IGF-IEc Expression Is Associated with Advanced Clinical and Pathological Stage of Prostate Cancer

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Abstract. *Background: Recent evidence suggests a role for the insulin-like growth factor-1Ec (IGF-IEc) transcript variant in cancer biology. The aim of the present study was to investigate whether IGF-IEc expression is associated with prostate cancer stage. Materials and Methods: Formalin-fixed and paraffin-embedded prostate cancer surgical specimens from 83 patients were assessed by immunohistochemistry for IGF-IEc expression. Results: Normal prostate epithelium was negative or demonstrated mild IGF-IEc cytoplasmic expression whereas prostate cancer exhibited mild to strong cytoplasmic immunoexpression. The mean IGF-1Ec expression, was significantly lower (p=0.004) in localized (stage ≤IIb) prostate cancer, compared to locally advanced tumors (stage ≥III). Only one out of 83 (1.2%) prostate cancer samples was completely negative for IGF-IEc. A weak-positive correlation was also observed between IGF-IEc expression levels and Gleason score (r=0.247; p=0.024). Conclusion: The present data demonstrate that the expression of IGF-IEc is positively-associated with more advanced stage and higher Gleason score of prostate carcinomas.*

Insulin-like growth factor-I (IGF-I) is encoded by the IGF-I gene (of six exons) that can generate multiple heterogeneous transcripts *via* an alternative splicing mechanism (1). The IGF-I transcripts differ at their C-terminal extensions known as the E domain (exons 5 and 6) which may contain either exon 5 (IGF-IEb transcript), or exon 6 (IGF-IEa transcript). A third IGF-I transcript, the IGF-IEc can also be generated by the insertion, prior to exon 6, of 49 base pairs which are part of the exon 5 (1). Mature IGF-I, a 70-amino-acid peptide, is encoded by exons 3 and 4 and represents the common bioactive product of all IGF-I transcripts (2). Mature IGF-I possesses potent growth and survival factor activity for practically all cell types, including human prostate cancer cells (PCa cells) (2, 3, 4, 5, 6). However, mature IGF-I is not the only bioactive product of *IGF-I* gene since bioactivity has also been associated with the E domain, particularly of the IGF-IEc transcript (1, 7). There is a growing interest in the potential role of IGF-IEc expression in muscular dystrophy, muscle hypertrophy, muscle remodelling after mechanical overloading and exercise-induced skeletal muscle damage, as well as in the process of myocardial remodelling, following experimental coronary artery ligation-induced myocardial infarction in rats (1, 8, 9).

To enable further characterization of the molecular physiology and differential expression of the IGF-IEc transcript at the protein level, a specific rabbit antibody to IGF-IEc, raised against a synthetic peptide (24 amino acids) corresponding to the insertion part (49 basis) of exon 5 in the E domain of IGF-IEc, was produced by our laboratory (10). This polyclonal antibody was recently utilized to demonstrate the overexpression of IGF-IEc mRNA in prostate cancer cell lines (PC-3 and LNCaP) and in paraffin sections of human prostate cancer as compared to normal prostate epithelial cell lines (HPrEC) and tissues (5). Indeed, IGF-IEc expression was remarkably higher in PCa and prostatic intra-epithelial neoplasia than the adjacent normal and benign prostate hyperplasia tissues, while the HPrEC cell line did not express IGF-IEc (5). Compelled by the above evidence, we undertook the present study to investigate whether such expression, as assessed by immunohistochemistry (IHC), is associated with the pathological and surgical stage of patients with newly-diagnosed prostate cancer.

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Key Words: Prostate cancer, immunohistochemistry, insulin-like growth factor 1 (IGF-1), IGF1Ec, mechano-growth factor (MGF).

Materials and Methods

Patients. Eighty-three paraffin tissue blocks from patients who underwent radical prostatectomy for histologically proven prostate adenocarcinoma between February 2002 and February 2008 were retrospectively selected from the archives of the Evangelismos General Hospital of Athens, Pathology Department. The samples were used in accordance with approval of the local Ethics Committee (National and Kapodistrian University, Medical School, approval number 2827/02-12-2011). The patients ranged in age from 51 to 78 years (mean age=65.4 years). None of the patients had received any hormonal or radiation therapy prior to surgery. A copy of the official pathology report from a certified pathologist was kept on record for all cases and all specimens were further reevaluated and confirmed by our expert pathologists.

