



## Mini Review

# Ec Peptide of the IGF-1Ec Isoform in Relation to Prostate Cancer: Brief Review and Latest Data

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## Abstract

Prostate cancer is a complex disease that affects millions of men worldwide, mainly in areas with high human growth rates; is a major cause of disease and mortality among men. The most common treatment is surgical or medical castration. The last few years, the role of IGF-1 is well established in cancer biology. In many studies Ec peptide is associated with prostate cancer and metastasis and it seems that it is an important progression factor for human prostate cancer cells. It is defined the potential role in tumor growth, progression and metastasis. This scientific article is about the Ec peptide of the IGF1Ec isoform and its association with prostate cancer, the proliferation of PC-3 by PEc through an autocrine/paracrine mode of action, the association of the Ec peptide with epithelial mesenchymal transition and finally the Ec peptide with prostate cancer metastasis.

**Keywords:** Prostate cancer; Ec peptide; Metastasis; Mesenchymal cells

## Introduction

Prostate cancer is a complex disease that affects millions of men worldwide, mainly in areas with a high human development index. Patients with localized disease at low to intermediate risk of recurrence generally have a favorable outcome with an overall survival of 99% over 10 years if the disease is detected and treated at an early stage. Key genetic alterations include fusions of TMPRSS2 with ETS family genes, amplification of the MYC oncogene, deletion and/or mutation of PTEN and TP53 and, in advanced disease, amplification and/or mutation of the androgen receptor (AR) [1].

Insulin-like growth factor 1 (IGF-1) is a key mediator in human physiology and pathophysiology, including cancer. The IGF-1 receptor type I (IGF-1R) mediates the effects of IGF-1 by activating two important intracellular signaling cascades: the phosphatidylinositol-3-kinase/AKT-kinase (PI3K/AKT) pathway

and the Raf/AKT kinase pathway. Mitogen-activated protein kinase (Raf/MAPK) [2,3]. IGF-1R-dependent signaling regulates a wide range of cellular responses, including cell proliferation. IGF-1 can act as both a capacity growth factor, stimulating the “G0 to G1 transition” of quiescent/dormant cells, and a progression growth factor, stimulating the “G1 to G2 transition” of somatic cells in the cell cycle [4,5].

By alternative splicing of exons 5 and 6, human igf1 produces three transcripts, namely IGF-1Ea, IGF-1Eb and IGF-1Ec [2]. Since they contain exons 3 and 4, all can produce mature IGF-1. However, translation of these transcripts produces different peptides of the E structure, namely Ea, Eb and Ec. It is, therefore, possible that the preferential expression of IGF-1Ec detected in various experimental settings after tissue injury supports the need of the injured tissue for an adjuvant of IGF-1, the Ec-related bioactivity [6,7]. Indeed, several studies have confirmed that synthetic human Ec peptide (hEc) and murine E peptide (mE) [product of the E domain of the mouse IGF-1Eb transcript of igf1] possess mitogenic, angiogenic, and migratory growth factor activity *in vitro* [8,9].

The role of IGF-1 as a potent growth and survival factor in human cancer has long been established. The IGF-1 gene produces multiple heterogeneous transcripts, which result in the mature form of IGF-1 [10,11]. Recent studies have indicated the preferential expression of the IGF-1Ec isoform in prostate cancer [6], whereas exogenous administration of a 24 amino acid synthetic peptide of the COO-terminal part of the Ec isoform (parts of exons 5 and 6 of IGF-1 gene), has been associated with a statistically significant increase in proliferation in PC-3 and LNCaP prostate cancer cells [6]. It has been suggested that the effects of this synthetic PEc are mediated by extracellular signal-regulated kinase (ERK) but not Akt, promoting prostate cancer cell growth *in vitro* [6].

