prevalence of disease are unknown, as is the basis for the marked regional specificity of prostate cancer for the periphery of the prostate gland.

LOI, or a relaxation of allele-specific expression, has been implicated as a nonmutational genetic event that may have an important role in the pathogenesis of cancers (3). Imprinting has been identified for the IGF-II gene, which demonstrates paternal allele-specific expression in most normal fetal and adult tissues (4, 5). Recently, researchers have demonstrated a relaxation of IGF-II imprinting in sporadic Wilm's tumors (3, 6) and lung carcinomas (7), and this epigenetic molecular event may contribute to the overexpression of IGF-II in these tumors. Additionally, a constitutional relaxation of IGF-II imprinting was also noted in a subgroup of patients with Beckwith-Wiedemann syndrome, a disease associated with somatic tissue overgrowth and predisposition to several embryonal tumors (8). The association of a relaxation of IGF-II imprinting with abnormal growth states might suggest that a LOI may be linked to the development of abnormal growth states in the prostate.

IGF-II, a major embryonic mitogen, has an important role in the adult prostate as a paracrine and autocrine regulator of cell proliferation (9). The production of IGF-II has been demonstrated in the media of cultured prostatic stromal cells (10) and is present in seminal plasma from men (11). Additionally, growth stimulation of the prostatic epithelium (12) and prostate cancer cell lines in vitro has been demonstrated (13). This mitogenic effect has been demonstrated in many other fetal and adult tissues, as well as multiple tumor types, including breast tumors (14). IGF-II has also been implicated in the neoplastic transformation of susceptible cells. Transgenic mice engineered to reexpress IGF-II as adults develop diverse carcinomas after a long latency period (15). Hyperproliferation associated with IGF-II expression has been demonstrated to be a rate-limiting step in the multistage neoplastic transformation of β-cell pancreatic islet tumors in mice (16). Thus, it appears that IGF-II participates as an important prostatic growth factor, and indirect evidence suggests that it may also contribute to the neoplastic transformation of a subpopulation of prostatic cells.

Received 6/13/95; revised 8/14/95; accepted 8/25/95.

1 Supported by the American Foundation for Urological Disease George Sheehan Fund (to D. J.), NIH Training Grant DK07552, National Cancer Institute Grant CA59457 (to G. B.), and Specialized Project of Research Excellence Grant CA 58236 (to W. I.).

2 To whom requests for reprints should be addressed, at Department of Urology, Brady Urological Institute, Marburg 105, 600 North Wolfe Street, Baltimore, MD 21287.

The abbreviations used are: BPH, benign prostatic hyperplasia; LOI, loss of imprinting; IGF-II, insulin-like growth factor II; RT, reverse transcription.
The IGF-II gene is located on chromosome 11p15, a region infrequently demonstrating genomic alterations in prostate cancer, and spans approximately 30 kb of genomic DNA (14). The complexity of IGF-II gene regulation is underscored by the fact that it can direct the synthesis of several different mRNAs that encode the same preproprotein (17, 18). The 5’ part of the gene consists of noncoding leader exons which are each preceded by a distinct promoter (P1–P4). Expression of promoters P2–P4 are usually coexpressed in fetal tissues and at a low level in adult nonliver tissues (18). Activity of P1 has been demonstrated in adult liver, the major source of IGF-II in the adult. The adult human liver also contains a relaxation of imprinting, and it has been proposed that this may be a result of activation of the P1 promoter from both IGF-II alleles (19). The status of promoter utilization for IGF-II in the prostate is unknown.

In this study, we examine the status of imprinting in the IGF-II gene, using a previously described Apal polymorphism (20), in normal peripheral, BPH, and adenocarcinomatous prostate tissues obtained from radical prostatectomy specimens. Furthermore, the expression of the IGF-II gene by Northern blot analysis and ISH is examined. We demonstrate the biallelic expression of IGF-II in prostate carcinoma and associated histologically normal peripheral prostate tissue. This pattern of expression was infrequently demonstrated in periurethral BPH tissues. Biallelic expression in the peripheral prostate may be associated with abnormal proliferation and the development of regional carcinogenesis.

Materials and Methods

Prostate Samples and Establishment of Cultures. Radial prostatectomy samples were obtained at surgery from men diagnosed with cancer, ranging in age from 54 to 68 years. Portions of each tumor, surrounding normal peripheral prostate tissue, and periurethral BPH specimens were immediately frozen at −70°C for DNA and RNA analysis. Frozen sections were stained with hematoxylin and eosin for histological evaluation of each tissue specimen. In the case of tumor tissue, tissue blocks were trimmed to yield samples containing ≥70% tumor nuclei. Normal prostate samples were also obtained and histologically analyzed from autopsy of men <30 years old.

