An Analysis of Growth, Differentiation and Apoptosis Genes with Risk of Renal Cancer

Linda M. Dong1*, Paul Brennan2, Sara Karami1, Rayjean J. Hung3, Idan Menashe1, Sonja I. Berndt1, Meredith Yeager4, Stephen Chanock4, David Zardzie5, Vsevolod Matveev4, Vladimir Janout6, Hellena Kollarova6, Vladimir Bencko7, Kendra Schwartz8, Faith Davis9, Marie Navratilova10, Neonia Szemes-Dabrowska11, Dana Mates12, Joanne S. Colt1, Ivana Holcatova7, Paolo Boffetta3, Nathaniel Rothman1, Wong-Ho Chow1, Philip S. Rosenberg1, Lee E. Moore1

1 Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, United States of America, 2 International Agency for Research on Cancer, Lyon, France, 3 Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, Canada, 4 Core Genotyping Facility, National Cancer Institute, Gaithersburg, Maryland, United States of America, 5 Institute of Carcinogenesis, Moscow, Russia, 6 Palacky University, Olomouc, Czech Republic, 7 Institute of Hygiene and Epidemiology, Prague, Czech Republic, 8 Department of Family Medicine and Public Health Sciences and Karmanos Cancer Institute, Wayne State University, Detroit, Michigan, United States of America, 9 Department of Biostatistics and Epidemiology, University of Illinois at Chicago, Chicago, Illinois, United States of America, 10 Masaryk Memorial Cancer Institute, Brno, Czech Republic, 11 Institute of Occupational Medicine, Lodz, Poland, 12 Institute of Public Health, Bucharest, Romania

Abstract

We conducted a case-control study of renal cancer (987 cases and 1298 controls) in Central and Eastern Europe and analyzed genomic DNA for 319 tagging single-nucleotide polymorphisms (SNPs) in 21 genes involved in cellular growth, differentiation and apoptosis using an Illumina Oligo Pool All (OPA). A haplotype-based method (sliding window analysis of consecutive SNPs) was used to identify chromosome regions of interest that remained significant at a false discovery rate of 10%. Subsequently, risk estimates were generated for regions with a high level of signal and individual SNPs by unconditional logistic regression adjusting for age, gender and study center. Three regions containing genes associated with renal cancer were identified: caspase 1/5/4/12(CASP1/5/4/12), epidermal growth factor receptor (EGFR), and insulin-like growth factor binding protein-3 (IGFBP3). We observed that individuals with CASP1/5/4/12 haplotype (spanning area upstream of CASP1 through exon 2 of CASP5) GGGCTCAGT were at higher risk of renal cancer compared to individuals with the most common haplotype (OR:1.40, 95% CI:1.10–1.78, p-value = 0.007). Analysis of EGFR revealed three strong signals within intron 1, particularly a region centered around rs759158 with a global p = 0.006 (GGG: OR:1.26, 95% CI:1.04–1.53 and ATG: OR:1.55, 95% CI:1.14–2.11). A region in IGFBP3 was also associated with increased risk (global p = 0.04). In addition, the number of statistically significant (p-value<0.05) SNP associations observed within these three genes was higher than would be expected by chance on a gene level. To our knowledge, this is the first study to evaluate these genes in relation to renal cancer and there is need to replicate and extend our findings. The specific regions associated with risk may have particular relevance for gene function and/or carcinogenesis. In conclusion, our evaluation has identified common genetic variants in CASP1, CASP5, EGFR, and IGFBP3 that could be associated with renal cancer risk.

Citation: Dong LM, Brennan P, Karami S, Hung RJ, Menashe I, et al. (2009) An Analysis of Growth, Differentiation and Apoptosis Genes with Risk of Renal Cancer. PLoS ONE 4(3): e4895. doi:10.1371/journal.pone.0004895

Editor: Amanda Ewart Toland, Ohio State University Medical Center, United States of America

Received November 24, 2008; Accepted February 19, 2009; Published March 24, 2009

This is an open-access article distributed under the terms of the Creative Commons Public Domain declaration which stipulates that, once placed in the public domain, this work may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose.

Funding: This work was supported in part by the Intramural Research Program of the US National Institutes of Health, National Cancer Institute, Division of Cancer Epidemiology and Genetics. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: donglm@mail.nih.gov

Introduction

Renal cancer is among the most commonly diagnosed cancers in men and women in the United States [1] and Eastern Europe [2]. The incidence of renal cell carcinoma (RCC), the most common malignancy of renal cancer, has increased rapidly worldwide over the past few decades [3,4] with some of the highest rates occurring in Central and Eastern Europe [2,5]. Only a few well-established lifestyle risk factors have been identified: cigarette smoking, obesity, hypertension and diabetes [6]. An increased risk observed among those with a family history of renal cancer and the identification of inherited forms of kidney cancer provide justification for evaluating the genetic susceptibility of this disease, which has not been fully investigated [6].

The mechanism by which a normal cell progresses to carcinoma customarily involves the disruption of critical molecular pathways in cellular growth, differentiation, and development [7]. Among the steps required for tumor cell growth and survival are the amplification of signals from growth factors and the interruption of signals promoting cell death or apoptosis [8,9]. Alterations in genes involved in such pathways are thus likely to contribute to cancer risk. Based on this logic, we identified genes involved in cell growth and differentiation (AKT1, EGF, EGFR, IGFBP3, IGFBP5, PPARG, TGFA, VCAM1, and VEGF) and apoptosis (CASP1, CASP2, CASP3, CASP4, CASP5, CASP6, CASP7, CASP8, CASP9, CASP10, CASP12, and CASP14; Table 1). Several of these genes have been associated with risk of cancer at other sites [10,11]; however, the role of these genes in the development of renal cancer remains unknown.
Table 1. Description of selected genes and tagging SNPs.