IHC methods. The Bondmax automated system (Leica Microsystems, New Castle, Newcastle Upon Tyne, UK) was used for the IHC staining on paraffin sections, using the IGF-IEc polyclonal antibody at 1:500 dilution (10). Skeletal muscle biopsies were used as positive controls (8, 9). Furthermore, negative controls were performed by omitting the primary antibody. Each slide was then evaluated independently by two blinded trained pathologists (A. S. and C. P.) using intermediate power light microscopy (11). One representative tumour section was stained and evaluated per case. IHC expression of IGF-IEc was mainly cytoplasmic and was categorized as either grade 1 (weak intensity), grade 2 (moderate intensity) or grade 3 (strong intensity). The H scoring system, a model that incorporates both intensity and distribution of staining (11, 12), was used for semiquantitative analysis of IGF-IEc immunoreactivity. More specifically, the percentage of positive tumour cells was measured in every section and multiplied by 1, 2 and 3, respectively (grade 1 score=percentage with grade 1 expression ×1; grade 2 score=percentage with grade 2 expression ×2; grade 3 score=percentage grade 3 expression ×3). A total score between 0 and 300 was obtained for each case (total score=grade 1 score + grade 2 score + grade 3 score).

Statistical analysis. All statistical analyses were performed using R (Foundation for Statistical Computing, Vienna, Austria) (13). The Shapiro-Wilk test was applied for analysis of variance for all continuous variables and the choice of methods for statistical testing of continuous variables was based on whether the data permitted parametric or non-parametric analysis. Comparisons of IGF-IEc expression between two groups were performed using Student's *t*-test. One-way analysis of variance (ANOVA) was used to assess for differences between more than two groups. Relationships between different continuous and categorical ordinal variables were assessed by the Spearman correlation coefficient. A p-value <0.05 was considered statistically significant. p-Values of *post-hoc* paired comparisons were adjusted with the Bonferroni method.

Results

In accordance with the American Joint Committee on Cancer (AJCC), four out of the 83 tumours (4.8%) were classified as stage IIa, 25 (30.1%) as stage IIb, 47 (56.6%) as stage III and 7 (8.4%) as stage IV. The vast majority of patients (83.1%) had a Gleason score of 7 (4+3) whereas only 7.2% and 9.6% had Gleason scores of 8 and 9 respectively. High-grade prostatic intra-epithelial neoplasia (PIN) was observed in 76 cases (91.6%), positive surgical margins in 46 cases (55.4%), vascular invasion in 59 cases (71.1%) and perineural invasion in 79 cases (95.2%) respectively.

Table I. *Relationship between insulin-like growth factor-IEc (IGF-IEc) status and other clinicopathological variables of the study.*

Variable		Association with IGF-Ec expression
Age, years[a]	65.4±5.9 (51-78)	r=0.027; p=0.81[c]
Gleason Score[b]		
7 (4+3)	69 (83.1%)	r=0.247; p=0.024[c]
8	6 (7.2%)	
9	8 (9.6%)	
AJCC stage[b]		p=0.004[d]
≤IIb	29 (34.9%)	
≥III	54 (65.1%)	
PIN[b]		p=0.153[d]
Absent	7 (8.4%)	
Present	76 (91.6%)	
Surgical margins[b]		p=0.95[d]
Negative	37 (44.6%)	
Positive	46 (55.4%)	
Vascular invasion[b]		p=0.347[d]
Absent	24 (28.9%)	
Present	59 (71.1%)	
Perineural invasion[b]		p=0.185[d]
Absent	4 (4.8%)	
Present	79 (95.2%)	
Tumor volume (%)[a]	27.4±20.8 (2-90)	r=0.103; p=0.18[c]

[a]Continuous variable; data are given as mean±standard deviation (range). [b]Categorical variable; data in parentheses represent the percentage of each group. [c]Calculated by Spearman correlation coefficient. [d]Calculated by Student's *t*-test. AJCC: American Joint Committee on Cancer. PIN: prostatic intraepithelial neoplasia.

The tumour volume ranged from 2% to 90% of the resected gland (mean value 27.4%). Thirty-four patients (41%) had tumours occupying less than 20% of the resected gland whereas 49 patients (59%) had tumors occupying ≥20% of the gland. The patients' clinicopathological characteristics in relationship to IGF-IEc expression are listed in Table I.

