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Expression of IGF-IEc Isoform in Renal Cell Carcinoma Tissues

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Abstract. *Background/Aim: Insulin-like growth factor-I (IGF-I) regulates various aspects of cancer biology. There is a growing body of evidence regarding the potential distinct role of IGF-I isoforms, particularly of IGF-IEc, in the pathophysiology of various human cancer types, however, there are no studies which examined the expression of the different IGF-I isoforms in renal cell carcinoma (RCC). This study aimed to characterize the expression of IGF-IEc in human RCC tissues and investigated whether its expression is associated with the histopathological type of RCC as well as with the overall survival of patients. Materials and Methods: Formalin-fixed paraffin-embedded renal tissue samples from 94 patients (58 males and 36 females) were assessed for IGF-IEc expression by immunohistochemistry. Results: RCC tissues showed mainly cytoplasmic IGF-IEc staining but immunoreactivity of IGF-IEc was also localized in the cell membrane. Significantly lower IGF-IEc expression was found in clear cell RCC vs. all other histological types ($p=0.010$), and this remained significant after adjusting for tumor size, grade, stage, and mitotic index ($p<0.05$). No association was found between IGF-IEc expression level and overall survival of patients with RCC. Conclusion: The differential expression of IGF-IEc isoform among the RCC histopathological types may indicate its histological type-specific regulation and possibly suggests a discrete biological role of this isoform in the pathophysiology of RCC.*

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Renal cell carcinoma (RCC) is the most common kidney malignancy, affecting men more than women, and metastatic disease at presentation occurs in up to one-third of patients. The pathological stage (size of the tumor and the extent of invasion), grade, and histological cell type are widely used in clinical practice for the prognosis of RCC (1). In particular, clear cell is the most common histological type of RCC accounting for 60-70% of cases (2). It represents a genetically and histologically diverse group of cancers that includes chromophobe, papillary, unclassified RCC, and other rare subtypes such as renal medullary carcinoma (3-6).

Insulin-like growth factor-I (IGF-I) plays an important role in various aspects of cancer biology, such as cell growth, apoptosis resistance, cell differentiation and migration (7-10) and, thus, it has been implicated in the pathophysiology and prognosis of several human cancer types (11-14), including RCC (2, 15, 16). IGF-I is not only secreted by the liver, but is also produced by other organs such as skeletal muscle, kidney and brain (17-27). The *IGF1* gene consists of six exons and can produce multiple heterogeneous transcripts *via* alternative splicing during its transcription, namely IGF-IEa, IGF-IEb, and IGF-IEc, which encode the corresponding IGF-I protein isoforms (24, 26). Mature IGF-I peptide represents the common bioactive product of all IGF-I isoforms (24, 28) and numerous studies have shown that this peptide is involved in cell survival and protection from apoptosis, as well as in the process of uncontrolled cell division, which generally characterizes cancer development (10, 29, 30). Various types of human cancer cells, such as prostate, breast and osteosarcoma (8, 31-35), have been found to be affected by these IGF-I functions, while IGF-I has also been shown to contribute to cancer cell migration (36), tumor aggressiveness (37, 38) and neovascularization (39).

Interestingly, a new component of the IGF bioregulatory system has recently been studied for its potential role in carcinogenesis, specifically, IGF-I isoforms (precursors).

These undergo post-translational cleavage, generating the common mature IGF-I peptide but also different carboxyl-terminal extension (E-) peptides (24, 40) and there is a growing body of evidence regarding the potential role of IGF-I isoforms and their respective post-translational IGF-I products, other than mature peptide, in the pathophysiology of different cancer models both *in vitro* and *in vivo* (11, 13, 33, 34, 41-43). The differential regulation of the IGF-I isoforms in the pathophysiology of cancer (13, 33, 41) may imply their discrete biological roles, potentially *via* the putative Ea, Eb and Ec peptides (11, 18, 20, 33-35, 40, 44, 45). In particular, Ec peptide is a bioactive product of the IGF-IEc isoform and its action has been shown to be mediated *via* an IGF-I receptor-independent mechanism (11, 18-20, 33, 44) and has been postulated to be oncogenic (46).

However, to our knowledge, there are no studies examining the expression of the different IGF-I isoforms in RCC. Given the potentially distinct biological role, particularly of the IGF-IEc isoform and its post-translational product Ec peptide in different types of cancer (13, 33, 37, 38, 46), this study aimed to characterize the expression/localization of IGF-IEc in human renal cell carcinoma tissues and investigate whether its expression is associated with the histopathological type of RCC as well as with the overall survival of patients.

Materials and Methods

Ethical approval. A retrospective selection of primary renal carcinoma tissue diagnostic biopsy samples was performed from the archives of the Pathology Department of a General Hospital and this research approach was approved by the Ethics Committee of the Medical School of the National and Kapodistrian University of Athens (approval number: 781/29-9-2009), while all experimental procedures conformed to the Declaration of Helsinki.