| Name of Gene | Function | Chromosome Location | Number of tagSNPs/1/ p-value min P | Adjusted p-value |
|--------------|----------|---------------------|----------------------------------|-----------------|
| **Growth and Differentiation Genes** | | | | |
| AKR1C3 - Aldo-keto Reductase Family 1, Member C3 | Catalyzes the reduction of prostaglandin (PGD). Role in cell differentiation by diverting conversion of PGD from PGJ2 to PGF2. | 10p15-p14 | 17/1 | 0.57 |
| EGF - Epidermal Growth Factor Receptor | A transmembrane growth factor receptor. EGF binding leads to the activation of major signal transduction pathways involved in regulating cell proliferation, differentiation, migration and survival. | 7p12 | 65/12* | 0.48 |
| EGF - Epidermal Growth Factor | EGF is a mitogenic factor and an important ligand for EGFR. Subsequently has a major effect on cell differentiation. | 4q25 | 16/0 | 0.90 |
| IGFBP3 - Insulin-like Growth Factor Binding Protein 3 | Main carrier of circulating IGFs. Independently, has a key role in regulating cell proliferation and apoptosis. | 7p13-p12 | 14/3* | 0.23 |
| IGFBP5 - Insulin-like Growth Factor Binding Protein 5 | Another carrier for IGF. Role in regulating cell survival, differentiation, and apoptosis. | 2q33-q36 | 17/1 | 0.34 |
| PPARG - Peroxisome Proliferator-activated Receptor Gamma | A nuclear hormone receptor. When dimerizes with retinoid X receptor, regulates transcription of numerous genes. Also a regulator of adipocyte differentiation. | 3p25 | 26/1 | 0.66 |
| TGFA - Transforming Growth Factor, alpha | Growth factor that competes with EGF in binding to EGFR | 2p13 | 39/1 | 0.76 |
| VCAM1 - Vascular Cell Adhesion Molecule 1 | Member of the immunoglobulin family and encodes a cell surface glycoprotein expressed by cytokine-activated endothelial cells | 1p32-p31 | 14/1 | 0.41 |
| VEGF - Vascular Endothelial Growth Factor A | Important growth factor in angiogenesis and tumor growth | 6p12 | 21/1 | 0.78 |
| **Apoptosis Genes** | | | | |
| CASP1/5/6/12 | A member of the cysteine-aspartic acid protease (caspase) family which plays an integral role in a complex cascade of events that regulate cell apoptosis | 11q22.2-22.3 | 24/5* | 0.26 |
| CASP2 | Member of the caspase family | 7q34-q35 | 6/0 | 0.90 |
| CASP3 | Member of the caspase family | 4q34 | 10/0 | 0.83 |
| CASP6 | Member of the caspase family | 4q25 | 7/0 | 0.77 |
| CASP7 | Member of the caspase family | 10q25 | 16/0 | 0.85 |
| CASP8/10 | Member of the caspase family | 2q33-q34 | 12/0 | 0.85 |
| CASP9 | Member of the caspase family | 1p36.3-p36.1 | 7/0 | 0.96 |
| CASP14 | Member of the caspase family | 19p13 | 8/1 | 0.25 |

1/rs numbers for SNPs can be found in supplementary table 1.
*TagSNPs with p-value for trend <0.05.
1/indicates genes where number of tagSNPs with p-value for trend <0.05 is more than one would expect to see by chance.

Given the importance of these pathways in carcinogenesis and the lack of studies evaluating genetic susceptibility and renal cancer, we evaluated whether polymorphisms in these 21 genes could alter the risk for developing renal cancer in a large multicenter case-control study based in Central and Eastern Europe. We hypothesized that common variation in genes involved in cellular growth, differentiation and apoptosis may increase genetic susceptibility to renal cancer.

**Methods**

**Study Population**

The Central and Eastern European Renal Cancer (CEERC) Study is a hospital-based case-control study of renal cancer (1,097 cases and 1,555 controls) that was conducted in seven centers in Eastern and Central Europe (Moscow, Russia; Bucharest, Romania; Lodz, Poland; and Prague, Olomouc, Ceske Budejovice and Brno, Czech Republic). Details of the study have been described previously [12]. Newly diagnosed and histologically confirmed cases of renal cancer (ICD-0-2 code C64) between the ages of 20 and 79 years were recruited from August 1999 through January 2003. Trained medical staff reviewed medical records and extracted information on date and method of diagnosis, histological classification, tumor location, stage and grade. Pathology data was available for 917 cases. RCC was defined as the following subtypes: clear cell, clear cell with papillary features, clear cell with sarcomatoid, papillary type I, papillary non-type I, papillary type II, chromophobe and hybrid subtype (n = 848). Clear cell renal cancer was defined as the first three clear cell subtypes (n = 760). Eligible controls were chosen from among patients admitted to the same hospital as cases for conditions unrelated to smoking or genitourinary disorders (except for benign prostatic hyperplasia) and were frequency-matched to cases on age (within 3 years), sex, and study center. Among controls, the disease conditions associated with hospitalization were the following: obstructive or perinatal (0.1%), infectious (1%), psychiatric (1%), endocrine (2%), hematologic (3%), dermatologic (3%), injury or poisoning (3%), genitourinary (benign prostatic hyperplasia (4%), pulmonary (4%), orthopedic or rheumatologic (9%), cardiovascular (10%), neurologic (11%), ophthalmologic or otorlogic (14%),...
gastrointestinal (19%), and other (16%). No single disease made up more than 20% of the control group. A portion of the controls were also recruited for a parallel study of lung cancer. All recruited cases and controls were Caucasian. Response rates at each center ranged from 90.0 to 90.6% for cases and from 90.3 to 96.1% for controls.

Interviews were conducted by trained personnel to collect standardized lifestyle and food frequency questionnaires. Data was collected on demographic characteristics, education, tobacco smoke exposures, alcohol consumption, dietary practices, anthropometry, medical history, family history, and occupational history.

Blood samples were collected and stored at approximately -80°C and shipped to the National Cancer Institute (NCI). Genomic DNA was extracted from whole blood buffy coat by the standard phenol chloroform method at the NCI laboratory. All subjects in this study provided written informed consent. This study was approved by the institutional review boards (IRB) at the NCI, International Agency for Research on Cancer (IARC), and each participating center.