IGF-IEc expression (mean±standard deviation) in stage IIa, IIb, III and IV tumours was 114.8±30.9%, 99.2±69.4%, 139.5±62.1% and 180±60.6%, respectively. The mean IGF-1Ec expression was significantly lower (p=0.004) in localized (stage ≤IIb) prostate carcinomas compared to locally advanced tumours (stage ≥III). Only one out of 83 (1.2%) prostate cancer samples was completely negative for IGF-IEc. A weak positive correlation was observed between IGF-IEc expression and Gleason score (r=0.247; p=0.024). However, there was no association between IGF-IEc

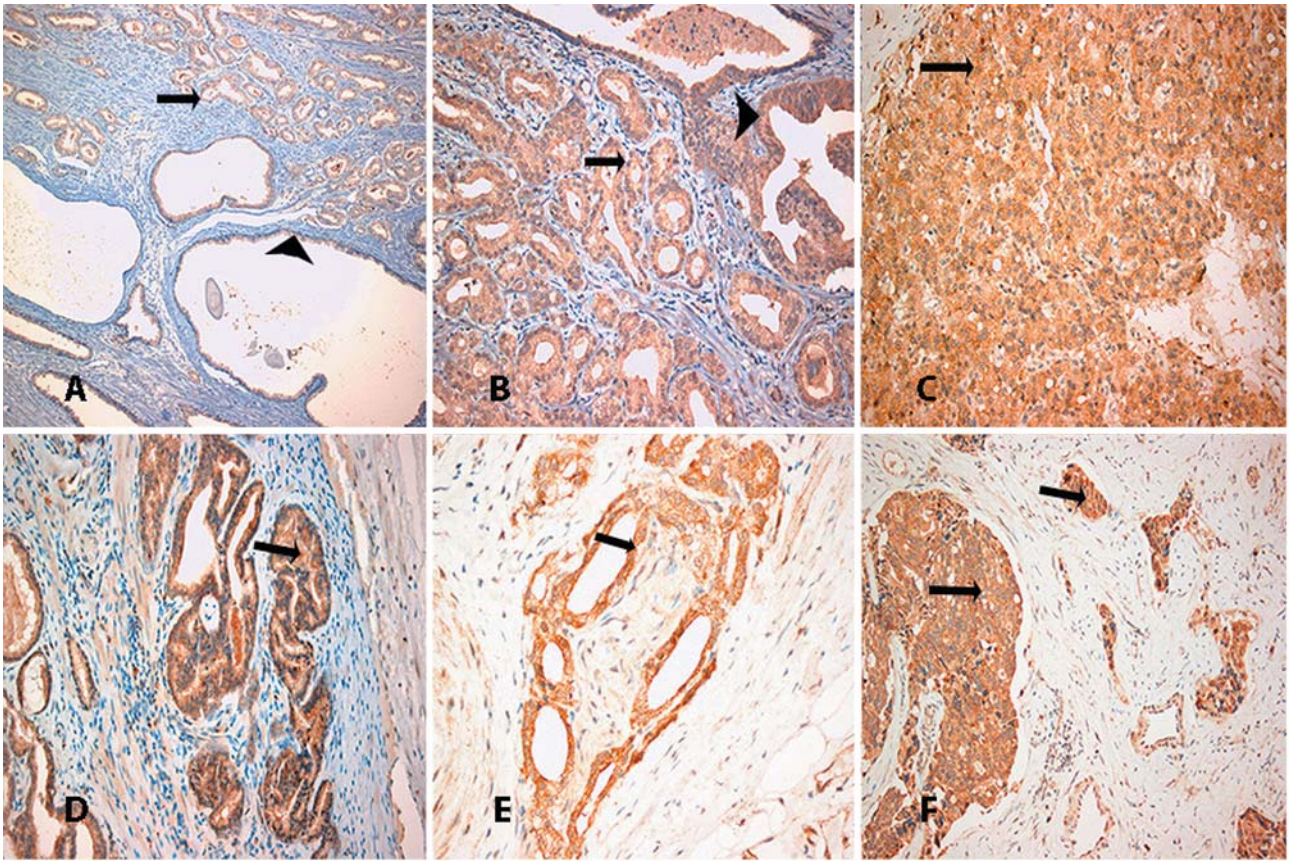

Figure 1. *Examples of the insulin-like growth factor-I (IGF-IEc) expression in prostate cancer tissues. A: We detected extensive to moderate cytoplasmic immunohistochemical expression [IE] of IGF-IEc in prostate cancer (arrow). However, no IE or very mild IE was detected in normal prostate epithelium (arrowhead). B: Further evidence for a strong cytoplasmic IGF-IEc IE in prostate cancer (arrow) and prostate intraepithelial neoplasia (arrowhead). C-F: Evidence for strong cytoplasmic IGF-IEc IE in high-grade (arrows) and advanced-stage (arrows) prostate cancer. All magnifications ×200, except A ×100 and E ×400.*

expression and patient age (p=0.81), presence of PIN (p=0.153), positive surgical margins (p=0.95), vascular invasion (p=0.347) or perineural invasion (p=0.185). In addition, tumour extent inside the prostate gland was not associated with IGF-IEc expression (r=0.103; p=0.18).