Patients. Formaldehyde-fixed and paraffin wax-embedded renal tissue samples, derived from 94 patients, 58 males and 36 females, who underwent radical nephrectomy for histologically proven renal carcinoma within the time period from 1986 to 1998 were retrospectively selected from the archives of the Pathology Department of the "Evangelismos" General Hospital of Athens. Based on the official pathology reports, detailed information was recorded and analyzed, while all tissue specimens were further re-evaluated and confirmed by another pathologist. The patients were between 36 and 82 years of age and divided into different subgroups based on their age, sex, tumor location, invasion and size, histological type, grade and stage according to the 2016 World Health Organization/International Society of Urologic Pathologists classification (47).

Immunohistochemical analysis. Paraffin wax-embedded renal tissue samples were processed for paraffin sections as described elsewhere (18) and a Bondmax automated system (Leica Microsystems, Newcastle upon Tyne, UK) was used for the immunohistochemical (IHC) staining of the sections. The sections were then incubated with specific anti-human IGF-IEc antibody (48) at a dilution of 1:1,000 in phosphate-buffered saline, as previously described (18).

Table I. *Descriptive statistics of the patients' clinicopathological characteristics*.

Characteristic		Value
Age (n=87), yrs	Mean±SD	63.0±9.9
IGF-IEc (n=89)	Mean±SD	171.7±55.9
Tumor size (n=94), cm	Mean±SD	8.0±3.5
Gender, n (%)	Female	36 (38.3%)
	Male	58 (61.7%)
Grade, n (%)	1	5 (5.3%)
	2	42 (44.7%)
	3	35 (37.2%)
	4	12 (12.8%)
Histological type, n (%)	Clear cell	65 (69.1%)
	Papillary type I	5 (5.3%)
	Papillary type II	5 (5.3%)
	Oncocytic	9 (9.6%)
	Chromophobe	5 (5.3%)
	Unclassifiable/sarcomatoid	5 (5.3%)
Mitotic index, n (%)	1	44 (46.8%)
	2	29 (30.9%)
	3	21 (22.3%)
Pathological stage, n (%)	T1A	10 (10.6%)
	T1B	25 (26.6%)
	T2	12 (12.8%)
	T3A	28 (29.8%)
	T3B	19 (20.2%)
Death, n (%)	No	52 (55.9%)
	Yes	35 (37.6%)
	Yes from other cause	6 (6.5%)

Secondary biotinylated goat anti-rabbit IgG antibody (Dako Real EnVision, Glostrup, Denmark) was used and tissue sections were visualized under light microscopy. Prostate cancer biopsy sections were used as positive control (33), while control for the specificity of the reactions obtained in immunohistochemical analysis was performed by substituting the primary IGF-IEc antibody with the antibody diluent (phosphate-buffered saline) only (negative control). One representative tumor section per case was evaluated independently by two blinded pathologists, using intermediate-power light microscopy. Expression/localization of IGF-IEc was assessed and categorized as either grade 1 (weak intensity), grade 2 (moderate intensity) or grade 3 (strong intensity) according to both the intensity and distribution of the staining, while absence of staining was categorized as grade 0 (IGF-Ec-negative). The percentage distribution of each grade was multiplied by the corresponding number (0, 1, 2, or 3) and the summation of those products comprised the total IGF-IEc expression score of the sample (range of expression level: 0-300).

Statistical analysis. Data analysis was performed using Stata 13 statistical software (StataCorp. LP, College Station, TX, USA). Comparison of IGF-IEc expression between groups was performed using *t*-test or Man–Whitney test. Kolmogorov–Smirnov or Shapiro–Wilk test was used to evaluate normality. Multiple linear regression was used to test differences of IGF-IEc expression between groups after controlling for potential confounders. Cox proportional hazards model was used to investigate association