Prostate cultures were established with fresh tissue after pathological analysis. Tissue cores were weighed, and cultures were established and passaged according to the method of Pechel (21). Cultures of stromal cells were established by inoculating single cells, obtained after collagenase digestion of minced tissue fragments, into DMEM containing 10% fetal bovine serum (22). Cultured cells were analyzed within the first passage.

Allele-specific Gene Expression. Genomic DNA was isolated using standard techniques involving proteinase K and phenol-chloroform separation (23). Heterozygosity for each specimen was determined at an Apal polymorphism located in exon 9 of the IGF-II gene as previously described (6, 20). DNA-PCR reactions were performed in 50-µl volumes for 30 cycles at 94°C (30 s), 55°C (2 min), and 72°C (3 min). This reaction was within the log range of amplification. The primers utilized were: sense 5’-CTTGGAGCTTTTGGATCAAATTGGA-3’ and antisense 5’-GGTCGTGCAATCTACAGGTTTCA-3’.

For informative specimens, total cellular RNA was prepared using standard guanidinium/thiocyanate techniques (24). Contaminating DNA was removed using a modification of a published method (25). In brief, total RNA (2 µg) was incubated with 20 units RNase-free DNase I (Boehringer) for 30 min at 37°C in Perkin Elmer/Cetus RT-PCR reaction buffer, which included 1 mm deoxyribonucleotides, 20 units RNasin, and 1 µmol of each primer. The DNase was then inactivated at 95°C for 10 min. Each RNA sample was then divided into two aliquots and incubated with or without 1 unit reverse transcriptase (Perkin Elmer/Cetus). The antisense primer was radioactively labeled with [γ-32P]ATP using T4 polynucleotide kinase (New England Nuclear). PCR of the cDNA was performed utilizing the primers listed above. The cDNA-PCR product of 292 bp was digested with 30 units Apal (Stratagene), and the products (292 bp or 61 bp and 231 bp) were quantitated using phosphorimaging (Molecular Dynamics) after PAGE separation. Experiments were performed in duplicate. To confirm the elimination of DNA from RNA preparations, the products plus and minus reverse transcriptase were analyzed in each experiment. RNA from Apal heterozygous renal tissue, an imprinted tissue (26) and cell line TSU-PRI, a homozygote for the cutting allele, were included in each experiment as controls. Because of heterodimer formation during PCR which is refractory to Apal digestion, a ~3:1 intensity ratio of the two polymorphic bands was theoretically expected from the expression of both alleles at a 1:1 ratio.

ISH of IGF-II. A IGF-II-specific antisense oligonucleotide was designed based on a previously published cDNA sequence (27) and was located within exon 8. The oligonucleotide 5’-TCTCGGACCTTGGCGGGGTACACA-3’ (64% GC content) was commercially hyperbutilated at the 3’ end with 6 biotin units using phosphoramidite chemistry (Research Genetics, Huntsville, AL). The probe was reconstituted to 1 mg/ml with TE buffer. The working hybridization dilution was obtained after titration of the probe with a known positive tissue (i.e., liver), and a concentration of 1:50 of the stock was utilized. A biotinylated poly(dT)30 single-stranded oligonucleotide (Research Genetics) diluted 1:100 was used to verify the integrity of mRNA in each sample. Fresh prostate specimens were sectioned, placed on ProbeOn Plus slides (Fisher Scientific, Pittsburgh, PA), and fixed with 4% paraformaldehyde in HBBS (BRL, Rockville Md) for 15 min. ISH was performed using the Microprobe Manual Staining System and incubator (Fisher Scientific). Hybridization with the biotinylated IGF-II probe was performed according to the ISH protocol (Research Genetics) at a temperature of 52°C for 1 h. The specificity of the hybridization signal was determined by: (a) RNase pretreatment of sections (100 µg RNase A/100 units RNase T/ml at 37°C) and (b) addition of the nonlabeled competing IGF-II oligonucleotide (concentration 1:10) to ablate the signal. Endogenous alkaline phosphatase was ablated using a blocker (Research Genetics). ISH with IGF-II and control probes was performed on sequential prostatic sections of normal, BPH, and tumor.
A. Representative autoradiograph showing monoallelic and biallelic expression of IGF-II in specimens heterozygous for the Apal polymorphism. First-strand cDNA synthesis, PCR, and product digestion with Apal (polymorphic bands 292 and 231 bp) were performed to analyze allele-specific expression. Biallelic expression of IGF-II is noted in normal peripheral prostate samples in Lanes 2 and 5 and tumor samples in Lanes 1 and 4 (A). Periurethral BPH samples in Lanes 3 and 6 demonstrate a nonsignificant LOI when compared to known imprinted tissues such as renal tissue (Lane 10). Lane 9, a homozygote for the cutting allele. TSU-PRI, that was included as a control for complete Apal digestion in each experiment. Normal prostate samples from men under 30 years old demonstrate variable biallelic expression in Lanes 7 and 8. B, cultured epithelial and stromal cells from one prostate specimen were isolated, grown, and harvested for RNA prior to passaging. Allele-specific IGF-II analysis revealed biallelic expression from epithelial cultures in Lanes 1-3 (Lane 1, tumor; Lane 2, normal; and Lane 3, BPH) and stromal cultures in Lanes 4-6 (Lane 4, tumor; Lane 5, normal; and Lane 6, BPH). No amplification was observed in any of the samples lacking reverse transcriptase (−), indicating the absence of DNA contamination in the RNA samples.

