## Title
Expression of IGF/insulin receptor in prostate cancer tissue and progression to lethal disease



## Journal
Carcinogenesis, 39(12)

## ISSN
0143-3334

## Authors
Ahearn, Thomas U
Peisch, Sam
Pettersson, Andreas
et al.

## Publication Date
2018-12-31

## DOI
10.1093/carcin/bgy112

Peer reviewed

*Carcinogenesis*, 2018, Vol. 39, No. 12, 1431–1437

**doi:10.1093/carcin/bgy112**
Advance Access publication August 24, 2018
Original Article

ORIGINAL ARTICLE

# Expression of IGF/insulin receptor in prostate cancer tissue and progression to lethal disease

Thomas U.Ahearn[1,†], Sam Peisch[1,†], Andreas Pettersson[1,2], Ericka M.Ebot[1], Cindy Ke Zhou[1], Rebecca E.Graff[1,3], Jennifer A.Sinnott[4], Ladan Fazli[5], Gregory L.Judson[1], Tarek A.Bismar[6], Jennifer R.Rider[1,7,8], Travis Gerke[1], June M.Chan[3], Michelangelo Fiorentino[9], Richard Flavin[10], Howard D.Sesso[1,11], Stephen Finn[10], Edward L.Giovannucci[1,7,12], Martin Gleave[5], Massimo Loda[13], Zhe Li[14], Michael Pollak[15,‡] and Lorelei A.Mucci[1,7,*,‡]; on behalf of the Transdisciplinary Prostate Cancer Partnership (ToPCaP)

[1]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA, [2]Clinical Epidemiology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden, [3]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA, [4]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA, [5]Vancouver Prostate Center, Department of Urologic Sciences, University of British Columbia, Vancouver, British Columbia, Canada, [6]Department of Pathology and Laboratory Medicine, University of Calgary and Calgary Laboratory Services, Calgary, Alberta, Canada, [7]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA, [8]Boston University School of Public Health, Boston, MA, USA, [9]Pathology Unit, Addarii Institute, S. Orsola-Malpighi Hospital, Bologna, Italy, [10]Department of Histopathology Research, Trinity College, Dublin, Ireland, [11]Divisions of Preventive Medicine and Aging, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA, [12]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA, [13]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA, [14]Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA and [15]Cancer Prevention Research Unit, Departments of Medicine and Oncology, Lady Davis Research Institute of the Jewish General Hospital and McGill University, Montreal, Quebec, Canada

[*]To whom correspondence should be addressed. Tel: +1 617 432 1732; Email: lmucci@hsph.harvard.edu
[†]These authors share first authorship equally.
[‡]These authors share senior authorship equally.

## Abstract

Circulating insulin-like growth factor-1 (IGF-1) is consistently associated with prostate cancer risk. IGF-1 binds to IGF-1 receptor (IGF1R) and insulin receptor (IR), activating cancer hallmark pathways. Experimental evidence suggests that *TMPRSS2:ERG* may interact with IGF/insulin signaling to influence progression. We investigated IGF1R and IR expression and its association with lethal prostate cancer among 769 men. Protein expression of IGF1R, IR and ERG (i.e. a surrogate of *ERG* fusion genes) were assayed by immunohistochemistry. Cox models estimated hazard ratios (HR) and 95% confidence intervals (CI) adjusted for clinical characteristics. Among patients, 29% had strong tumor IGF1R expression and 10% had strong IR expression. During a mean follow-up of 13.2 years through 2012, 80 men (11%) developed lethal disease. Tumors with strong IGF1R or IR expression showed increased cell proliferation, decreased apoptosis and a higher prevalence of ERG. In multivariable models, strong IGF1R was associated with a borderline increased risk of lethal prostate cancer (HR 1.7; 95% CI 0.9–3.1). The association appeared greater in ERG-positive tumors (HR 2.8; 95% CI 0.9–8.4) than in ERG-negative tumors (HR 1.3; 95% CI 0.6–3.0, p-heterogeneity 0.08). There was no association between IR and lethal prostate cancer (HR 0.8; 95% CI 0.4–1.9). These results suggest that tumor IGF1R expression may play a role in prostate cancer progression to

Received: February 13, 2018; Revised: August 2, 2018; Accepted: August 22, 2018

a lethal phenotype and that ERG-positive tumors may be more sensitive to IGF signaling. These data may improve our understanding of IGF signaling in prostate cancer and suggest therapeutic options for disease subtypes.

**Abbreviations**

| | |
|---|---|
| BMI | body mass index |
| CI | confidence intervals |
| HR | hazard ratios |
| IGF-1 | insulin-like growth factor-1 |
| IGF1R | IGF-1 receptor |
| IR | insulin receptor |
| IR-A | IR isoform A |
| IR-B | IR isoform B |
| PI3K | phosphoinositide 3-kinase |

## Introduction

There is ample evidence implicating the insulin-like growth factor (IGF) and insulin signaling pathways in prostate cancer initiation and progression (1,2). Molecular epidemiological studies consistently find that high prediagnostic circulating levels of the ligand IGF-1 are associated with an increased risk of prostate cancer, particularly advanced disease (3–6). Experimentally, IGF-1 binds to both the IGF-1 receptor (IGF1R) and insulin receptor (IR) to subsequently promote mitogenic signaling events, increase cell proliferation and inhibit apoptosis (1). Both IGF1R and IR are overexpressed in prostate tumor tissue (7), and therapeutic agents targeting the IGF1R/IR pathway are under development (8,9).