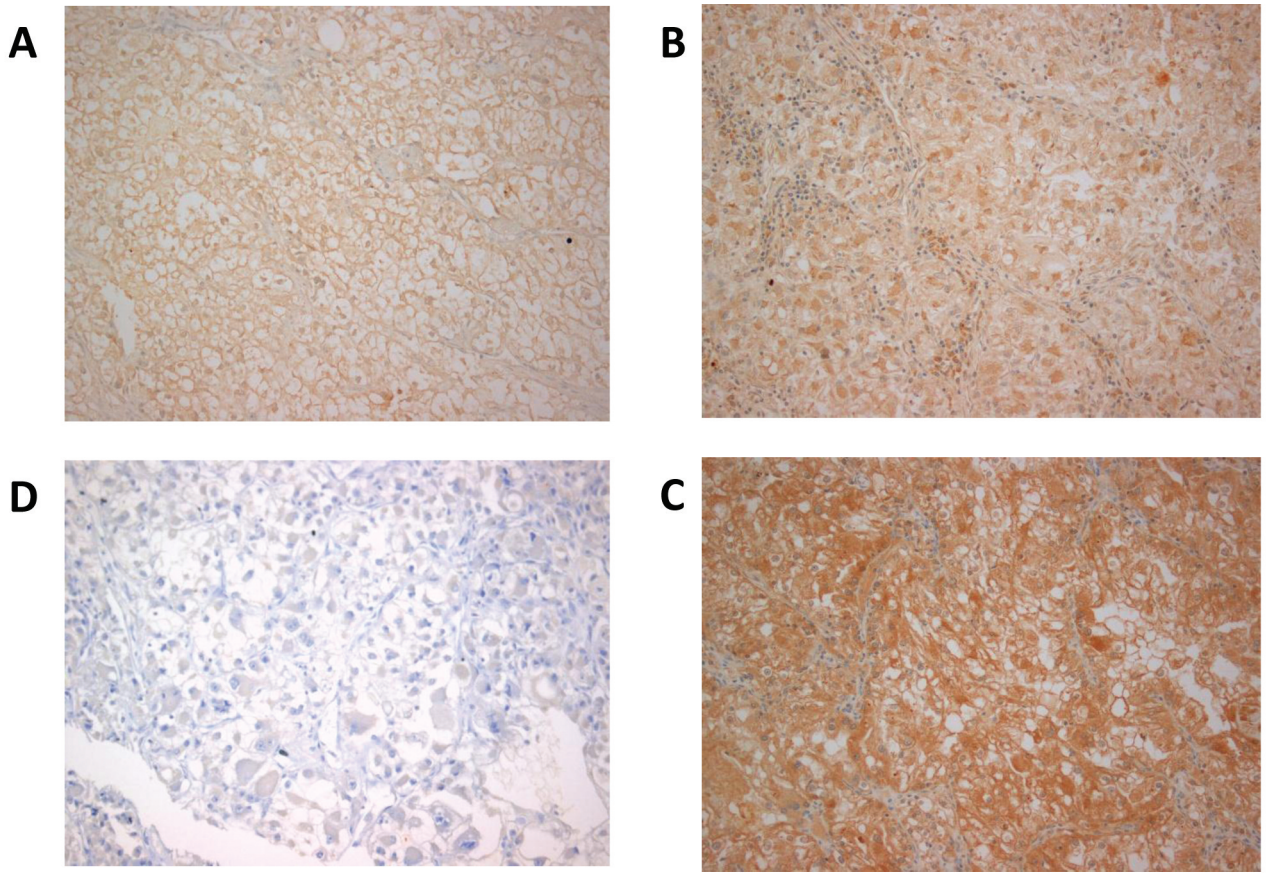

Figure 1. *Representative images of weak (A), moderate (B) and strong (C) immunohistochemical staining of renal cell carcinoma tissue sections using the specific antibody to human insulin-like growth factor (IGF)-IEc. Note the positive (brown) staining with mainly cytoplasmic localization. Specificity of the immunohistochemical detection of IGF-IEc was confirmed by the absence of immunoreactivity in the control (D) (see text for details; magnification ×200).*

between IGF-IEc and risk of death from cancer. Fine and Grey competing risks model (49) was applied to assess confounding by other causes of death. The shape of the association between IGF-IEc and risk of death from cancer was evaluated using natural cubic splines with four knots. All analyses were performed at a=5% level of statistical significance.

Results

Table I lists the clinicopathological characteristics of the patients included in the present study. Representative samples stained with the IGF-IEc specific antibody are shown in Figure 1. RCC tissues showed mainly cytoplasmic IGF-IEc staining but immunoreactivity of IGF-IEc was also localized in the cell membrane. The median IGF-IEc expression score overall was 184 (range=40-300; 25th and 75th percentile values of 125 and 215, respectively). Five out of 94 samples (~5%) were completely negative for IGF-IEc.

IGF-IEc expression in clear cell RCC *versus* all other histological types was significantly lower (Table II, p=0.010; Figure 2). This finding remained significant after adjusting for tumor size, grade, stage, and mitotic index (p<0.05). Higher IGF-IEc levels were found in sarcomatoid (five cases) compared to other cell types although not a statistically significant finding (p=0.420) (Table II).

Table II. *Comparison of insulin-like growth factor (IGF)-IEc expression score (mean±SD) by histological type.*

Histological type		Other	p-Value
Clear cell (n=60)	161.1±57.3	193.5±46.7 (n=29)	0.010*
Sarcomatoid (n=5)	191.4±53.9	170.5±56.1 (n=84)	0.420

*After adjusting for size, grade, stage or mitotic index.

The Cox proportional hazards as well as Fine and Grey competing risks models did not suggest a clinically meaningful association between IGF-IEc level and risk of death from cancer [hazard ratio (95% confidence interval) of

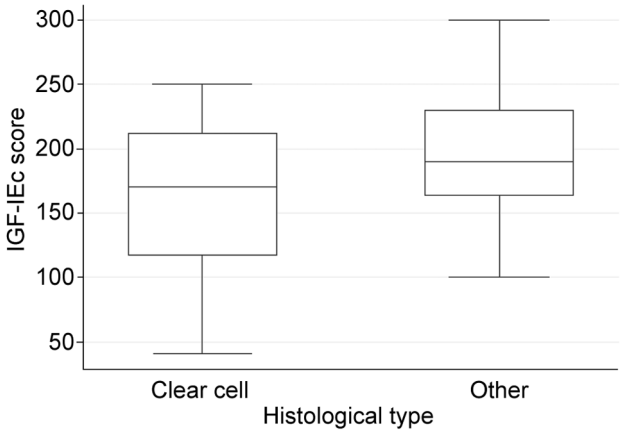

Figure 2. *Box plots representing the distribution of insulin-like growth factor (IGF)-IEc expression values by cell type. The line within each box is the median value, upper and lower whiskers represent the minimum and the maximum values, and the box shows the interquartile range.*