Genotyping

We analyzed 319 tagging single-nucleotide polymorphisms (SNPs) in 21 genes involved in cell growth, differentiation (AKR1C3, EGFR, EGF, IGFBP3, IGFBP5, PPARG, TGFα, VCA1, VEGF) and apoptosis (CASP1, CASP3, CASP4, CASP12, CASP2, CASP5, CASP6, CASP7, CASP8, CASP10, CASP9, CASP11) that we hypothesized could increase RCC risk (Table 1). Since several of the caspase genes were located relatively close to each other, tagSNPs were selected from among common variants (minor allele frequencies ≥5%) found in Caucasians using a tagSNP method [13] with an r²>0.80 to provide high genomic coverage. In addition, important nonsynonymous SNPs or those with potential functional significance were included. All SNPs are reported in the NCI SNP500Cancer database (http://snp500cancer.nci.nih.gov)[14]. Genotyping was conducted at NCI’s Core Genotyping Facility where staff was blinded to case/control status and duplicate quality control samples (5% samples) interspersed among plates. All genotyping was performed using an Illumina GoldenGate® Oligo Pool All (OPA) assay, which was designed using publicly available sequencing information. The genotype frequencies among controls showed no deviation from the expected Hardy-Weinberg equilibrium proportions (p>0.05). All SNPs had a genotyping completion rate >98% except for rs3770472 (96%). The quality control concordance rates were >97% for all SNPs except for rs10885493 (92%), rs12416109 (92%), rs10752001 (94%), and rs13392762 (94%).

Replication Study

The US Kidney Cancer Study is a population-based case-control study conducted in Detroit and Chicago. Cases were residents of the study areas, aged 20 to 79 years who were newly diagnosed with histologically confirmed renal cell carcinoma (ICD-O2 C64.9) from February 2002 through January 2007. Controls were frequency-matched to cases by study center, race, age, and sex. Controls aged 63 years and older were identified from Medicare files, and those under age 63 years were identified from Division of Motor Vehicle records. African American cases and controls were over-sampled. Written informed consent was obtained from all participants, and IRB approvals were obtained from all participating study centers.

Participants were interviewed by trained interviewers to elicit information on demographic factors, use of tobacco and alcohol, diet, occupational history, height and weight history, family history of cancer, reproductive history among women, medical history, and medication history including the use of diet pills and antihypertensives. A total of 1568 Caucasians (856 cases and 712 controls) and 804 African-Americans (523 cases and 361 controls) were interviewed. Of these subjects, 1109 cases and 1106 controls provided DNA that was extracted using standard procedures. Genotyping data was available for 966 cases and 977 controls with sufficient quality and quantity of DNA. Subjects were predominantly recruited from the Detroit area (84%), and were similar in age (76%>50 years) and sex (57% male) to those in the CEERC study.

Statistical Analyses

Of the 987 cases and 1298 controls that had valid study data and provided genomic DNA, analyses were based on the 777 cases and 1035 controls that had adequate quality DNA and were successfully genotyped on the OPA platform in the CEERC study. Associations were evaluated through several methods. Global p-values were evaluated using the minimum-p value permutation test [15]. A haplotype-based method called HaploWalk, conducted in Matlab, was used to identify chromosome regions of interest by examining regional associations rather than effects from an individual SNP. For a gene with K SNPs, the HaploWalk procedure considered a 3 SNP sliding window for each SNP from SNP 2 through SNP K-1. To account for multiple testing across the K SNPs, the K-2 p-values (one for each window) were adjusted for multiple comparisons using the False Discovery Rate (FDR)-controlling procedure of Benjamini and Hochberg [16]. Windows that remained significant at a FDR level of 10% were considered to be a candidate region of interest. If adjacent windows were significant, they were amalgamated into a single candidate region of interest. Haplotypes in the candidate block were then reconstructed and effects evaluated using Haplostats (Version 1.3.1) in R (version 2.4.1). The most common haplotype was used as the reference group and haplotypes with frequencies less than 1% were combined into one category for testing. Subsequently, unadjusted and adjusted (age, sex, and study center) odds ratios (OR) and 95% confidence intervals (95% CI) using the log-additive model were generated for regions with a high level of signal.

The association between individual SNPs and risk of renal cancer were estimated by unconditional logistic regression, adjusted for age, sex, and study center. Genotypes were evaluated by coding the homozygous common allele as the referent group and separately comparing the heterozygous and homozygous rare allele genotypes to the referent group. Linear tests for trends were conducted by including a variable coded 0, 1, and 2 corresponding to the number of rare alleles. Associations for SNPs were considered robust if they were significant (based on the p-value of the test for trend) with a FDR level of 20% or less. A more liberal FDR level was chosen at this stage of analysis in order to guide us toward SNPs that may be of interest within previously identified regions of interest. FDR adjustment was based on the number of SNPs within each gene region. Additional adjustment for potential confounders (body mass index [BMI], self-reported hypertension, and smoking) did not result in meaningful changes of the risk estimates and were not included in the analyses. In addition, we investigated multiplicative interaction between individual SNPs and age, sex, and BMI, using the likelihood ratio test to compare the fit of models with and without interaction terms. Heterogeneity of genotype frequencies among countries was evaluated by using the likelihood ratio test to compare the fit of models with and without interaction terms, but we did not find
any evidence of heterogeneity. Analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC).

Results

A large proportion of the study population was from the Czech Republic, with a slightly higher proportion among cases (Table 2). Controls were more likely to be male, but were similar to cases in age distribution. Cases were more likely than controls to have higher BMI, have a family history of cancer, and report hypertension.