Emerging evidence suggests the IGF/insulin signaling pathway may be relevant to the *TMPRSS2:ERG* gene fusion, the most common somatic event in primary prostate cancer (10,11). We previously reported that men with positive ERG protein expression (an established marker of the *TMPRSS2:ERG* fusion) have significantly higher protein expression of IGF1R and IR compared with men with ERG-negative disease (12). *In vitro* studies support this epidemiological finding by showing direct regulation of IGF1R by *TMPRSS2:ERG* (13). Taken together, these data suggest that ERG-positive tumors may be more sensitive to IGF/insulin signaling, which could promote prostate cancer progression.

There is limited evidence to date on IGF1R and IR expression in tumor tissue and prostate cancer progression in patient cohorts, and none have used lethal disease as an end point (14). We hypothesized that higher tumor expression of IGF1R and IR would be associated with an increased risk of lethal prostate cancer, with a stronger effect in men whose tumors were positive for ERG. Lethal prostate cancer was defined as prostate cancer death or distant metastases to bone or other organs. We undertook a prospective study of 769 men who were diagnosed with prostate cancer with long-term follow-up for metastasis and cancer death to investigate associations of tumor protein expression of IGF1R and IR with lethal prostate cancer.

## Materials and methods

### Study population

This study was nested within the Physicians' Health Study (PHS) and Health Professionals Follow-up Study (HPFS). The PHS I and II were randomized trials of aspirin and vitamin supplements in the prevention of cardiovascular disease and cancer among 29 071 US male physicians (15,16). The HPFS is an ongoing cohort of 51 529 male health professionals followed with biennial questionnaires since 1986 (17). In both cohorts, men were cancer free at baseline. Incident prostate cancers (ICD-9: 185) were initially identified through self-report and confirmed through medical record and pathology report review.

Archival prostate tumor tissue specimens have been collected from participants diagnosed with prostate cancer when available. For this analysis, we focused on men diagnosed with prostate cancer between 1983 and 2004 who had archival radical prostatectomy or transurethral resection of the prostate tissue on constructed tumor tissue microarrays. Tumor tissue microarrays were constructed from the archival materials by taking at least three 0.6 mm cores of tumor tissue per case from the primary tumor nodule or the nodule with the highest Gleason grade. Our success rate in collecting tumor tissue materials from pathology departments has been 69%. The clinical and demographic characteristics of men with and without available tissue materials are similar.

### Clinical and follow-up data

Data on tumor stage, prostate-specific antigen levels at diagnosis and primary treatments were abstracted from medical records and pathology reports. Standardized histopathologic review of hematoxylin and eosin slides was performed by study pathologists to provide uniform Gleason grading (International Society of Urologic Pathology, 2014) and denote areas for construction of tissue microarrays (M.F., S.F.) (18). Prostate cancer patients have been followed prospectively with biennial questionnaires to collect detailed information regarding additional cancer treatments and development of metastases. For this analysis, lethal prostate cancer was defined as prostate cancer death or distant metastases to bone or other organs. Causes of death are determined by an end points review committee using all available data including medical records, death certificates and a search of the National Death Index. The completeness of follow-up for mortality in the cohorts is >98%.

### Tumor biomarkers

We assessed protein expression of IGF1R and IR by immunohistochemistry on 5 μm sections of tumor tissue microarrays constructed from prostatectomy ($n$ = 710) and transurethral resection of the prostate ($n$ = 59) specimens. Details of the immunohistochemical methods were previously reported (7,12,19–22). Briefly, IGF1R expression was assessed using an IGF1Rβ polyclonal rabbit antibody (sc-713; Santa Cruz Biotechnology Inc., Santa Cruz, CA) and IR expression using an IRβ rabbit immunoaffinity purified IgG antibody (#07-724; Upstate Cell Signaling Solutions, Lake Placid, NY). For both IGF1R and IR, tumor staining intensity expression was semiquantitatively scored by study pathologists (L.F., T.A.B.) ranging from 0 to 3 as follows: no staining in tumor cells = 0, faint = 1, moderate = 2 and intense staining = 3. In a subset of samples, IR ($n$ = 328) and IGF1R ($n$ = 336) expression were also scored in tumor-adjacent 'normal' prostate tissue. We used the mean staining intensity across TMA cores as our primary exposures for both IGF1R and IR.

We characterized *TMPRSS2:ERG* status using a validated protocol for ERG protein expression (monoclonal antibody clone EPR3864, Epitomics Inc.) (22). We classified tumors as ERG positive if at least one core stained positive for ERG and as ERG negative if all cores stained negative for ERG, using ERG-positive nuclear staining of normal endothelial cells as internal controls. ERG tumor status was available among 739 of the 753 patients with IGF1R and among 707 of the 718 patients with IR.