Representative samples stained with anti-IGF-1Ec antibody are shown in Figure 1. Normal columnar prostate epithelium was negative or exhibited mild IGF-IEc cytoplasmic expression. Basal cells were negative. High-grade PIN and prostate cancer exhibited a variably mild to strong cytoplasmic immunoexpression.

Discussion

The present study investigated the IHC expression of IGF-1Ec in prostate cancer and established the positive correlation between expression levels of this molecule and prognostic clinicopathological variables. Previous work by our group indicated a potential role of IGF-1Ec in cancer biology (5). As far as we know, the present study is the first to study the expression patterns of IGF-1Ec in prostate cancer samples and to find an association between higher IGF-1Ec expression levels and advanced prostate cancer stage and Gleason score.

IGF-I activity is mediated by high affinity binding to IGF-IR, a tyrosine kinase receptor, while IGF-IIR, a non tyrosine kinase receptor, mediates mainly internalization and metabolic processes (1, 2). Notably, IGF-I can act also *via* its low-affinity binding to insulin receptor (INSR; A and B isoforms), another tyrosine kinase receptor, as well as *via* hybrid IGF-IR/INSR, which is composed of an INSR hemi-receptor linked to an IGF-1R hemi-receptor (14). The IGFs are found in various biological fluids bound to specific IGF-binding proteins from which they are dissociated by the action of specific serine proteases (15, 16). Accumulating evidence has demonstrated the significant role of the IGF axis in human cancer development and progression (16, 17, 18). The pathophysiology of the IGF system in cancer appears to be a complex and multi-

factorial process whereby different components of the IGF axis may play more prominent roles in some types of cancer compared to others.

Previous studies have documented the potential role of an E domain-related product of the IGF-IEc transcript in prostate cancer (5). The mechanism by which IGF-IEc regulates prostate tumour growth remains unclear. The IGF-IEc E domain-related peptide has been shown to stimulate the proliferation of human PC-3 (androgen receptor-negative) and LNCaP (androgen receptor-positive) human prostate cancer cells, as well as HPrEC cells *via* an IGF-IR-independent, INSR-independent and hybrid IGF-IR/INSR-independent mechanism (5). The latter was evidenced by the fact that synthetic IGF-1Ec peptide, a synthetic peptide of the C-terminal product of the IGF-IEc transcript, was bioactive on PC-3 and LNCaP cells, as well as on *IGF-IR* knock-out and *INSR* knock-out PC-3 and LNCaP transfectants (5). In addition, synthetic IGF-1Ec peptide phosphorylated ERK1/2 in both the unaffected and in the IGF-IR knockout and INSR knockout PC-3 and LNCaP cells. Interestingly, ERK activation was not associated with Akt phosphorylation (5). These data suggested that the IGF-IEc E domain can generate a novel bioactive product which may play a significant role in cancer biology. This E peptide action may not be mediated by the known receptors involved in the IGF-I system (IGF-IR and INSR) and may thus be acting *via* an as yet unidentified receptor.

Novel anticancer treatments targeting the IGF system are currently under intense pre-clinical and clinical investigation (14, 16, 18). Thus, a better understanding of the complex biological role of the IGF transcripts and biological components in specific types of neoplasia can have a significant impact on the design, testing and clinical applications of newly-developed drugs. Herein, we have shown that IGF-IEc expression is associated with more aggressive prostate cancer phenotypes, as evidenced by higher Gleason scores and advanced cancer stages. Of note, however, we did not find any significant association between IGF-IEc expression and vascular or perineural invasion, the percentage of tumour in the surgical specimens and positive surgical margins. This may indicate that while IGF-IEc-expressing tumours are frequently of higher Gleason score and advanced clinical stage, these associations may be independent of the relationship of IGF-IEc with mechanisms involving the invasion of adjacent structures.

It should be noted that the studied population may have been biased toward more advanced prostate cancer cases referred from rural areas to our Institution as evidenced by the fact that the Gleason scores of all our samples were 7 or higher. Thus, further multicenter studies are required to provide a more generalizable account of IGF-IEc expression in prostate cancer. In addition, future studies should investigate the potential independent association between IGF-1Ec and survival in prostate cancer.

In conclusion, the present study demonstrated that the expression of IGF-IEc was positively-associated with more advanced stage and higher Gleason score of prostate cancer tumours, as analyzed in a cohort of 83 patients, with newly-diagnosed prostate cancer who underwent treatment with curative intent by radical prostatectomy. These results further support the role of IGF-IEc expression in prostate cancer biology. Furthermore, our data set the foundation for additional investigation of IGF-IEc expression as both a potential clinical biomarker and novel therapeutic target.

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Received March 13, 2013
Revised April 28, 2013
Accepted April 30, 2013