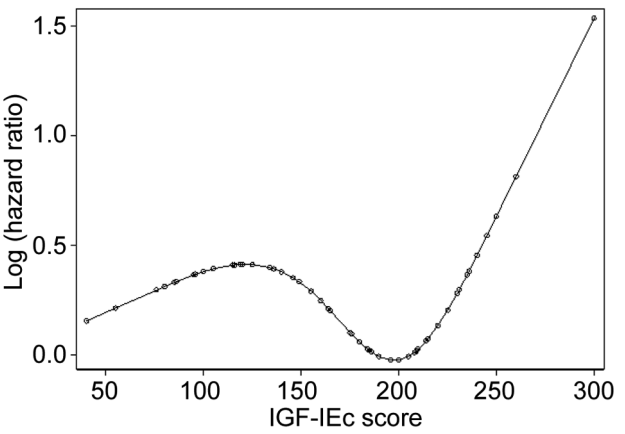

Figure 3. *The polynomial shape of the association revealed between score for insulin-like growth factor (IGF)-IEc isoform expression and risk of cancer death in patients with renal cell carcinoma.*

1.001 (0.994-1.007) and 1.000 (0.994-1.007), respectively] under assumption of a linear association. When the linear association assumption was relaxed, a non-linear, non-significant (p=0.470) association was identified between IGF-IEc level and overall survival, the shape of which was polynomial as depicted in Figure 3.

Discussion

The present study investigated the IHC expression/localization of the IGF-IEc isoform in different histological types of RCC tissues, revealing significant differences in the IGF-IEc levels between clear cell RCC and all other histological types and these differences remained significant after adjusting for tumor size, grade, stage or mitotic index, alternatively. To our best knowledge, this is the first study investigating the expression pattern of IGF-IEc isoform in different types of renal cancer, showing an association between low expression of IGF-IEc and specific RCC pathology. Moreover, it was demonstrated that its expression/localization in RCC tissues was mainly cytoplasmic, while no significant association was found between IGF-IEc level and overall survival of patients with RCC.

IGF-I has been shown to play an important role in cell proliferation and protection from apoptosis in a wide variety of cancer types such as of the prostate, breast, lung, colon, stomach, esophagus, sarcoma, leukemia, liver, pancreas, thyroid, brain, ovary and uterus (cervix and endometrium) (50-53). In particular, studies that have addressed the role of IGF-I or its receptor (IGF-IR) in RCC (2, 15, 16, 54-56) implicated the IGF-I system in the development and progression of RCC, specifically reporting IGF-IR expression to be increased in RCC, which in its turn was associated with poorer cancer-specific survival and increased risk of death compared to patients who had tumors with low IGF-IR expression (2, 15).

Interestingly, there has been a growing interest in the investigation of the expression pattern of the IGF-I isoforms in cancer, as well as in other conditions and pathologies (13, 18-20, 25, 33, 57, 58), since a differential regulation of the IGF-I isoforms in such pathologies may indicate a distinct biological role for the different IGF-I precursor polypeptides or their extension peptides (*i.e.* Ea, Eb and Ec). The role of the IGF-I E peptides remains as yet unclear (24), nevertheless initial evidence was provided recently that Ec peptide is differentially regulated during muscle regeneration in humans (40), while synthetic Ec peptide was documented to possess bioactivity (11, 18-20, 44, 59) and its overexpression in PC-3 prostate cancer cells was found to increase their oncogenic potential in mice (46).

Specifically, the mitogenic effect of an E domain-related product of the IGF-IEc isoform in human prostate cancer, breast cancer, endometrial and osteosarcoma cells has been documented in previous studies of our group *in vitro* (18, 33-35, 46). Moreover, a potential role of the IGF-IEc isoform in the pathophysiology of bladder cancer (13), thyroid cancer (38) and neuroendocrine neoplasms (37) has also been shown, *in vivo*. In particular, differential expression of the IGF-IEc isoform was detected in PCa and prostatic intraepithelial neoplasia, as well as in bladder cancer (13, 33), compared to the corresponding normal tissues. In addition, IGF-IEc expression was found to be significantly related to TNM staging and the presence of muscular and capsule

cancerous invasion in thyroid cancer, exhibiting increased levels in more aggressive compared with the non-aggressive types of cancer. Moreover, there was a positive association between the expression level of this IGF-I isoform and the risk of disease recurrence (38). Furthermore, these findings were in line with previous *in vivo* studies showing that IGF-IEc expression was increased in secondary compared to primary foci in neuroendocrine neoplasms (37), and that its expression was positively correlated with prostate cancer stage and Gleason's score (31). Overall, these findings imply a possible gradual increase of IGF-IEc expression during the progress of this disease.

In the present study, we did not find any association between IGF-IEc expression and overall survival of patients with RCC, nevertheless we did demonstrate that its expression is associated with clear cell RCC independently of tumor pathological stage, grade, size, and mitotic index.