IGF1R/IR pathway is a regulator of the phosphoinositide 3-kinase (PI3K) signaling pathway and is involved in apoptosis and cell proliferation. As such, we leveraged data on additional tumor markers characterizing these features. Specifically, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was used to identify the percentage of tumor cells undergoing apoptosis using the Apoptag Peroxidase In Situ Kit (Chemicon International), Ki67 for cell proliferation using the polyclonal anti-Ki67 antibody (Vector Labs) and endothelial cell marker CD34 for angiogenesis using a primary mouse monoclonal antibody anti-CD34

(QBEND10; Biogenex, San Ramon, CA) (19). For the PI3K pathway, we evaluated three markers: PTEN using a rabbit monoclonal anti-PTEN antibody (#9188; Cell Signaling Technologies), pAKT expression using a rabbit polyclonal anti-pAKT (#4060; Cell Signaling Technologies) and pS6 expression using a rabbit polyclonal anti-pS6 antibody (#2215S; Cell Signaling Technologies).

### Statistical methods

We compared demographic and clinical characteristics according to IGF1R and IR mean tumor intensity categorized as weak to none (0–1), moderate (>1–2) or strong (>2). We used the Kruskal–Wallis test and the Cochran–Armitage trend test to assess associations across the biomarkers of IGF1R and IR with the tumor markers. Cox proportional hazards models estimated hazard ratios (HR) and 95% confidence intervals (95% CI) of associations between IGF1R and IR with time to lethal prostate cancer, defined as prostate cancer death or distant metastases to bone or other organs. Person-time was calculated from the date of cancer diagnosis to the earliest of the following time points: development of lethal prostate cancer, censored at time of death from other causes or end of follow-up at the end of 2012.

Cox models were adjusted for age at diagnosis (years, continuous) and additionally adjusted for body mass index (BMI) (kg/m$^2$, continuous), Gleason score (≤6, 3 + 4, 4 + 3, 8–10) and clinical tumor stage (T1/T2 N0/Nx M0/Mx, T3 N0/Nx M0/Mx, and T4/N1). To test for linear trends, we alternatively fitted continuous mean IGF1R and IR expression. We stratified Cox models according to tumor ERG status and tested the multiplicative interaction terms of ERG with IGF1R and IR in separate Cox models using Wald tests. Missing data on clinical ($n$ = 24) or pathologic tumor stage ($n$ = 18; only among prostatectomy cases) were replaced with the most common category of clinical (T1/T2 N0/Nx M0/Mx) or pathologic TNM stage (T2 N0/Nx M0/Mx). The proportional hazards assumption held when we tested the interaction between IGF1R and IR expression with follow-up time in Cox models.

Analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC), and all statistical tests were two sided with $P$-values < 0.05 considered statistically significant. The research project was approved by the Institutional Review Boards at Partners Healthcare and the Harvard T.H. Chan School of Public Health.

## Results

Selected clinical and demographic characteristics of the patient cohorts are presented in Table 1. The cohorts are >95% white. There was strong tumor staining of IGF1R in 29% of patients and tumor staining of IR in 10% of patients. IGF1R and IR expression were significantly higher in tumor tissue compared with normal appearing tissue (data not shown). Tumor IGF1R and IR expression were not associated with age, BMI, prostate-specific antigen at diagnosis, Gleason score or pathologic/clinical tumor stage at diagnosis. The prevalence of ERG-positive tumors was 30% in patients with absent to weak IGF1R expression, 48% in tumors with moderate IGF1R expression and 66% in tumors with strong IGF1R expression. Similarly, the prevalence of ERG increased with higher IR protein expression.

Table 2 presents associations between tumor expression of IGF1R and IR with a range of tumor markers. Tumors with strong IGF1R and IR also exhibited increased tumor proliferation and lower apoptosis. In addition, IGF1R and IR protein expression were positively associated with expression of the PI3K markers pAKT and pS6. Strong IGF1R was suggestively associated with a more angiogenic profile ($P$ = 0.06), whereas no association for IR and angiogenesis was observed. There was no association between either IR or IGF1R and PTEN status.

During a mean follow-up of 13.2 years, 80 lethal events occurred among men with measured IGF1R expression ($n$ = 753) and 79 lethal events among men with measured IR ($n$ = 718). In the fully adjusted models, strong IGF1R expression was associated with a borderline significant increased risk of lethal prostate cancer (HR 1.7; 95% CI 0.9–3.1) (Table 3). In contrast, there was no association between IR expression and lethal prostate cancer. When we restricted to patients treated with radical prostatectomy alone and adjusted for pathologic tumor stage in place of clinical tumor stage, the results did not appreciably change (data not shown).