B. PCR-cDNA analysis of IGF-II spanning exons 1-3 was then performed using primers based on the Genbank DNA sequence of the IGF-II gene (Accession L15440). Sense primer 5'-AGCCGTCCTAGGGAACA-3' and antisense primer 5'-GGGGCTGGGAAT-CCACCTC-3' were utilized under the PCR conditions previously described for the IGF-II Apal polymorphism. PCR-cDNA analysis of IGF-II exons 5-8, corresponding to the P3 promoter was then performed (sense 5'-GCCCGTGGTGCAGAG-3' and antisense 5'-GGTCCCCACAGAC-GACGAACTG-3') using primers based on the Genbank DNA sequence of the IGF-II gene (Accession no. M17426).

Results

Relaxation of IGF-II Imprinting in Prostate Specimens. We examined the status of genomic imprinting for IGF-II in the prostate by RT-PCR using an Apal polymorphism in exon 9. Heterozygosity for the polymorphism was assessed initially from DNA extracted from 35 radical prostatectomy specimens containing normal peripheral tissue, tumor, and periurethral BPH tissue. Thirteen specimens were found to be heterozygous and were therefore informative for assay of biallelic expression. IGF-II mRNA transcripts were readily detected after RT-PCR in all normal, tumor, and BPH specimens. Monoallelic expression (i.e., imprinting) of the IGF-II gene is seen in virtually all adult tissues with the exception of liver. Indeed, imprinting was observed in virtually all periurethral BPH specimens (Fig. 1), with only 2 of 11 samples demonstrating any detectable biallelic expression (<10% of the total sample). In contrast, biallelic expression was readily demonstrated in 10 of 12 prostate tumor specimens and 8 of 11 matched normal peripheral prostate tissue samples. Two tumor cases (specimens 358 and 478) demonstrated a clear increase in the degree of biallelic expression...
when compared to normal peripheral tissue (Table 1). An analysis of normal prostate tissue from 10 autopsy specimens (men under 30 years old) demonstrated only 2 to be heterozygous, and these revealed a variable loss of imprinting (Fig. 1).

Prostate cancer cell lines were examined, including PC3, Du145, LNCaP, and TSU-PR1; only PC3 was found to be informative for the Apal polymorphism. Extensive biallelic expression was demonstrated for the PC3 cultured cell line.

This assay of allele-specific expression is critically dependent upon the ability to completely digest the RT-PCR products. To control for complete digestion of PCR products by Apal, cell line TSU-PR1, a homozygote for the cutting allele, was included in each experiment and demonstrated >99% digestion in each case. The extent of PCR heterodimer formation (±:3:1) that is refractory to Apal digestion was also considered. Biallelic expression in prostate tissues was determined after comparison upon the ability to completely digest the RT-PCR products. A value of 0 equals complete monoallelic (imprinted) expression. A value of +, 1-25%, ++, 25-50%, +++, 50-75%, ++++, 75-100% (with 100% representing equal expression from both alleles). LOI not expressed in kidney or bladder.