Previous studies have reported that IGF-IR overexpression is associated with increased risk of death in patients with clear cell RCC compared to those who had tumors without IGF-IR expression, while the chemosensitivity of clear cell RCC cells was found to increase after silencing of IGF-IR (2, 60). However, the IGF system components have been shown to be differentially expressed among specific tumor types and particularly in clear cell RCC, where IGF-IR expression was found to be expressed in a disproportionally lower percentage of cases compared to the percentage with IGF-I expression (61), while a recent study further confirmed that the expression of IGF-IR is not related to the expression of its ligands, neither in papillary nor in clear cell RCC tumors (54). Moreover, the expression of IGF-I system components was not found be related to tumor stage, grade, or prognosis of the disease in clear cell RCC (61). Our study, for the first time, revealed a differential regulation of the specific IGF-IEc isoform among different histological types of RCC, while we found no associations of this particular component with the prognosis of the disease, specifically with the overall survival of patients with RCC.

In conclusion, the present study characterized the expression/localization of IGF-IEc isoform in human RCC tissues, showing a significantly lower expression in clear cell RCC *versus* all other RCC histological types. This differential expression of IGF-IEc remained significant after adjusting for other clinicopathological characteristics, such as tumor grade or stage, while it was not found to be associated with the overall survival of those patients. Further studies are needed to investigate the possible tumor type-specific regulation of the IGF-I isoforms in the pathophysiology of RCC, as previously shown in other cancer types.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

FM contributed to the experimental design, carried out the experiments, analyzed data and reviewed the article; CP analyzed data and reviewed the article; AP analyzed data and wrote the article; AA analyzed data, performed the statistical analysis and reviewed the article; PM analyzed data, contributed to the statistical analysis and reviewed the article; MK conceived and designed the study, analyzed data and reviewed the article. All Authors read and approved the final version of article.

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References

1 Semeniuk-Wojtas A, Stec Rand Szczylik C: Are primary renal cell carcinoma and metastases of renal cell carcinoma the same cancer? Urol Oncol *34:* 215-220, 2016. PMID: 26850779. DOI: 10.1016/j.urolonc.2015.12.013

2 Tracz AF, Szczylik C, Porta C and Czarnecka AM: Insulin-like growth factor-1 signaling in renal cell carcinoma. BMC Cancer *16:* 453, 2016. PMID: 27405474. DOI: 10.1186/s12885-016-2437-4

3 Storkel S and van den Berg E: Morphological classification of renal cancer. World J Urol *13:* 153-158, 1995. PMID: 7550386. DOI: 10.1007/BF00184870

4 Jonasch E, Gao J and Rathmell WK: Renal cell carcinoma. BMJ *349:* g4797, 2014. PMID: 25385470. DOI: 10.1136/bmj.g4797

5 Shuch B, Amin A, Armstrong AJ, Eble JN, Ficarra V, Lopez-Beltran A, Martignoni G, Rini BI and Kutikov A: Understanding pathologic variants of renal cell carcinoma: distilling therapeutic opportunities from biologic complexity. Eur Urol *67:* 85-97, 2015. PMID: 24857407. DOI: 10.1016/j.eururo.2014.04.029

6 Msaouel P, Zurita AJ, Huang S, Jonasch E and Tannir NM: Plasma cytokine and angiogenic factors associated with prognosis and therapeutic response to sunitinib *vs*. everolimus in advanced non-clear cell renal cell carcinoma. Oncotarget *8:* 42149-42158, 2017. PMID: 28178674. DOI: 10.18632/oncotarget.15011

7 Reyes-Moreno C, Sourla A, Choki I, Doillon C and Koutsilieris M: Osteoblast-derived survival factors protect PC-3 human prostate cancer cells from adriamycin apoptosis. Urology *52:* 341-347, 1998. PMID: 9697810. DOI: 10.1016/s0090-4295(98)00182-4

8 Koutsilieris M, Mitsiades C and Sourla A: Insulin-like growth factor I and urokinase-type plasminogen activator bioregulation system as a survival mechanism of prostate cancer cells in osteoblastic metastases: Development of anti-survival factor therapy for hormone-refractory prostate cancer. Mol Med *6:* 251-267, 2000. PMID: 10949907.

9 Mitsiades CS, Mitsiades N and Koutsilieris M: The AKT pathway: Molecular targets for anti-cancer drug development. Curr Cancer Drug Targets *4:* 235-256, 2004. PMID: 15134532. DOI: 10.2174/1568009043333032

10 Samani AA, Yakar S, LeRoith D and Brodt P: The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocr Rev *28:* 20-47, 2007. PMID: 16931767. DOI: 10.1210/er.2006-0001

11 Philippou A, Armakolas A and Koutsilieris M: Evidence for the possible biological significance of the *IGF-1* gene alternative splicing in prostate cancer. Front Endocrinol *4:* 31, 2013. PMID: 23519101. DOI: 10.3389/fendo.2013.00031

12 Philippou A, Christopoulos PF and Koutsilieris M: Clinical studies in humans targeting the various components of the IGF system show lack of efficacy in the treatment of cancer. Mutat Res Rev Mutat Res *772:* 105-122, 2016. PMID: 28528684. DOI: 10.1016/j.mrrev.2016.09.005

13 Mourmouras N, Philippou A, Christopoulos P, Kostoglou K, Grivaki C, Konstantinidis C, Serafetinides E, Delakas D and Koutsilieris M: Differential expression of IGF-I transcripts in bladder cancer. Anticancer Res *38:* 3453-3459, 2018. PMID: 29848696. DOI: 10.21873/anticanres.12614

14 Gkioka E, Msaouel P, Philippou A, Vlaghogiannis NI, Vogkou CT, Margiolis A and Koutsilieris M: Review: The role of insulin-like growth factor-1 signaling pathways in uterine leiomyoma. In Vivo *29:* 637-649, 2015. PMID: 26546520.