**Table 1.** Clinical characteristics among men diagnosed with prostate cancer between 1983 and 2004 in the Health Professionals Follow-up Study and the Physicians' Health Study, by IGF1R or IR

| | IGF1R | | | | IR | | | |
|---|---|---|---|---|---|---|---|---|
| Characteristic | N | Weak to none ($n$ = 173) | Moderate ($n$ = 376) | Strong ($n$ = 204) | N | Weak to none ($n$ = 348) | Moderate ($n$ = 300) | Strong ($n$ = 70) |
| Age at diagnosis, years, mean (SD) | 753 | 66.7 (6.3) | 65.3 (6.4) | 65.9 (6.4) | 718 | 66.5 (6.3) | 65.0 (6.4) | 65.4 (5.9) |
| BMI at diagnosis, kg/m$^2$, mean (SD) | 753 | 25.6 (3.4) | 25.7 (3.8) | 25.5 (3.0) | 718 | 25.5 (3.1) | 25.6 (4.0) | 25.8 (3.2) |
| Prostate-specific antigen at diagnosis, ng/ml, median (quartile 1, quartile 3) | 640 | 7.4 (5.1, 12.0) | 7.0 (5.0, 10.4) | 7.0 (5.0, 11.7) | 609 | 7.0 (5.0, 10.0) | 6.9 (4.8, 10.8) | 7.0 (5.1, 12.3) |
| ERG positive, $n$ (%) | 739 | 51 (30) | 178 (48) | 133 (66) | 707 | 123 (36) | 181 (61) | 44 (64) |
| Gleason score, $n$ (%) | | | | | | | | |
| <7 | 160 | 43 (25) | 87 (23) | 30 (15) | 148 | 79 (23) | 54 (18) | 15 (21) |
| 3 + 4 | 266 | 50 (29) | 131 (35) | 85 (42) | 257 | 116 (33) | 115 (38) | 26 (37) |
| 4 + 3 | 182 | 40 (23) | 91 (24) | 51 (25) | 174 | 85 (24) | 75 (25) | 14 (20) |
| ≥8 | 145 | 40 (23) | 67 (18) | 38 (19) | 139 | 68 (20) | 56 (19) | 15 (21) |
| Pathologic TNM, $n$ (%) | | | | | | | | |
| T2 N0/Nx | 482 | 98 (65) | 251 (73) | 133 (73) | 455 | 204 (70) | 201 (71) | 50 (74) |
| T3 N0/Nx | 173 | 49 (32) | 81 (24) | 43 (24) | 168 | 85 (29) | 67 (24) | 16 (24) |
| T4/N1 | 22 | 4 (3) | 11 (3) | 7 (4) | 21 | 3 (1) | 16 (6) | 2 (3) |
| Clinical TNM, $n$ (%) | | | | | | | | |
| T1/T2 N0/Nx | 686 | 155 (90) | 347 (95) | 184 (93) | 650 | 313 (93) | 270 (92) | 67 (97) |
| T3 N0/Nx | 34 | 13 (8) | 10 (3) | 11 (6) | 33 | 15 (4) | 17 (6) | 1 (2) |
| T4/N1 | 15 | 3 (2) | 9 (2) | 3 (1) | 15 | 9 (3) | 5 (2) | 1 (1) |

**Table 2.** Association[a] between tumor expression of IGF1R and IR with additional tissue markers among men diagnosed with prostate cancer in the Health Professionals Follow-up Study and the Physicians' Health Study

| | IGF1R expression | | | | | IR expression | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | Weak to none | Moderate | Strong | *P*[b] | *N* | Weak to none | Moderate | Strong | *P*[b] |
| IR | 702 | 0.3 (0, 1.0) | 1.3 (1.0, 2.0) | 2.0 (1.3, 2.0) | <0.0001 | — | — | — | | |
| IGF1R | — | — | — | | | 702 | 1.7 (1.0, 2.0) | 2.0 (2.0, 2.7) | 2.3 (2.0, 3.0) | <0.0001 |
| Ki67[c] | 726 | 0.04 (0, 0.3) | 0.1 (0, 0.4) | 0.2 (0, 0.8) | <0.0001 | 692 | 0 (0, 0.3) | 0.1 (0, 0.6) | 0.1 (0, 0.5) | 0.01 |
| TUNEL[d] | 638 | 1.0 (0.5, 3.0) | 0.5 (0, 2.0) | 0.5 (0, 2.0) | 0.03 | 607 | 1.0 (0.5, 3.0) | 0.5 (0, 2.0) | 0.5 (0, 0.5) | <0.0001 |
| PTEN loss, %[e] | 570 | 17 | 22 | 16 | 0.72 | 537 | 18 | 22 | 20 | 0.32 |
| pAKT[f] | 682 | 0.02 (0, 0.07) | 0.04 (0.01, 0.09) | 0.08 (0.03, 0.17) | <0.0001 | 656 | 0.02 (0.01, 0.07) | 0.06 (0.02, 0.14) | 0.09 (0.04, 0.18) | <0.0001 |
| pS6[f] | 717 | 0.09 (0.04, 0.19) | 0.14 (0.05, 0.30) | 0.16 (0.07, 0.35) | <0.0001 | 684 | 0.10 (0.04, 0.21) | 0.16 (0.07, 0.34) | 0.21 (0.09, 0.59) | <0.0001 |
| Vessel area[g] | 300 | 482 (324, 662) | 491 (387, 672) | 428 (302, 597) | 0.06 | 280 | 459 (358, 620) | 457 (341, 684) | 470 (310, 600) | 0.85 |

[a]Values are presented as median (quartile 1, quartile 3) unless otherwise indicated.
[b]P-values calculated by Kruskal–Wallis test except for PTEN, which is calculated by the Cochran–Armitage trend test.
[c]Ki67 percent positive nuclear staining.
[d]TUNEL percent positive nuclear staining.
[e]A tissue core was considered to have PTEN protein loss if the intensity of cytoplasmic and nuclear staining was markedly decreased or entirely negative across >10% of tumor cells compared with surrounding benign glands and/or stroma.
[f]Expressed as mean area values are median (quartile 1, quartile 3) unless otherwise indicated.
[g]Vessel area = area composed of microvessels (in square micrometers); smaller vessels are more angiogenic.