Unlike IGF-1, the effects of PEc are not mediated through the IGF-1 receptor (IGF-1R), the insulin receptor (IR), or any of the hybrid receptors (IGF-1R/IR) [6,12]. Mechanistically, synthetic PEc has been suggested to be involved in mouse mesenchymal cell migration and invasion [11,13-15]. Despite this evidence, some studies show conflicting results regarding the action of synthetic PEc on muscle cells [12,15]. Therefore, the role of Ec in cancer biology remains to be elucidated.

### **The Ec Peptide of the IGF1Ec Isoform in Relation to Prostate Cancer**

IGF-1Ec expression levels were examined in randomly selected prostate cancer sections. In a work carried out in our country, seventy-eight patients with prostate cancer were examined and the mean immunohistochemical expression of IGF-1Ec was found to be significantly lower in patients with prostate cancer stage  $\leq$  IIb (AJCC) compared to the tissues of stage III and IV patients ( $p < 0.004$ ). Mean IGF1Ec expression was significantly lower in stage IIa-IIb prostate tumors compared to stage III (mean IGF1Ec expression was 101.3 vs. 144.7,  $p = 0.005$ ). These results are in agreement with those previously described [6] and suggest that IGF-1Ec expression is related to prostate cancer stage. Overexpression of Ec peptide leads to increased proliferation in PC-3 cells.

Thus, the wild-type prostate cancer cell line PC-3 (wtPC-3) was selected, which expresses only a small amount of IGF-1Ec compared to LNCaP prostate cancer cells and to human prostate epithelial cells (HPrEc under tissue culture [6]). PEc-overexpressing PC-3 cells (PC-3PEc) were generated and compared with control wild-type PC-3 (mPC-3) cells. mPC-3 cells were compared with PC-3 wild type in terms of cell proliferation and ERK1/2 phosphorylation and showed no statistically significant differences.

### **PEc Induces PC-3 Multiplication through an Autocrine/Parecrine Mode of Action**

To determine whether PEc in PC-3PEc cells becomes extracellular, PEc levels were examined in the media of PC-3PEc

cells and mPC-3 cells by multiple reaction (MRM) analysis. PC-3PEc cells were found to secrete a significant amount of PEc in contrast to mPC-3 cells [6].

### **Relationship of the Ec Peptide with the Phenomenon of Epithelial-Mesenchymal Transition**

PC-3PEc cells grown *in vitro* exhibited a fibroblast-like morphology under light microscopy compared to mPC-3 epithelial cells. Recent evidence shows that the IGF-1 pathway induces the EMT effect through the IGF-1 receptor (IGF-1R) by regulating the zinc ring enhancer-binding protein 1 (ZEB1) in prostate carcinoma cells, which is stimulated by ERK1/2 phosphorylation [16-18]. ZEB1 represses E-cadherin and is involved in further chromatin condensation and gene silencing [19-21]. Loss of E-cadherin is associated with increased tumor migration and invasion both *in vitro* and *in vivo*. The decrease in E-cadherin occurs gradually and the increase in vimentin starts at 48 hours, synchronously, with a peak effect within 72 hours [22]. Treatment of PC-3PEc cells with the anti-PEc antibody (administered in medium) results in reversal of the mesenchymal phenotype (increased E-cadherin expression and decreased vimentin expression) at 24 h. This finding reinforces the idea of an autocrine/paracrine mode of action of PEc. In addition PC-3PEc cells showed a more than fivefold increase in ZEB1 expression level compared to mPC-3 cells. On the other hand, it has also been suggested that, in cancer, EMT can also be promoted by the direct effect of cdc6 (cell division cycle 6), a protein involved in the replication licensing mechanism, at the E-cadherin locus, as a molecular switch [22].