15 Solarek W, Czarnecka AM, Escudier B, BieleckaZF, Lian F and Szczylik C: Insulin and IGFs in renal cancer risk and progression. Endocr Relat Cancer *22:* R253-264, 2015. PMID: 26330483. DOI: 10.1530/ERC-15-0135

16 Cao Q, Liang C, Xue J, Li P, Li J, Wang M, Zhang Z, Qin C, Lu Q, Hua L, Shao P and Wang Z: Genetic variation in *IGF1* predicts renal cell carcinoma susceptibility and prognosis in Chinese population. Sci Rep *6:* 39014, 2016. PMID: 27976731. DOI: 10.1038/srep39014

17 Moschos MM, Armakolas A, Philippou A, Pissimissis N, Panteleakou Z, Nezos A, Kaparelou M and Koutsilieris M: Expression of the insulin-like growth factor 1 (IGF-1) and type I IGF receptor mRNAs in human HLE-B3 lens epithelial cells. In Vivo *25:* 179-184, 2011. PMID: 21471532.

18 Milingos DS, Philippou A, Armakolas A, Papageorgiou E, Sourla A, Protopapas A, Liapi A, Antsaklis A, Mastrominas M and Koutsilieris M: Insulin-like growth factor-1Ec (MGF) expression in eutopic and ectopic endometrium: Characterization of the MGF E-peptide actions *in vitro*. Mol Med *17*: 21-28, 2011. PMID: 20844834. DOI: 10.2119/molmed.2010.00043

19 Stavropoulou A, Halapas A, Sourla A, Philippou A, Papageorgiou E, Papalois Aand Koutsilieris M: IGF-1 expression in infarcted myocardium and MGF E peptide actions in rat cardiomyocytes *in vitro*. Mol Med *15:* 127-135, 2009. PMID: 19295919. DOI: 10.2119/molmed.2009.00012

20 Philippou A, Papageorgiou E, Bogdanis G, Halapas A, Sourla A, Maridaki M, Pissimissis N and Koutsilieris M: Expression of IGF-1 isoforms after exercise-induced muscle damage in humans: Characterization of the MGF E peptide actions *in vitro*. In Vivo *23:* 567-575, 2009. PMID: 19567392.

21 Philippou A and Barton ER: Optimizing IGF-I for skeletal muscle therapeutics. Growth Horm IGF Res *24:* 157-163, 2014. PMID: 25002025. DOI: 10.1016/j.ghir.2014.06.003

22 Barton ER, Park S, James JK, Makarewich CA, Philippou A, Eletto D, Lei H, Brisson B, Ostrovsky O, Li Z and Argon Y: Deletion of muscle *GRP94* impairs both muscle and body growth by inhibiting local IGF production. FASEB J *26:* 3691-3702, 2012. PMID: 22649033. DOI: 10.1096/fj.11-203026

23 Durzynska J, Philippou A, Brisson BK, Nguyen-McCarty M and Barton ER: The pro-forms of insulin-like growth factor I (IGF-I) are predominant in skeletal muscle and alter IGF-I receptor activation. Endocrinology *154:* 1215-1224, 2013. PMID: 23407451. DOI: 10.1210/en.2012-1992

24 Philippou A, Maridaki M, Pneumaticos S and Koutsilieris M: The complexity of the *IGF1* gene splicing, posttranslational modification and bioactivity. Mol Med *20:* 202-214, 2014. PMID: 24637928. DOI: 10.2119/molmed.2014.00011

25 Thomas CG, Psarros C, Gekas A, Vandoros GP, Philippou A and Koutsilieris M: Alternative splicing of *IGF1* gene as a potential factor in the pathogenesis of Peyronie's disease. In Vivo *30:* 251-256, 2016. PMID: 27107083.

26 Philippou A, Maridaki M, Halapas A and Koutsilieris M: The role of the insulin-like growth factor 1 (IGF-1) in skeletal muscle physiology. In Vivo *21:* 45-54, 2007. PMID: 17354613.

27 Bikle DD, Tahimic C, Chang W, Wang Y, Philippou A and Barton ER: Role of IGF-I signaling in muscle bone interactions. Bone *80:* 79-88, 2015. PMID: 26453498. DOI: 10.1016/j.bone.2015.04.036

28 Philippou A, Halapas A, Maridaki M and Koutsilieris M: Type I insulin-like growth factor receptor signaling in skeletal muscle regeneration and hypertrophy. J Musculoskelet Neuronal Interact *7:* 208-218, 2007. PMID: 17947802.

29 Nagel JM, Geiger BM, Karagiannis AK, Gras-Miralles B, Horst D, Najarian RM, Ziogas DC, Chen X and Kokkotou E: Reduced intestinal tumorigenesis in APCmin mice lacking melanin-concentrating hormone. PLoS One *7:* e41914, 2012. PMID: 22848656. DOI: 10.1371/journal.pone.0041914

30 Hakam A, Yeatman TJ, Lu L, Mora L, Marcet G, Nicosia SV, Karl RC and Coppola D: Expression of insulin-like growth factor-1 receptor in human colorectal cancer. Hum Pathol *30:* 1128-1133, 1999. PMID: 10534157. DOI: 10.1016/s0046-8177(99)90027-8

31 Savvani A, Petraki C, Msaouel P, Diamanti E, Xoxakos I and Koutsilieris M: IGF-IEc expression is associated with advanced clinical and pathological stage of prostate cancer. Anticancer Res *33:* 2441-2445, 2013. PMID: 23749893.