**Table 1 Analysis of IGF-II imprinting at the Apal polymorphism**

<table>
<thead>
<tr>
<th>Prostate</th>
<th>Tumor</th>
<th>Normal</th>
<th>BPH</th>
<th>Tumor stage*</th>
</tr>
</thead>
<tbody>
<tr>
<td>329</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>T1</td>
</tr>
<tr>
<td>330</td>
<td>nd*</td>
<td>nd</td>
<td>0</td>
<td>T1x</td>
</tr>
<tr>
<td>338</td>
<td>++</td>
<td>+</td>
<td>0</td>
<td>T1</td>
</tr>
<tr>
<td>339</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>T1</td>
</tr>
<tr>
<td>340</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>T1</td>
</tr>
<tr>
<td>345</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>T1</td>
</tr>
<tr>
<td>347</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>T1</td>
</tr>
<tr>
<td>358</td>
<td>++</td>
<td>+</td>
<td>0</td>
<td>T1</td>
</tr>
<tr>
<td>393</td>
<td>+</td>
<td>-</td>
<td>0</td>
<td>T1</td>
</tr>
<tr>
<td>427</td>
<td>0</td>
<td>nd</td>
<td>nd</td>
<td>TNM*</td>
</tr>
<tr>
<td>478</td>
<td>++++</td>
<td>0</td>
<td>0</td>
<td>T2</td>
</tr>
<tr>
<td>489</td>
<td>++++</td>
<td>+++</td>
<td>nd</td>
<td>T2</td>
</tr>
<tr>
<td>558</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>T3</td>
</tr>
<tr>
<td>LOI</td>
<td>10/12*</td>
<td>8/11*</td>
<td>2/11</td>
<td></td>
</tr>
</tbody>
</table>

* Expression from the imprinted allele. A value of 0 equals complete monoallelic (i.e., imprinted) expression. A value of +, 1-25%, ++, 25-50%, +++, 50-75%, ++++, 75-100% (with 100% representing equal expression from both alleles). LOI not expressed in kidney or bladder.

**Analysis of IGF-II Regulation in the Prostate.** Transcription of the IGF-II gene can occur at multiple promoters (P1–P4), giving rise to transcripts of different, promoter-specific sizes. To determine if the LOI in prostate tissue is related to promoter usage, an analysis of the IGF-II gene transcripts was performed. In all prostate tissues examined, IGF-II mRNA species were found by Northern blot analysis (Fig. 2) using a 1.0-kb probe (phIGF2) complimentary to exon 7, which is present in all IGF-II transcripts (28). Two promoter-specific bands of 6.0 kb and 5.0 kb were observed in each prostate tissue sample examined. Based on these sizes, these transcripts originate from promoters P3 and P2, respectively (17). In addition, cell line TSU-PR1 and several prostate epithelial cultures demonstrated a faint 4.8-kb mRNA, representing the P4 promoter. No 5.3-kb species, corresponding to P1, was seen in any prostate sample. To confirm this lack of usage of P1, cDNA-PCR amplification spanning exons 1–3 of the IGF-II gene was performed. These exons are represented only in transcripts from P1. A ~320-bp product was demonstrated from cDNA obtained from adult liver; however, 0 of the 10 matched tumor, normal, or BPH prostate tissues analyzed contained P1 transcripts. Therefore, the mechanism for biallelic expression in the prostate does not result from utilization of the P1 promoter, as had been proposed for the adult liver (19). PCR amplification spanning exons 5–8, corresponding to the P3 promoter transcript, demonstrated a 209-bp product in all prostate tissues, indicating utilization of this promoter.

**Localization of IGF-II mRNA Production.** To determine the origin of IGF-II production in prostate tissue, an ISH method for IGF-II was performed (30). To confirm that the mRNA in each section was intact, we used a dT(30) oligonucleotide probe. ISH with the biotinylated antisense IGF-II probe produced distinct cytochemical staining in all tumor specimens as well as in normal and BPH tissues (Fig. 3). The IGF-II probe bound to both the nuclei periphery and cytoplasm of stromal and epithelial tissues. Of interest, intense cell specific staining of the epithelium was noted along the periphery of the prostatic acini. Stromal cells, which are known to express IGF-II in culture (10), appeared to react with the biotinylated probe at a lower level than epithelial cells. Differences in ISH staining between normal, BPH, and tumor were less pronounced; however, all stained positively. Some tumor heterogeneity with respect to staining intensity was noted within samples (Fig. 3b). Pretreatment of matched sections with RNase abrogated the signal, as did a 1:10 dilution of the biotinylated probe with unlabeled IGF-II oligonucleotide. Reagent control hybridization using no probe showed no endogenous reactivity.