**Table 3.** HR and 95% CI of the association between IGF1R and IR and risk of lethal prostate cancer among men in the Health Professionals Follow-up Study and the Physicians' Health Study

| | N[a] | Lethal events | Person-years | Model 1[b] | Model 2[c] |
|---|---|---|---|---|---|
| IGF1R expression | | | | | |
| Weak to none | 173 | 18 | 2176 | Reference | Reference |
| Moderate | 376 | 34 | 4973 | 0.9 (0.5–1.6) | 1.1 (0.6–2.0) |
| Strong | 204 | 28 | 2763 | 1.3 (0.7–2.4) | 1.7 (0.9–3.1) |
| Trend *P*-value | | | | 0.30 | 0.07 |
| IR expression | | | | | |
| Weak to none | 348 | 40 | 4343 | Reference | Reference |
| Moderate | 300 | 32 | 4091 | 1.0 (0.6–1.5) | 0.9 (0.5–1.4) |
| Strong | 70 | 7 | 974 | 0.8 (0.4–1.9) | 0.8 (0.4–1.9) |
| Trend *P*-value | | | | 0.71 | 0.51 |

[a]Total number of cases.
[b]Adjusted for age at diagnosis.
[c]Adjusted for age at diagnosis, BMI at diagnosis, Gleason score and clinical TNM stage.

Table 4 shows associations of IGF1R and IR with the risk of lethal prostate cancer according to ERG status. The positive association between IGF1R and lethal prostate cancer was restricted to men with ERG-positive tumors (HR 2.8; 95% CI 0.9–8.4), whereas there was no association between IGF1R and lethal cancer in ERG-negative diseases (HR 1.3; 95% CI 0.6–3.0) (Table 4). There was no association between IR expression in prostate cancer tissue and lethal prostate cancer risk regardless of ERG status.

## Discussion

In this prostate cancer cohort with long-term follow-up, tumor expression of IGF1R was positively associated with the risk of developing lethal prostate cancer after adjusting for clinical factors. Moreover, we found strong correlations between expression of IGF1R and expression of other tissue markers including PI3K activation, cell proliferation and reduced tumor apoptosis. Although IR tumor expression was also correlated with a range of biomarkers, it was not associated with lethal disease.

The association between IGF1R and an increased risk of lethal prostate cancer is supported by experimental and epidemiological evidence that IGF signaling plays a role in prostate cancer progression. Circulating IGF-1 levels are mainly produced in the liver under the regulation of growth hormone, and normal prostatic stromal cells can produce IGF-I and IGF binding proteins locally in an autocrine/paracrine fashion. IGF-1 binds to IGF1R, IR, as well as to hybrid IR/IGF1R, activating the PI3K–AKT–TOR and RAF–MAPK pathways, which promote cell survival and proliferation (1,23). Molecular epidemiological studies have found higher prediagnostic circulating IGF-1 levels to be consistently associated with an increased risk of incident prostate cancer (6,24–27), in particular some reported an increased risk of advanced disease or disease-specific mortality (25,28–30). Genetic variations in 26 IGF pathway-related genes were also reported to be significantly associated with prostate cancer mortality among 5887 prostate cancer cases in the Breast and Prostate Cancer Cohort Consortium (BPCCC) (31).

Data from experimental studies indicate that insulin signaling influences prostate carcinogenesis and tumor progression independently from IGF signaling pathways (32). For example, *in vitro* studies reported that overexpression of IR was associated with increased proliferation, migration, angiogenesis and decreased apoptosis (33,34), whereas knockdown of IGF1R and IR resulted in reduced cell growth and proliferation and

**Table 4.** HR and 95% CI of the association between IGF1R and IR with lethal prostate cancer stratified by ERG tumor status among men in the Health Professionals Follow-up Study and the Physicians' Health Study

| | Lethal prostate cancer | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ERG negative | | | | ERG positive | | | | |
| | Lethal events | Person-years | Model 1[a] | Model 2[b] | Lethal events | Person-years | Model 1[a] | Model 2[b] | *P* interaction[c] |
| IGF1R | | | | | | | | | |
| Weak to none | 13 | 1411 | Reference | Reference | 4 | 712 | Reference | Reference | 0.08 |
| Moderate | 17 | 2474 | 0.8 (0.4–1.6) | 1.2 (0.6–2.6) | 16 | 2407 | 1.3 (0.4–3.8) | 1.3 (0.4–3.9) | |
| Strong | 10 | 872 | 1.3 (0.6–2.9) | 1.3 (0.6–3.0) | 18 | 1843 | 1.9 (0.6–5.5) | 2.8 (0.9–8.4) | |
| Trend *P*-value | | | 0.65 | 0.52 | | | 0.18 | 0.02 | |
| IR | | | | | | | | | |
| Weak to none | 24 | 2676 | Reference | Reference | 14 | 1596 | Reference | Reference | 0.45 |
| Moderate | 14 | 1540 | 1.1 (0.6–2.1) | 0.9 (0.4–1.7) | 18 | 2496 | 0.9 (0.5–1.9) | 1.0 (0.5–2.0) | |
| Strong | 3 | 342 | 1.0 (0.3–3.4) | 0.6 (0.2–1.9) | 4 | 614 | 0.8 (0.3–2.4) | 1.4 (0.4–4.3) | |
| Trend *P*-value | | | 0.86 | 0.36 | | | 0.68 | 0.72 | |