32 Koutsilieris M and Polychronakos C: Proteinolytic activity against IGF-binding proteins involved in the paracrine interactions between prostate adenocarcinoma cells and osteoblasts. Anticancer Res *12:* 905-910, 1992. PMID: 1377896.

33 Armakolas A, Philippou A, Panteleakou Z, Nezos A, Sourla A, Petraki C and Koutsilieris M: Preferential expression of *IGF-1Ec* (MGF) transcript in cancerous tissues of human prostate: evidence for a novel and autonomous growth factor activity of MGF E peptide in human prostate cancer cells. Prostate *70:* 1233-1242, 2010. PMID: 20564425 DOI: 10.1002/pros.21158

34 Philippou A, Armakolas A, Panteleakou Z, Pissimissis N, Nezos A, Theos A, Kaparelou M, Armakolas N, Pneumaticos SG and Koutsilieris M: IGF1Ec expression in MG-63 human osteoblast-like osteosarcoma cells. Anticancer Res *31:* 4259-4265, 2011. PMID: 22199289.

35 Christopoulos PF, Papageorgiou E, Petraki C and Koutsilieris M: The COOH-terminus of the IGF-1Ec isoform enhances the proliferation and migration of human MCF-7 Breast Cancer Cells. Anticancer Res *37:* 2899-2912, 2017. PMID: 28551627. DOI: 10.21873/anticanres.11643

36 Brodt P, Fallavollita L, Khatib AM, Samani AA and Zhang D: Cooperative regulation of the invasive and metastatic phenotypes by different domains of the type I insulin-like growth factor receptor beta subunit. J Biol Chem *276:* 33608-33615, 2001. PMID: 11445567. DOI: 10.1074/jbc.M102754200

37 Alexandraki KI, Philippou A, Boutzios G, Theohari I, Koutsilieris M, Delladetsima IK and Kaltsas GA: IGF-IEc expression is increased in secondary compared to primary foci

in neuroendocrine neoplasms. Oncotarget *8:* 79003-79011, 2017. PMID: 29108282. DOI: 10.18632/oncotarget.20743

38 Karagiannis AK, Philippou A, Tseleni-Balafouta S, Zevolis E, Nakouti T, Tsopanomichalou-Gklotsou M, Psarras V and Koutsilieris M: IGF-IEc expression is associated with advanced differentiated thyroid cancer. Anticancer Res *39:* 2811-2819, 2019. PMID: 31177118. DOI: 10.21873/anticanres.13409

39 Shigematsu S, Yamauchi K, Nakajima K, Iijima S, Aizawa T and Hashizume K: IGF-1 regulates migration and angiogenesis of human endothelial cells. Endocr J *46 Suppl*: S59-62, 1999. PMID: 12054122. DOI: 10.1507/endocrj.46.suppl_s59

40 Vassilakos G, Philippou A and Koutsilieris M: Identification of the IGF-1 processing product human Ec/rodent Eb peptide in various tissues: Evidence for its differential regulation after exercise-induced muscle damage in humans. Growth Horm IGF Res *32:* 22-28, 2017. PMID: 27836414. DOI: 10.1016/j.ghir.2016.11.001

41 Christopoulos PF, Philippou A and Koutsilieris M: Pattern of *IGF-1* variant expression in human cancer cell lines using a novel q-RT-PCR approach. Anticancer Res *35:* 107-115, 2015. PMID: 25550540.

42 Kasprzak A, Szaflarski W, Szmeja J, Andrzejewska M, Przybyszewska W, Kaczmarek E, Koczorowska M, Kościński T, Zabel M and Drews M: Differential expression of *IGF-1* mRNA isoforms in colorectal carcinoma and normal colon tissue. Int J Oncol *42:* 305-316, 2012. PMID: 23165777. DOI: 10.3892/ijo.2012.1706

43 Christopoulos PF, Msaouel P and Koutsilieris M: The role of the insulin-like growth factor-1 system in breast cancer. Mol Cancer *14:* 43, 2015. PMID: 25743390. DOI: 10.1186/s12943-015-0291-7

44 Papageorgiou E, Philippou A, Armakolas A, Christopoulos PF, Dimakakos A, and Koutsilieris M: The human Ec peptide: The active core of a progression growth factor with species-specific mode of action. Hormones *15:* 423-434, 2016. PMID: 27838607. DOI: 10.14310/horm.2002.1699

45 Vassilakos G, Philippou A, Tsakiroglou P andKoutsilieris M: Biological activity of the e domain of the IGF-1Ec as addressed by synthetic peptides. Hormones *13:* 182-196, 2014. PMID: 24776619. DOI: 10.1007/BF03401333