Northern hybridization with the IGF-II-specific probe phIGF2 demonstrated readily observed signals corresponding to IGF-II transcripts in epithelial cultures (Fig. 2B). Similarly, stroma established in culture demonstrated IGF-II expression similar to that of previous reports (9). Northern blot analysis of 14 matched specimens of prostate tumor, normal peripheral, and periurethral BPH tissues failed to demonstrate any consistent cell line E3, and these also demonstrated a marked biallelic expression of IGF-II in tumor (92%), normal (93%), and BPH (93%) stromal cultures (Fig. 1B). The imprinted pattern of IGF-II observed in in vivo periurethral BPH tissue was not maintained in culture.
Fig. 2 Northern blot analysis of IGF-II mRNA expression in human prostate cancer cell lines and matched peripheral normal (N), benign prostatic hypertrophy (C), and tumor (T) tissues from surgical specimens. Ten µg of total RNA were applied to each lane and subjected to Northern blot analysis using an IGF-II-radiolabeled probe (pHIGF2) specific for the IGF-II coding region. A. radiograph demonstrates the presence of multiple bands (6.0 kb and 5.0 kb) indicating the utilization of several leader sequences corresponding to promoters 3 and 2, respectively, in the prostate. No significant alterations in IGF-II production were demonstrated in prostate cancer (Lanes 1, 4, and 6) or BPH (Lanes 3 and 8) when compared to matched normal peripheral tissues (Lanes 2, 5, and 7). B. abundant production of IGF-II mRNA in cell line TSU-PRI (Lane 2) and the presence of an additional 4.8-kb leader sequence (promoter 4) in this metastases-derived cell line. Lane 1, liver cell line Hep3A which expresses 6.0-kb and 5.0-kb bands. Northern blot analysis of epithelial cultures (Lanes 3 and 4) harvested in an initial passage readily demonstrate IGF-II RNA transcripts.

**Discussion**

We have analyzed the status of IGF-II imprinting in prostate tissues using an Apal polymorphism, and demonstrate that a loss of imprinting occurs regionally in the peripheral prostate and in associated prostate cancers. For the customarily imprinted IGF-II gene, the paternally inherited allele is expressed, while the maternally inherited allele is silent (5). This imprint is strictly maintained in most adult tissues, with the exception of the adult liver (29), a tissue that abundantly elaborates IGF-II. In contrast, biallelic expression was rarely demonstrated in BPH specimens (18%), a tissue of inner zone origin, that is generally believed not to be a precursor for prostate adenocarcinoma (31). The significant variation in LOI between prostate cancers and BPH tissues imply striking differences in the origin and growth of these two common prostatic diseases. During embryogenesis, the inner zone of the prostate, giving rise to BPH, appears to arise from a mesodermal origin, while the peripheral zone originates from endoderm (2). This marked contrast in imprinting for the IGF-II gene provides molecular data to support a separate embryological origin for these two regions.

The biological consequences of a biallelic expression of IGF-II are unknown in the prostate. However, there are a number of indications that a relaxation of genomic imprinting may be important in the development of carcinogenesis in selected tissues. A LOI, when compared to normal associated tissues, has been demonstrated to be an early epigenetic molecular event in the development of Wilms’ tumors (3, 6, 26) and lung carcinomas (7), both abnormal growth states in which IGF-II is expressed in high levels. We did not note this switch to biallelic activation in our study of the prostate because, remarkably, biallelic expression of IGF-II was already present in the histologically normal peripheral prostate. The demonstration of biallelic expression in normal prostate tissue from younger men suggests that this is not a de novo change, but may be a tissue-specific pattern of gene expression. Early neoplastic alterations in β-cell pancreatic islet tumors correlate with focal activation of IGF-II (16). Both alleles are activated during this switch to hyperproliferation in the oncogene-expressing β-islet cells. Similarly, the demonstration of biallelic expression in
histologically normal peripheral prostate tissues may herald altered molecular control mechanisms that reflect a susceptibility for neoplasia among subpopulations of prostate cells. Indeed, prostate cancer has been demonstrated to arise as numerous separate tumor foci in specimens removed at prostatectomy, suggesting a regional susceptibility for tumor development in the peripheral prostate (32).