[a]Adjusted for age at diagnosis.
[b]Adjusted for age at diagnosis, BMI at diagnosis, Gleason score and clinical TNM.
[c]P-value based on Wald test of multiplicative interaction term between continuous IGF1R or IR and ERG status; adjusted for age at diagnosis, BMI at diagnosis, Gleason score and clinical TNM.

increased apoptosis (35). However, epidemiological evidence for circulating insulin and prostate cancer risk is inconsistent. A study within the Health Professionals Follow-up Study used prediagnostic C-peptide levels as a marker of insulin and reported no association for risk total or aggressive prostate cancer (36). However, other prospective studies showed that C-peptide was positively associated with the aggressive prostate cancer (3,4,37,38). Intratumoral correlations observed between IR expression and apoptosis, cell proliferation and AKT expression imply the involvement of other pivotal markers in the complex signaling network.

The IGF1R and IR downstream signaling events are similar, but not identical. IGF1R signaling has been associated with increased mitogenesis, whereas IR signaling has been associated with metabolic events (1,39), potentially explaining the lack of association between IR and lethal progression. The null association between IR and lethal prostate cancer may also be explained by the inability of our immunohistochemical assay to distinguish between IR isoform A (IR-A) and IR isoform B (IR-B). Experimental evidence suggests that IR-B is predominantly responsible for the metabolic effects of insulin, whereas IR-A has a high affinity of IGF-1 and IGF-2 and has a mitogenic response when activated (39). Activation of IR-A, but not IR-B, has been associated with proliferation, migration and resistance to apoptosis (33). In our cohort, any potential association between IR-A with lethal progression may have been obscured by IR-B.

Our results support the hypothesis that synergy between IGF1R expression and ERG status may be related to prostate cancer progression, as men with ERG-positive tumors and upregulated expression of IGF1R had a 2.8-fold increased risk of lethal prostate cancer. Our previous study found higher IGF1R and IR tumor expression in ERG-positive tumors compared with ERG-negative tumors (12). IGF1R interacts with androgen signaling potentially by activating androgen receptor cofactors that promote nuclear androgen receptor localization (40,41). A survey of ChIP-Seq data showed extensive ERG binding to the IGF1R promotor, thus regulating *IGF1R* expression (42). In the *ERG*-positive VCaP prostate cancer cell line, *IGF1R* is highly expressed compared with cell lines lacking ERG. Moreover, in cell lines exposed to IGF1R inhibitors, only VCaP cell lines showed strong growth inhibition (42). IGF-1 signaling may also be a mechanism for the formation of Ewing's sarcoma, which contains a high prevalence of ETS-associated fusion proteins, including ERG (43,44). If replicated, these findings may help improve our understanding of different biological pathways associated with ERG prostate cancer. It may also highlight an opportunity to develop anti-IGF1R therapies that target ERG-positive tumors. These findings may also provide *in vivo* insights into the role of IGF1R in driving prostate cancer progression of ERG-positive tumors.

The only other cohort study that has investigated cancer outcomes examined mRNA expression of *IGF1R* and *IR* in among 270 patients and used biochemical recurrence rather than lethal prostate cancer as the end point (14). Although they found a positive correlation between *IGF1R*, *IR* and ERG, they found that *IR* and *IGF1R* expression were downregulated in tumor versus benign tissue, which contrasts with our study findings. Moreover, high *IGF1R* expression was inversely associated with biochemical recurrence, with a stronger association between *IGF1R* and ERG-negative cancer. Several possibilities may explain the divergent differences in the previous study and our results and the hypothesized association based on laboratory data. First, *IGF1R* and *IR* were measured at the mRNA level as opposed to protein expression in our study. Second, this cohort was smaller and had shorter follow-up than our study. Third, the risk of recurrence was surprisingly high in their cohort, with almost half of men experiencing recurrence during <2 years of follow-up, despite the relatively low-risk features of the cohort at baseline.

The validity of our study should be considered in the context of strengths and limitations. First, we had a large, well-annotated cohort with long and complete follow-up, which allowed us to investigate the most clinically relevant end point, lethal prostate cancer. Our study pathologists centrally reviewed and scored Gleason for all patients, minimizing measurement errors.

We used an established immunohistochemical approach for assessing the tumor biomarkers of interest. However, our assay is unable to distinguish the two isoforms of IR, of which IR-A may be most relevant in prostate cancer (39). Finally, white men (>95%) primarily comprise our cohort. Given the lower prevalence of *TMPRSS2:ERG* in black and Asian men (45), it will be important to investigate our results in large and diverse cohorts with lethal prostate cancer as the end point.