Results from global gene-based tests of association are included in Table 1. Among results from the minimum p-value test, CASP1/5/4/12, CASP14, and IGFBP3 were the most promising gene regions, but were not significant after adjustment for multiplicity (total number of SNPs) over the entire gene (Table 1). However, regions, but were not significant after adjustment for multiplicity window method, we identified the same genes with regions that were associated with renal cancer risk at a FDR level. In addition, with a haplotype-based sliding window method, we identified the same genes with regions that were associated with renal cancer risk at a FDR level <10%: 0.05) than one would expect to see by chance. In addition, with a haplotype-based sliding window method, we identified the same genes with regions that were associated with renal cancer risk at a FDR level <10%:

| Table 2. Distribution of demographic variables among subjects in the Central and Eastern European Renal Cancer study. |
|-----------------|-----------------|-----------------|-----------------|
|                  | Cases           | Controls        | p-value         |
|-----------------|-----------------|-----------------|-----------------|
| N               | %               | N               | %               |<0.0001          |
| All subjects    | 987             | 1298            |
| Center          |                 |                 |                 |
| Bucharest, Romania | 91             | 132             | 10.2            |
| Lodz, Poland    | 81              | 197             | 15.2            |
| Moscow, Russia  | 288             | 368             | 28.4            |
| Czech Republic1 | 527             | 601             | 46.3            |
| Sex             |                 |                 |                 |
| Male            | 589             | 838             | 64.6            |
| Female          | 398             | 460             | 35.4            | 0.02            |
| Age at Interview (y) |             |                 |                 |
| <50             | 163             | 228             | 17.6            |
| ≥50             | 824             | 1070            | 82.4            | 0.51            |
| Smoking Status  |                 |                 |                 |
| Never           | 454             | 528             | 40.7            |
| Former          | 225             | 316             | 24.4            |
| Current         | 305             | 452             | 34.9            | 0.03            |
| Body Mass Index (kg/m²) |             |                 |                 |
| <25             | 288             | 457             | 35.4            |
| 25–30           | 429             | 556             | 43.1            |
| 30+             | 270             | 278             | 21.5            | 0.001           |
| Family history of cancer2 |             |                 |                 |
| No              | 654             | 932             | 71.8            |
| Yes             | 333             | 366             | 28.2            | 0.004           |
| Self-Reported Hypertension |             |                 |                 |
| No              | 539             | 800             | 61.7            |
| Yes             | 447             | 497             | 38.3            | 0.001           |

1Four centers: Brno, Olomuc, Prague, and Ceske.
2First degree relative with any cancer.
doi:10.1371/journal.pone.0004895.t002

An interesting region was detected that spans over the area upstream of CASP1 through exon 2 of CASP5 (Supplementary Figure S1). At this region, individuals with a specific variant haplotype GGCTCAGT (OR: 1.40; 95% CI: 1.10–1.70) had a 1.4 fold higher risk of renal cancer compared to those with the most common haplotype (Table 3). Concordant with the haplotype analysis, several individual variants within this haplotype also had nominal statistically significant associations with renal cancer risk (Table 4). After applying FDR adjustment, four CASP5 and CASP5 SNPs (rs1785883, rs568910, rs4928539 and rs507879) were considered significant at a FDR level <20%. The strongest association among individual SNPs was rs507879 (Thr90Ala), located in exon 2 of CASP5. The ORs (95% CI) for heterozygote and homozygote rare genotypes compared to the homozygote common genotypes were 1.29 (1.03–1.60) and 1.39 (1.07–1.82; p-value for trend = 0.01), respectively. The OR p-value of the specific variant haplotype were stronger than the associations (p-value for trend) observed for any of the individual SNPs in this region, suggesting that the causal variant within this haplotype may not have been genotyped.

We had the opportunity to conduct a quick replication of our most statistically significant finding, CASP5 SNP rs507879 in the US Kidney Cancer Study population (Table 5). Although results from the US Kidney Cancer Study were not statistically significant, the point estimates were in the same direction as those from the CEERC study. A pooled estimate of 1.22 (95% CI: 1.04–1.42) was observed for those with at least one copy of the rare allele of rs507879 among Caucasian participants. A pooled estimate including both Caucasians and African-Americans from both studies was not noticeably different from the estimate restricted to Caucasians (OR: 1.29, 95% CI: 1.05–1.41; Table 5).

A sliding window analysis over EGFR revealed three signals within intron 1 (Supplementary Figure S2). In particular, two haplotypes centered on rs759158 (region 3) were associated with a higher risk of renal cancer (GGG: OR: 1.26, 95% CI: 1.04–1.53 and ATG: OR: 1.55, 95% CI: 1.14–2.25) when compared to the common haplotype (Table 3). In the second EGFR region, variant haplotype TGA was associated with an increased risk of renal cancer compared to the common haplotype (OR: 1.32, 95% CI: 1.02–1.70), (Table 4). The ORs (95% CI) for heterozygote and homozygote rare genotypes compared to the homozygote common genotypes were 1.29 (1.03–1.60) and 1.39 (1.07–1.82; p-value for trend = 0.01), respectively. The OR p-value of the specific variant haplotype were stronger than the associations (p-value for trend) observed for any of the individual SNPs in this region, suggesting that the causal variant within this haplotype may not have been genotyped.