In the prostate, it is apparent that degrees of biallelic expression exist for IGF-II and that variable expression of the otherwise silent allele may occur. Incomplete or "leaky" imprinting has been noted from the normally imprinted gene H19 and may result from a partial transcriptional repression of the imprinted maternal allele (33), possibly due to incomplete methylation (34). Alternatively, a subset of cells, either epithelium or...
stroma, may express the IGF-II gene biallelically, while other populations within the tissue maintain the monoallelic imprint. We examined epithelial and stromal cultures from each region of the prostate and determined that the imprinted pattern of IGF-II observed in BPH was not maintained in culture. Our analysis of cultures demonstrated biallelic expression from all regions of prostate epithelial and stromal cells. Imprinting patterns may not be stable in vitro since patterns of methylation have been commonly observed to alter in cell culture (35).

The mechanism responsible for the relaxation of IGF-II imprinting is unknown. The regulation of the IGF-II gene is complex and arranged in a tissue-specific and developmental stage-specific fashion (36–38). Both development-specific promoters and alternative splicing generate mRNAs containing different 5′ untranslated sequences; however, the final IGF-II protein is invariable. Our study demonstrates that the prostate utilizes multiple IGF-II promoters, with primarily the 6.0-kb and 5.0-kb transcripts being produced, corresponding to the P3 and P2 promoters. No significant differences were noted between leader sequences in prostate tumors and other prostate tissues examined. Although others have suggested that the recruitment of P1 is required for a LOI in the IGF-II gene in liver and other tissues (19, 29, 38), utilization of the P1 promoter by RT-PCR analysis was not demonstrated in the presence of LOI in the prostate. Biallelic expression in the prostate may involve other factors, such as the binding and activation of response elements (39) or alterations in CpG methylation of one or more promoter regions (34, 40).

IGF-II, a major embryonic mitogen, has been considered a candidate gene responsible for the development and promotion of neoplasia in several tumor systems (15, 16, 41). In the prostate, IGF-II has important mitogenic properties in both normal epithelium (12) and in prostate cancer, including cell line Du145 (13) and the PA-III rat prostate adenocarcinoma line (42). By Northern blot analysis, we find RNA transcripts are abundantly expressed in the prostate. Although overexpression has been demonstrated in many tumors (43, 44), our study did not demonstrate a significant alteration in the production of IGF-II transcripts by Northern blot analysis when histologically normal peripheral tissue was compared to prostate cancer or BPH. However, the expression of IGF-II in all tissues removed for cancer may reflect a global overexpression throughout the prostate. Although a direct causal role of IGF-II in malignant transformation has yet to be established, there are a number of indications that its presence may be important. Induction of the expression of IGF-II by transfection in MCF-7 cells leads to an altered morphology and anchorage-independent growth (45), and a reexpression of IGF-II in adult transgenic mice induced the development of varied carcinomas after a long latency period (15). The abundant presence of IGF-II in the adult human prostate may cause subtle alterations over time in the rate of cell proliferation, possibly through an alteration in the apoptotic index similar to B-cell pancreatic islet tumors (16). These studies and others (41, 46) suggest that chronic IGF-II exposure has the capacity to act in some tumor systems as an early initiation factor in the development of neoplasia.

The cellular origin of IGF-II production and expression in the prostate is indeterminate. The prostate is a composite organ composed of both epithelial and stromal elements. In culture, Cohen et al. (10) detected IGF-II in the media from cells of stromal origin; utilizing a RIA however, no IGF-II was noted from prostate epithelial cell cultures. Utilizing ISH, we demonstrate that IGF-II mRNA is produced in both the epithelia and stroma in these specimens removed for prostate cancer. Additionally, prostate epithelial cells, in an initial passage, are demonstrated to readily elaborate IGF-II mRNA by Northern blot analysis. These discrepancies in the cellular origin of IGF-II may reflect differences in culture conditions, lack of correlation between mRNA and protein levels, frequency of cell passage, or the difficulties of assaying IGF-II protein in conditioned media. The IGF-II axis also includes many binding proteins and multiple receptors that may alter the local effect of the polypeptide in the prostate (9). All of these factors contribute to our understanding of the complex regulation of this critical gene product in prostate pathology.

The demonstration of biallelic IGF-II expression in both cancerous and associated histologically normal prostate suggests a tissue-specific, regional pattern of gene expression that may, we hypothesize, predispose peripheral prostate tissue over a long latency period to the development of carcinogenesis. The absence of a LOI in BPH suggests a fundamental molecular difference in the origin and development of this entity. Additional work to determine the ultimate implications of this alteration in the prostate is in progress.

References

Regional loss of imprinting of the insulin-like growth factor II gene occurs in human prostate tissues.


Updated version  Access the most recent version of this article at:  
http://clincancerres.aacrjournals.org/content/1/12/1471

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.