In conclusion, our epidemiological findings support the hypothesis that IGF signaling in prostate tumors plays a role in the progression of prostate cancer. Our findings align with previously published experimental studies and both highlight a potential mechanism of ERG formation and suggest that a subset of prostate cancers could be treated by therapeutic agents targeting the IGF/insulin signaling pathway.

## Funding

This work was supported in part by National Institutes of Health {grant numbers R01CA136578, R01CA141298, T32CA09001 [to (T.U.A. and C.K.Z.)], R25CA112355 [to (R.E.G.)], P50CA090381 [to (L.A.M. and E.L.G.)]}; Prostate Cancer Foundation Young Investigators Awards to L.A.M., J.R.R., S.F. and T.A.B.; T.U.A. was supported by an American Cancer Society—Ellison Foundation Postdoctoral Fellowship (PF-14-140-01-CCE). The Health Professionals Follow-up Study is supported by U01CA167552 from the National Cancer Institute. The tissue microarrays were constructed by the Tissue Microarray Core Facility at the Dana-Farber/Harvard Cancer Center (P30CA06516).

## Acknowledgements

We are grateful to the ongoing participation of the men in the Physicians' Health Study and Health Professionals Follow-up Study. We thank M.J.Stampfer for his guidance and review of the manuscript; L.Bondarenko, L.Moy and H.Mandel for collection of tumor specimens; B.Frost-Hawes and S.Saint-Surin for project management; C.Li for her expert tissue microarray construction; and E.Nuttall and J.Huth for assistance with data management and manuscript preparation. In addition, we thank the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data.

*Conflict of Interest Statement:* None declared.

## References

1. Pollak, M. (2008) Insulin and insulin-like growth factor signalling in neoplasia. Nat. Rev. Cancer, 8, 915–928.
2. Pollak, M.N. *et al.* (2004) Insulin-like growth factors and neoplasia. Nat. Rev. Cancer, 4, 505–518.
3. Ma, J. *et al.* (2008) Prediagnostic body-mass index, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis. Lancet Oncol., 9, 1039–1047.
4. Neuhouser, M.L. *et al.* (2010) Finasteride modifies the relation between serum C-peptide and prostate cancer risk: results from the Prostate Cancer Prevention Trial. Cancer Prev. Res. (Phila.), 3, 279–289.
5. Nimptsch, K. *et al.* (2011) Plasma insulin-like growth factor 1 is positively associated with low-grade prostate cancer in the Health Professionals Follow-up Study 1993–2004. Int. J. Cancer, 128, 660–667.
6. Price, A.J. *et al.* (2012) Insulin-like growth factor-I concentration and risk of prostate cancer: results from the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol. Biomarkers Prev., 21, 1531–1541.
7. Cox, M.E. *et al.* (2009) Insulin receptor expression by human prostate cancers. Prostate, 69, 33–40.
8. Fahrenholtz, C.D. *et al.* (2013) Targeting IGF-IR with ganitumab inhibits tumorigenesis and increases durability of response to androgen-deprivation therapy in VCaP prostate cancer xenografts. Mol. Cancer Ther., 12, 394–404.
9. Ibuki, N. *et al.* (2014) The tyrphostin NT157 suppresses insulin receptor substrates and augments therapeutic response of prostate cancer. Mol. Cancer Ther., 13, 2827–2839.
10. Tomlins, S.A. *et al.* (2009) ETS gene fusions in prostate cancer: from discovery to daily clinical practice. Eur. Urol., 56, 275–286.
11. Abeshouse, A. *et al.* (2015) The molecular taxonomy of primary prostate cancer. Cell, 163, 1011–1025.
12. Pettersson, A. *et al.* (2013) Modification of the association between obesity and lethal prostate cancer by *TMPRSS2:ERG*. J. Natl Cancer Inst., 105, 1881–1890.
13. Meisel Sharon, S. *et al.* (2016) TMPRSS2-ERG fusion protein regulates insulin-like growth factor-1 receptor (IGF1R) gene expression in prostate cancer: involvement of transcription factor Sp1. Oncotarget, 7, 51375–51392.
14. Mancarella, C. *et al.* (2017) Insulin-like growth factor 1 receptor affects the survival of primary prostate cancer patients depending on TMPRSS2-ERG status. BMC Cancer, 17, 367.
15. Gaziano, J.M. *et al.* (2009) Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. JAMA, 301, 52–62.
16. Hennekens, C.H. *et al.* (1985) A randomized trial of aspirin and beta-carotene among U.S. physicians. Prev. Med., 14, 165–168.
17. Giovannucci, E. *et al.* (2007) Risk factors for prostate cancer incidence and progression in the health professionals follow-up study. Int. J. Cancer, 121, 1571–1578.
18. Stark, J.R. *et al.* (2009) Gleason score and lethal prostate cancer: does 3 + 4 = 4 + 3? J. Clin. Oncol., 27, 3459–3464.
19. Mucci, L.A. *et al.* (2009) Prospective study of prostate tumor angiogenesis and cancer-specific mortality in the health professionals follow-up study. J. Clin. Oncol., 27, 5627–5633.
20. Zu, K. *et al.* (2013) Protein expression of PTEN, insulin-like growth factor I receptor (IGF-IR), and lethal prostate cancer: a prospective study. Cancer Epidemiol. Biomarkers Prev., 22, 1984–1993.
21. Ahearn, T.U. *et al.* (2016) A prospective investigation of PTEN loss and ERG expression in lethal prostate cancer. J. Natl Cancer Inst., 108, pii: djv346.
22. Pettersson, A. *et al.* (2012) The *TMPRSS2:ERG* rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. Cancer Epidemiol. Biomarkers Prev., 21, 1497–1509.
23. Pollak, M. (2008) Targeting insulin and insulin-like growth factor signalling in oncology. Curr. Opin. Pharmacol., 8, 384–392.
24. Roddam, A.W. *et al.* (2008) Insulin-like growth factors, their binding proteins, and prostate cancer risk: analysis of individual patient data from 12 prospective studies. Ann. Intern. Med., 149, 461–471, W83.
25. Rowlands, M.A. *et al.* (2009) Circulating insulin-like growth factor peptides and prostate cancer risk: a systematic review and meta-analysis. Int. J. Cancer, 124, 2416–2429.
26. Travis, R.C. *et al.* (2016) A meta-analysis of individual participant data reveals an association between circulating levels of IGF-I and prostate cancer risk. Cancer Res., 76, 2288–2300.
27. Cao, Y. *et al.* (2015) Prediagnostic plasma IGFBP-1, IGF-1 and risk of prostate cancer. Int. J. Cancer, 136, 2418–2426.
28. Rowlands, M.A. *et al.* (2012) Serum insulin-like growth factors and mortality in localised and advanced clinically detected prostate cancer. Cancer Causes Control, 23, 347–354.
29. Allen, N.E. *et al.* (2007) Serum insulin-like growth factor (IGF)-I and IGF-binding protein-3 concentrations and prostate cancer risk: results from the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol. Biomarkers Prev., 16, 1121–1127.
30. Chan, J.M. *et al.* (2002) Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. J. Natl Cancer Inst., 94, 1099–1106.
31. Cao, Y. *et al.* (2014) Insulin-like growth factor pathway genetic polymorphisms, circulating IGF1 and IGFBP3, and prostate cancer survival. J. Natl Cancer Inst., 106, dju085.
32. Lubik, A.A. *et al.* (2013) IGF2 increases de novo steroidogenesis in prostate cancer cells. Endocr. Relat. Cancer, 20, 173–186.