Among common haplotypes, the effect estimates for haplotypes containing GGG or GTG appear to be consistently above 1.0. It is interesting to note that among common haplotypes in the integrated region, the variant haplotype TGA from the second region is present only with either variant haplotype GGG or GTG, the statistically significant haplotypes from the third region. This suggests that the two sets of haplotypes may be reflecting the same signal. A strong haplotype effect was observed for the variant haplotype TGA-A-GGG, with an OR of 1.84 (95% CI: 1.25–2.71) and a p-value of 0.002. This effect was stronger than those observed for the individual regions and reinforces the idea that these two regions are related. A second variant haplotype in the integrated region was also statistically significant (OR:1.60; 95%
| Gene/Haplotypes | Cases (%) | Controls (%) | OR | 95% CI | Unadjusted p-value | Adjusted p-value |
|----------------|-----------|--------------|----|--------|-------------------|-----------------|
| **CASP1/5/4/12 haplotype** | | | | | | |
| Region 1 (chr1: 104429456-104390522) | | | | | | |
| G-G-T-T-A-C-G-T | 41.8 | 45.0 | 1.00 | | | |
| G-G-T-C-A-C-A-C | 28.1 | 26.7 | 1.14 | 0.97–1.34 | 0.12 | |
| G-G-G-C-T-C-A-G-C | 10.6 | 8.1 | 1.40 | 1.10–1.78 | 0.007 | |
| A-G-G-T-T-A-C-G-T | 5.5 | 7.4 | 0.80 | 0.60–1.07 | 0.13 | |
| Global p-value | 0.14 | | | | | |
| **EGFR haplotype** | | | | | | |
| Region 1 (chr7 55122130-55123913) | | | | | | |
| C-G-T | 36.8 | 36.0 | 1.00 | | | |
| C-A-A | 21.8 | 24.7 | 0.87 | 0.73–1.03 | 0.09 | |
| T-G-T | 20.1 | 21.2 | 0.93 | 0.78–1.12 | 0.20 | |
| C-G-A | 21.4 | 18.1 | 1.13 | 0.94–1.36 | 0.40 | |
| Global p-value | 0.04 | | | | | |
| Region 2 (chr 7 55129830-55138684) | | | | | | |
| C-G-G | 29.5 | 29.9 | 1.00 | | | |
| T-A-G | 25.0 | 25.9 | 0.99 | 0.82–1.18 | 0.68 | |
| T-G-G | 16.7 | 17.3 | 1.00 | 0.79–1.26 | 0.98 | |
| C-A-G | 11.3 | 12.1 | 0.94 | 0.72–1.24 | 0.67 | |
| T-G-A | 11.1 | 8.5 | 1.32 | 1.02–1.70 | 0.03 | |
| C-G-A | 6.4 | 6.4 | 1.07 | 0.76–1.52 | 0.70 | |
| Global p-value | 0.31 | | | | | |
| Region 3 (chr 7 55143370-55147338) | | | | | | |
| A-T-G | 34.4 | 38.4 | 1.00 | | | |
| A-G-G | 22.6 | 22.7 | 1.11 | 0.91–1.34 | 0.30 | |
| G-G-G | 20.8 | 18.7 | 1.26 | 1.04–1.53 | 0.02 | |
| A-T-A | 12.3 | 11.8 | 1.14 | 0.90–1.43 | 0.28 | |
| G-T-G | 9.7 | 7.0 | 1.55 | 1.14–2.11 | 0.005 | |
| Global p-value | 0.007 | | | | | |
| **IGFBP3 haplotype** | | | | | | |
| Region 1 (chr 7 45940583-45921554) | | | | | | |
| T-A-A-T-C-A-G | 38.5 | 38.1 | 1.00 | | | |
| T-A-A-G-C-C-A-A | 19.8 | 21.5 | 0.94 | 0.78–1.13 | 0.52 | |
| T-A-A-T-C-C-A-G | 15.3 | 17.9 | 0.87 | 0.71–1.05 | 0.15 | |
| T-A-G-T-C-G-A-A | 19.6 | 16.9 | 1.17 | 0.98–1.41 | 0.08 | |
| T-G-A-T-T-C-A-G | 3.6 | 3.3 | 1.07 | 0.73–1.58 | 0.73 | |
| A-A-G-T-C-G-A-A | 1.3 | 1.0 | 1.13 | 0.60–2.11 | 0.70 | |
| Global p-value | 0.19 | | | | | |
| Region 2 (chr 7 45918779-45916095) | | | | | | |
| T-G-C | 48.6 | 51.3 | 1.00 | | | |
| A-G-C | 18.6 | 15.2 | 1.27 | 1.06–1.54 | 0.01 | |
| T-G-T | 13.8 | 15.4 | 0.93 | 0.75–1.16 | 0.54 | |
| T-A-C | 14.3 | 14.5 | 1.02 | 0.82–1.27 | 0.83 | |
| T-A-T | 4.7 | 3.1 | 1.62 | 1.05–2.51 | 0.03 | |
| Global p-value | 0.03 | | | | | |
| **VCAM1 haplotype** | | | | | | |
| Region 1 (chr 1: 100961998-100966793) | | | | | | |
| C-A-C | 45.3 | 45.9 | 1.00 | | | |
| C-C-C | 18.8 | 17.4 | 1.10 | 0.89–1.35 | 0.40 | |
| C-A-T | 20.3 | 16.5 | 1.25 | 1.01–1.54 | 0.04 | |
Table 3. cont.

| Gene/Haplotypes | Cases (%) | Controls (%) | OR¹ | 95% CI  | Unadjusted p-value | Adjusted p-value¹ |
|-----------------|-----------|--------------|------|---------|--------------------|------------------|
| T-A-C           | 8.3       | 10.6         | 0.81 | 0.63–1.04 | 0.09               |                  |
| C-C-T           | 6.1       | 8.6          | 0.75 | 0.55–1.02 | 0.07               |                  |
| Global p-value  |           |              | 0.02 |         | 0.03               |                  |

SNPs included within haplotype regions:
- CASP1/3/4/12: region 1: rs1785883, rs508760, rs7934239, rs501626, rs11821722, rs568910, rs492859, rs3181318, rs507879.
- EGFR: region 1: rs759169, rs11238349, rs12535226; region 2: rs11977660, rs6593025, rs6954351; region 3: rs7796139, rs759158, rs7796872.
- IGFBP3: region 1: rs10235181, rs13232606, rs2453836, rs903889, rs924140, rs2471551, rs9282734, rs3110697; region 2: rs6670, rs13223993, rs2270628.
- VCAM1 chr1: region 1: rs3917009, rs3917010, rs3176867.

¹ Adjusted for age, sex, and center.

doi:10.1371/journal.pone.0004895.t003

CI: 1.09–2.37, but we were unable to determine what was driving this association.