46 Armakolas A, Kaparelou M, Dimakakos A, Papageorgiou E, Armakolas N, Antonopoulos A, Petraki C, Lekarakou M, Lelovas P, Stathaki M, Psarros C, Donta I, Galanos PS, Msaouel P, Gorgoulis VG and Koutsilieris M: Oncogenic role of the Ec peptide of the IGF-1Ec isoform in prostate cancer. Mol Med *21:* 167-179, 2015. PMID: 25569803. DOI: 10.2119/molmed.2014.00222

47 Moch H, Cubilla AL, Humphrey PA, Reuter VE and Ulbright TM: The 2016 WHO classification of tumours of the urinary system and male genital organs-part A: Renal, penile, and testicular tumours. Eur Urol *70(1)*: 93-105, 2016. PMID: 26935559 DOI: 10.1016/j.eururo.2016.02.029

48 Philippou A, Stavropoulou A, Sourla A, Pissimissis N, Halapas A, Maridaki M and Koutsilieris M: Characterization of a rabbit antihuman mechano growth factor (MGF) polyclonal antibody against the last 24 amino acids of the E domain. In Vivo *22:* 27-35, 2008. PMID: 18396778.

49 Fine JP and Gray RJ: A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc *94:* 496–509, 1999. DOI: 10.2307/2670170

50 Singh P, Dai B, Yallampalli U, Lu X and Schroy PC: Proliferation and differentiation of a human colon cancer cell line (CaCo2) is associated with significant changes in the expression and secretion of insulin-like growth factor (IGF) IGF-II and IGF binding protein-*4:* Role of IGF-II. Endocrinology *137:* 1764-1774, 1996. PMID: 8612513. DOI: 10.1210/endo.137.5.8612513

51 LeRoith D, Baserga R, Helman L and Roberts CT, Jr: Insulin-like growth factors and cancer. Ann Intern Med *122:* 54-59, 1995. PMID: 7619109. DOI: 10.7326/0003-4819-122-1-199501010-00009

52 Yaginuma Y, Nishiwaki K, Kitamura S, Hayashi H, Sengoku K and Ishikawa M: Relaxation of insulin-like growth factor-II gene imprinting in human gynecologic tumors. Oncology *54:* 502-507, 1997. PMID: 9394848. DOI: 10.1159/000227610

53 Frostad S and Bruserud O: *In vitro* effects of insulin-like growth factor-1 (IGF-1) on proliferation and constitutive cytokine secretion by acute myelogenous leukemia blasts. Eur J Haematol *62*: 191-198, 1999. PMID: 10089897. DOI: 10.1111/j.1600-0609.1999.tb01743.x

54 Solarek W, Koper M, Lewicki S, Szczylik C and Czarnecka AM: Insulin and insulin-like growth factors act as renal cell cancer intratumoral regulators. J Cell Commun Signal *13:* 381-394, 2019. PMID: 30929166. DOI: 10.1007/s12079-019-00512-y

55 Xu J, Chang WH, Fong LWR, Weiss RH, Yu SL and Chen CH: Targeting the insulin-like growth factor-1 receptor in MTAP-deficient renal cell carcinoma. Signal Transduct Target Ther *4*: 2, 2019. PID: 30701095. DOI: 10.1038/s41392-019-0035-z

56 Cheung CW, Vesey DA, Nicol DL and Johnson DW: The roles of IGF-I and IGFBP-3 in the regulation of proximal tubule, and renal cell carcinoma cell proliferation. Kidney Int *65:* 1272-1279, 2004. PMID: 15086466. DOI: 10.1111/j.1523-1755.2004.00535.x

57 Armakolas N, Armakolas A, Antonopoulos A, Dimakakos A, Stathaki M and Koutsilieris M: The role of the IGF-1 Ec in myoskeletal system and osteosarcoma pathophysiology. Crit Rev Oncol Hematol *108:* 137-145, 2016. PMID: 27931832. DOI: 10.1016/j.critrevonc.2016.11.004

58 Armakolas N, Dimakakos A, Armakolas A, Antonopoulos A and Koutsilieris M: Possible role of the Ec peptide of IGF1Ec in cartilage repair. Mol Med Rep *14*: 3066-3072, 2016. PMID: 27571686. DOI: 10.3892/mmr.2016.5627

59 Armakolas A, Dimakakos A, Loukogiannaki C, Armakolas N, Antonopoulos A, Florou C, Tsioli P, Papageorgiou P, Alexandrou TP, Stathaki M, Spinos D, Pektasides D, Patsouris E and Koutsilieris Ml: IL-6 is associated with IGF-1Ec up-regulation and Ec peptide secretion, from prostate tumors. Mol Med *24:* 6, 2018. PMID: 30134795. DOI: 10.1186/s10020-018-0003-z

60 Parker A, Cheville JC, Lohse C, Cerhan JR and Blute ML: Expression of insulin-like growth factor I receptor and survival in patients with clear cell renal cell carcinoma. J Urol *170:* 420-424, 2003. PMID: 12853790. DOI: 10.1097/01.ju.0000071474.70103.92

61 Schips L, Zigeuner R, Ratschek M, Rehak P, Ruschoff J and Langner C: Analysis of insulin-like growth factors and insulin-like growth factor I receptor expression in renal cell carcinoma. Am J Clin Pathol *122:* 931-937, 2004. PMID: 15539386. DOI: 10.1309/G7PY-0RE7-T86H-HQYV

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