In PC-3 IGF-1R KD cells it has been found that, although exogenous administration of IGF-1 did not affect ZEB1 expression, exogenous administration of PEc increased ZEB1 expression. These data suggest that PEc induces EMT in PC-3 cells through a different receptor molecule than IGF-1R. Furthermore, IGF-1R silencing in PC-3PEc cells did not affect their mesenchymal phenotype (morphology and E-cadherin and vimentin patterns). The effect of PEc on the migration ability of mPC-3 prostate cancer cells was also examined by migration assays, where PC-3PEc cells and mPC-3 cells were examined under the influence of exogenous synthetic PEc. It was observed that PC-3PEc cells showed an increased migration rate compared to mPC-3 cells similar to that of mPC-3 cells under the influence of exogenous synthetic PEc. It was also observed that in both cases (PC3PEc cells and mPC-3 cells after PEc administration) anti-IGF-1Ec antibody led to a decrease in their migration rate [6].

### **Association of Ec Peptide with Prostate Cancer Metastasis**

Subcutaneous injection of mPC-3 cells into SCID mice is mainly associated with lymph node metastases [23]. Approximately 80% of men are diagnosed with organ-confined disease, 15%

with local metastases, and 5% with distant metastases [24]. Men diagnosed with advanced stage disease (distant metastases) have a poor overall survival of only 30% at 5 years [24]. Early detection of local disease may also be instrumental in efforts to increase the life expectancy of prostate cancer patients, while also preventing the occurrence of metastatic disease [25]. Furthermore, tailoring treatment to men who are likely to benefit from immediate definitive treatment and those who are not remains a key clinical challenge [26].

## Discussion

Prostate cancer is the most common cancer in older men. Cells within the prostate often form tumors, most often in mid- and late life. The adult human prostate can be divided into a central, transitional, and peripheral zone, and also contains fibromuscular and periurethral regions. In young adult men, the distal zone constitutes >70% of prostatic glandular tissue and contributes the most to normal prostate function. It is also the most common site of origin for neoplasms in the aged prostate, as nearly 80% of prostate tumors arise from this region [27,28].

There are no curative treatments for metastatic prostate cancer. The most common treatment for recurrent disease is surgical or medical castration. However, more commonly, patients progress and develop castration-resistant, metastatic growth [29-31]. Our results suggest that PEc is a key molecule in tumor growth and survival and is also involved in the Epithelial-Mesenchymal Transition (EMT) phenomenon of prostate cancer cells leading to metastasis. In a mouse study, endogenous PEc overproduction in PC-3 cells was associated with increased ERK1/2-driven proliferation (similar to exogenous PEc administration) and larger tumor formation in SCID mice compared to those generated by mPC-3 cells. Furthermore, the fact that PEc becomes extracellular and its biological effects are inhibited by anti-IGF-1Ec antibody suggests an autocrine/paracrine mode of action. The importance of PEc in tumor growth and progression is also indicated by inoculation of PC-3 IGF-1Ec KD cells, where only two of 10 (20%) mice developed palpable tumors ten weeks after subcutaneous injection. Our data also show that PEc expression is associated with the EMT phenomenon. Administration of exogenous PEc to mPC-3 cells, as well as overexpression and secretion of PEc from PC-3PEc cells, induces vimentin expression and suppresses E-cadherin expression. These *in vitro* results were consistent in mPC-3 and PC-3PEc-induced tumors in SCID mice. Furthermore, although IGF-1 induces EMT in a manner involving ERK1/2 activation and ZEB1 expression via IGF-1R, PEc appears to induce the same effect, but using an IGF-1R-independent mechanism. Further *in vivo* evidence for the involvement of PEc in the metastatic process of prostate cancer came from the orthotopic injection of immortalized HPrEcPEc cells into SCID mice, where four of seven

mice developed metastases to nearby tissues [32]. These results are in agreement with those obtained from immunohistochemical analysis of tumors from prostate cancer patients, where the IGF-1Ec isoform was found to be positively associated with prostate cancer stage and grade. These observations provide new insights into prostate cancer biology, whereby peptide E (PEc) resulting from proteolytic cleavage of the IGF-1Ec isoform induces prostate cancer progression, metastasis, and repair. Accordingly, Ec peptide may be an attractive candidate target for the treatment of prostate cancer without affecting the actions of mature IGF-1.

## Conflict of Interest

All authors declare no conflict of interest.

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