33. Heidegger, I. *et al.* (2014) Oncogenic functions of IGF1R and INSR in prostate cancer include enhanced tumor growth, cell migration and angiogenesis. Oncotarget, 5, 2723–2735.
34. Heidegger, I. *et al.* (2012) Diverse functions of IGF/insulin signaling in malignant and noncancerous prostate cells: proliferation in cancer cells and differentiation in noncancerous cells. Endocrinology, 153, 4633–4643.
35. Ofer, P. *et al.* (2015) Both IGF1R and INSR knockdown exert antitumorigenic effects in prostate cancer *in vitro* and *in vivo*. Mol. Endocrinol., 29, 1694–1707.
36. Lai, G.Y. *et al.* (2014) Association of C-peptide and leptin with prostate cancer incidence in the Health Professionals Follow-up Study. Cancer Causes Control, 25, 625–632.
37. Lai, G.Y. *et al.* (2010) Association between C-peptide concentration and prostate cancer incidence in the CLUE II cohort study. Cancer Prev. Res. (Phila.), 3, 1334–1341.
38. Stocks, T. *et al.* (2007) Insulin resistance is inversely related to prostate cancer: a prospective study in Northern Sweden. Int. J. Cancer, 120, 2678–2686.
39. Belfiore, A. *et al.* (2009) Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. Endocr. Rev., 30, 586–623.
40. Wu, J.D. *et al.* (2006) Interaction of IGF signaling and the androgen receptor in prostate cancer progression. J. Cell Biochem., 99, 392–401.
41. Sayeed, A. *et al.* (2012) Insulin-like growth factor 1 stimulation of androgen receptor activity requires β(1A) integrins. J. Cell. Physiol., 227, 751–758.
42. Mancarella, C. *et al.* (2015) ERG deregulation induces IGF-1R expression in prostate cancer cells and affects sensitivity to anti-IGF-1R agents. Oncotarget, 6, 16611–16622.
43. Martins, A.S. *et al.* (2008) A pivotal role for heat shock protein 90 in Ewing sarcoma resistance to anti-insulin-like growth factor 1 receptor treatment: *in vitro* and *in vivo* study. Cancer Res., 68, 6260–6270.
44. Rikhof, B. *et al.* (2009) The insulin-like growth factor system and sarcomas. J. Pathol., 217, 469–482.
45. Magi-Galluzzi, C. *et al.* (2011) *TMPRSS2-ERG* gene fusion prevalence and class are significantly different in prostate cancer of Caucasian, African-American and Japanese patients. Prostate, 71, 489–497.