For IGFBP3, a large region across the gene was considered noteworthy using a sliding window analysis (Supplementary Figure S3). Two regions were defined by evaluating linkage disequilibrium across the identified area. The second region, spanning the area of exon 5 to 3’ downstream of IGFBP3, was associated with a global p-value of 0.04. Among haplotypes in this region, variant haplotype AGC (OR: 1.27, 95% CI: 1.06–1.54) and TAT (OR: 1.62, 95% CI: 1.05–2.53) were associated with increased renal cancer risk (Table 3). Among SNPs in the haplotype, rs6670 was statistically significantly associated with renal cancer risk at a FDR level <20%. We observed a positive association between renal cancer risk among subjects that had at least one copy of the rare allele with an OR of 1.27 (95% CI: 1.04–1.56). The association for haplotype AGC, which contains the rare allele for rs6670, was slightly stronger than the effect observed for the individual SNP and appears to be driven primarily by rs6670. The causal variant for haplotype TAT, however, is not apparent, suggesting that the causal variant was not genotyped in this study.

In VCAM1, a variant haplotype centered on rs3917010 was also associated with an increased risk of renal cancer (CAT OR: 1.25, 95% CI: 1.01–1.54; Table 3). However, none of the VCAM1 SNPs were significantly associated with renal cancer risk after FDR adjustment. Although a statistically significant association was observed, this association could be spurious as the effects observed for the haplotype are not concordant with the individual SNP associations within this haplotype (Supplementary Table S1). Results for individual analyses of all SNPs can be found in Supplemental Table S1. No statistically significant interactions between our statistically significant SNPs and potential effect modifiers (age, sex, and BMI) were detected (data not shown). Additional sensitivity analyses restricted to RCC (n = 627 cases) and clear cell RCC (n = 564 cases) did not meaningfully change any of the previously detected associations (data not shown).

Discussion

In this study, we conducted an exploratory analysis of 319 SNPs in or around 21 genes involved in cell growth/differentiation and apoptosis pathways in relation to renal cancer risk. We identified both haplotypes and SNPs in CASP1/3/4/12, EGFR, and IGFBP3 that were statistically significantly associated with risk of renal cancer. Associations between SNPs in the other investigated cell growth/differentiation and apoptosis pathway genes were weak and less promising.

There is strong evidence supporting the biological relevance of genetic variants in EGFR and IGFBP3 and renal cancer risk. EGFR encodes for a transmembrane growth factor receptor that plays a critical role in the signal transduction pathway regulating cell proliferation, differentiation, and survival [17,18]. A recent study has proposed an additional role for EGFR of interacting with and stabilizing the sodium/glucose cotransporter 1 (SGLT1), thus helping to maintain intracellular glucose levels in low extracellular glucose environments and prevent cell death from occurring [19]. This is especially relevant to renal cancer, as both EGFR and SGLT1 are expressed in the kidney, where glucose uptake is important [20]. Altered glucose metabolism is one of the major hypotheses thought to explain the association between diabetes and renal cancer. Thus far, most studies have focused on evaluating EGFR in relation to cancer progression and targeted treatment [21,22]. It is interesting to note that the first intron of EGFR (>120 kb) has been implicated as an important regulatory area [21,23]. A highly polymorphic (CA)n repeat, in repeat 1 of EGFR, about 1.5 kb downstream of exon 1, has been associated with decreased EGFR transcription in multiple studies [24,25]. This microsatellite appears to be in linkage disequilibrium with several SNPs of unknown function in the promoter region of this gene, as well[26]. One of these variants (rs759171) was also genotyped in this study, but not associated with renal cancer risk (Supplementary Table S1). In this study, three SNPs (rs11238349, rs6954351, and rs7796139) from intron 1 of EGFR and identified through our initial screen were statistically significantly associated with risk of renal cancer. Among these three SNPs, only rs6954351 and rs7796139 were moderately correlated (r² = 0.47) with one another. Subsequent analyses suggest that perhaps a haplotype that includes these two SNPs may be driving the association found in this region. The mechanism through which these intronic SNPs (or variants in linkage disequilibrium with these SNPs) might affect renal cancer risk is unknown but they do reside within a functionally relevant region of EGFR that has been associated with decreased EGFR transcription and protein expression in humans.

Similar to our findings for EGFR, the IGFBP3 regions associated with modified risk appear to be functionally important in cancer. IGFBP3 encodes for IGF-binding protein 3 and is the primary carrier of circulating IGF-1. A reduction in the amount of IGFBP3 available results in an increase in levels of free IGF-1, a factor associated with growth, proliferation, and an elevated risk of several cancers [27,28]. Independent of IGF-1, IGFBP3 has also been shown to affect cell proliferation and apoptosis through its interactions with several signaling pathways [29,30]. In relation to renal cancer, experimental studies have demonstrated that IGFBP3 expression is increased among both clear cell renal tumors and renal cancer cell lines [31,32]. The promoter region of IGFBP3 has also been observed to be frequently hypermethylated in primary renal cell tumors, but unmethylated among normal cells [33]. We

PLoS ONE | www.plosone.org 6 March 2009 | Volume 4 | Issue 3 | e4895
observed a statistically significant increase in renal cancer risk with rs6670 located in the 3' untranslated region (UTR) of *IGFBP3*. Variants in the 3'UTR could be involved in the stability and

| Table 4. Association between Selected Polymorphisms and Renal Cancer Risk. |

| SNP/Genotypes | Cases | Controls | OR | 95% CI | p-trend |
|---------------|-------|----------|----|--------|---------|
| CASP 1/5/4/12 |       |          |    |        |         |
| CASP1 rs1785883 (12058T>C) |       |          |    |        |         |
| GG            | 688   | 880      |     | 1.00   |         |
| AG            | 85    | 145      |     | 0.75   | 0.56    | 1.00    |
| AA            | 2     | 8        |     | 0.34   | 0.07    | 1.60    | 0.02   |
| AG+AA         |       |          |    | 0.75   | 0.57    | 1.00    |        |
| rs6670 (Ex5-411A>T) |       |          |    |        |         |
| TT            | 514   | 741      |     | 1.00   |         |
| CT            | 314   | 405      |     | 1.11   | 0.92    | 1.34    |
| CC            | 77    | 91       |     | 1.21   | 0.75    | 2.05    | 0.03   |
| AC+CC         |       |          |    | 1.28   | 1.05    | 1.57    |        |
| CASP5         |       |          |    |        |         |
| rs4925859 (5645T>G) |       |          |    |        |         |
| CC            | 514   | 741      |     | 1.00   |         |
| AC            | 323   | 427      |     | 1.29   | 1.05    | 1.58    |
| AA            | 30    | 35       |     | 1.21   | 0.73    | 2.00    | 0.02   |
| AC+AA         |       |          |    | 1.29   | 1.05    | 1.58    |        |
| rs3181318 (373 C>T) |       |          |    |        |         |
| GG            | 337   | 472      |     | 1.00   |         |
| AG            | 349   | 457      |     | 1.21   | 0.88    | 1.65    | 0.21   |
| AA            | 89    | 106      |     | 1.12   | 0.93    | 1.35    |
| AG+AA         |       |          |    | 1.12   | 0.93    | 1.35    |        |
| rs507879 (Ex2-118A>G, T90A) |       |          |    |        |         |
| TT            | 212   | 337      |     | 1.00   |         |
| CT            | 381   | 481      |     | 1.29   | 1.03    | 1.60    |
| CC            | 179   | 209      |     | 1.39   | 1.07    | 1.82    | 0.01   |
| CT+CC         |       |          |    | 1.31   | 1.07    | 1.61    |        |
| EGFR          |       |          |    |        |         |
| rs11977660 (IVS1-47643T>G) |       |          |    |        |         |
| TT            | 220   | 285      |     | 1.00   |         |
| CT            | 381   | 499      |     | 0.98   | 0.79    | 1.23    |
| CC            | 176   | 251      |     | 0.88   | 0.68    | 1.15    | 0.37   |
| CT+CC         |       |          |    | 0.95   | 0.77    | 1.17    |

| Table 4. Cont. |

| SNP/Genotypes | Cases | Controls | OR | 95% CI | p-trend |
|---------------|-------|----------|----|--------|---------|
| rs6593205 (IVS1-41287A>G) |       |          |    |        |         |
| GG            | 310   | 414      |     | 1.00   |         |
| AG            | 368   | 456      |     | 1.09   | 0.89    | 1.34    |
| AA            | 98    | 164      |     | 0.80   | 0.60    | 1.07    | 0.37   |
| AG+AA         |       |          |    | 1.02   | 0.84    | 1.23    |
| rs6953431 (IVS1-38789G>A) |       |          |    |        |         |
| GG            | 522   | 752      |     | 1.00   |         |
| AG            | 236   | 257      |     | 1.34   | 1.08    | 1.65    |
| AA            | 18    | 26       |     | 1.04   | 0.56    | 1.93    | 0.03   |
| AG+AA         |       |          |    | 1.31   | 1.07    | 1.61    |
| rs7796139 (IVS1-34103A>G) |       |          |    |        |         |
| AA            | 370   | 543      |     | 1.00   |         |
| AG            | 337   | 423      |     | 1.17   | 0.96    | 1.42    |
| GG            | 70    | 68       |     | 1.52   | 1.06    | 2.18    | 0.02   |
| AG+GG         |       |          |    | 1.21   | 1.00    | 1.46    |
| rs759158 (IVS1-30770G>T) |       |          |    |        |         |
| TT            | 253   | 351      |     | 1.00   |         |
| GT            | 372   | 502      |     | 1.03   | 0.83    | 1.27    |
| GG            | 152   | 181      |     | 1.16   | 0.88    | 1.52    | 0.33   |
| GT+GG         |       |          |    | 1.06   | 0.87    | 1.30    |
| rs7796872 (IVS1-30135G>A) |       |          |    |        |         |
| GG            | 593   | 780      |     | 1.00   |         |
| AG            | 170   | 236      |     | 0.94   | 0.75    | 1.18    |
| AA            | 11    | 18       |     | 0.76   | 0.36    | 1.63    | 0.43   |
| AG+AA         |       |          |    | 0.94   | 0.75    | 1.17    |
| IGFBP3        |       |          |    |        |         |
| rs6670 (Ex5-411A>T) |       |          |    |        |         |
| TT            | 514   | 740      |     | 1.00   |         |
| AT            | 227   | 261      |     | 1.24   | 1.00    | 1.53    |
| AA            | 30    | 31       |     | 1.40   | 0.84    | 2.36    | 0.03   |
| AT+AA         |       |          |    | 1.27   | 1.04    | 1.56    |
| rs13223993 (Ex5+615C>T) |       |          |    |        |         |
| GG            | 506   | 702      |     | 1.00   |         |
| AG            | 247   | 294      |     | 1.15   | 0.94    | 1.42    |
| AA            | 24    | 37       |     | 0.87   | 0.51    | 1.49    | 0.46   |
| AG+AA         |       |          |    | 1.11   | 0.91    | 1.36    |
| rs2270628 (4848 bp 3' of STPG.A) |       |          |    |        |         |
| GG            | 506   | 702      |     | 1.00   |         |
| AG            | 247   | 294      |     | 1.15   | 0.94    | 1.42    |
| AA            | 24    | 37       |     | 0.87   | 0.51    | 1.49    | 0.46   |
| AG+AA         |       |          |    | 1.11   | 0.91    | 1.36    |

1Adjusted for age, sex, and center.

1 doi:10.1371/journal.pone.0004895.t004
expression of mRNA [34]. IGFBP2 variation has been evaluated with several other cancer sites [35], but this is the first study to evaluate SNPs in relation to renal cancer. In association studies, SNPs in IGFBP3 and IGF related genes (IGF-1 and IGFBP1) have been related to circulating IGF-1 and IGFBP-3 levels [36,37]. IGFBP3 SNP rs6670 (A allele) was not directly associated with IGFBP-3 levels but was weakly associated with a decreasing trend in circulating IGF-1 levels [36]. This is not entirely consistent with the positive association we observed with renal cancer in our study, but suggests that further study is needed to clarify the associations observed.

CASP1, CASP4, CASP5 and CASP12 belong to a caspase subfamily called the inflammatory caspases, which are involved in the maturation of inflammatory cytokines (IL-1β and IL-18) in addition to their role in apoptotic pathways [9,38,39]. Despite their involvement in two key carcinogenic pathways, inflammation and apoptosis, few published reports have evaluated genetic variation in these four caspase genes in relation to cancer. In our study, three CASP1/5/4/12 SNPs (rs568910, rs492859, rs507879) were associated with an increased risk of renal cancer, while one SNP (rs1785883) was associated with a decreased risk. The four SNPs were only weakly correlated with each other (r² < 0.5), except for rs492859 and rs568910 which were strongly correlated (r² = 0.99) within our data. The strongest individual SNP association with renal cancer was observed with rs507879, located within exon 2 of CASP5 and results in a missense mutation and amino acid substitution (Thr90Ala). The function of this particular exon 2 SNP is unclear and is predicted to be a benign mutation by PolyPhen. However, a common somatic mutation in exon 2 has also been identified in leukemias and, gastric, colon, and lung cancers, but has not yet been examined in renal tumors [40–43]. A somatic mutation in a mononucleotide repeat (A)10 in exon 2 produces a shift in the reading frame during transcription resulting in a premature stop and a truncated protein. This suggests that this region in CASP5 may be particularly important for carcinogenesis.

To our knowledge, this is the first study to evaluate SNPs in all but two of these growth/differentiation and apoptosis genes in relation to renal cancer. The primary focus so far in the area of renal cancer susceptibility has been on genetic variants in xenobiotic metabolism genes [12,44] and the von Hippel-Lindau (VHL) gene, which leads to an increased risk of the hereditary form of renal cancer [45]. Only three small studies have evaluated variants in PPARG and VEGF in relation to renal cancer. Smith et al. (n = 40 cases) observed that the rare allele of the PPARG P12A polymorphism [rs1801282] was underrepresented among RCC patients compared to controls, with an OR for trend of 0.28 (0.08–1.01) [46]. This finding is consistent with results from our analysis (OR for trend: 0.80; 95% CI: 0.67–0.96), but this SNP was not considered statistically significant after FDR adjustment. Kawai et al. (n = 213 cases) [47] observed a weak association between three VEGF promoter polymorphisms (rs1570360, rs2010963, rs699947) and renal cancer progression and prognosis; and Abe et al. (n = 145 cases) [48] observed a nonsignificant association between three VEGF 3’UTR polymorphisms (C702T → dbSNP identifier number is unknown, rs3025039, rs10434) and renal cancer risk in Japanese populations. Three of these SNPs were genotyped in our study (rs2010963, rs699947, rs3025039), but only SNP rs699947 demonstrated a weak but nonsignificant association with renal cancer risk. Our analysis of VEGF revealed only one nominally significant SNP in the promoter region (rs833058; Supplemental Table S1) which is correlated with rs699947 (r² = 0.65).

A strength of our study is the large sample size which provides sufficient statistical power to detect associations between SNPs and renal cancer risk. Hospital-based controls in our study could potentially cause selection bias if carrying specific genetic variants were somehow related to hospitalization or if the controls were somehow not representative of the general population. However, the high participation and response rates among both cases and controls minimize the potential for selection bias. Given the multiple centers and countries in our study, the potential for population stratification exists; however, we found no evidence of heterogeneity. Population stratification may still be present, but the likelihood of this is small among European populations [49]. Although tagSNP selection was not based on resequencing data, the strategy for selecting tagSNPs allowed a more comprehensive analysis of common genetic variation in these genes than the traditional candidate SNP approach. Given the large number of associations investigated, additional examination of statistically significant associations using FDR control helped us to evaluate the potential for chance findings due to multiple testing. Results from the replication conducted within the US Kidney Cancer study for rs507879 were not statistically significant on their own, but the study (696 cases and 593 controls) was underpowered (40%) to detect an association of 1.3. Point estimates calculated by pooling data from the two studies may better represent the true association between rs507879 and renal cancer.

In summary, the results from this study suggest that genetic polymorphisms and haplotypes within the CASP1, CASP5, EGFR,
and IGFBP3 genes are associated with renal cancer risk. The regions identified in this study appear to have functional relevance in renal and other types of cancer. To our knowledge, this is one of the largest evaluations of genetic susceptibility and renal cancer conducted to date, but there is need to replicate and extend our findings in other populations.

**Supporting Information**

**Figure S1** Sliding window results and linkage disequilibrium plot of CASP1/5/4/12 region. SNPs associated or located within a CASP gene are indicated by their respective lines. The haplotype results reported in Table 3 are indicated by a line depicting Region1. Upper portion of figure presents global p-value associated with each 3 SNP sliding window, unadjusted and FDR-adjusted. Lower portion of figure presents linkage disequilibrium plot with color scheme based on D' and logarithm of the odds of linkage (LOD) scores. Numbers in the squares are r² values.

Found at: doi:10.1371/journal.pone.0004895.s001 (4.14 MB TIF)

**Figure S2** Sliding window results and linkage disequilibrium plot of EGFR region. The haplotype results for EGFR reported in Table 3 are indicated by lines depicting each region. Upper portion of figure presents global p-value associated with each 3 SNP sliding window, unadjusted and FDR-adjusted. Lower portion of figure presents linkage disequilibrium plot with color scheme based on D' and logarithm of the odds of linkage (LOD) scores. Numbers in the squares are r² values.

Found at: doi:10.1371/journal.pone.0004895.s004 (2.66 MB TIF)

**Figure S3** Sliding window results and linkage disequilibrium plot of IGFBP3 region. The haplotype results reported in Table 3 are indicated by a line depicting each region. Upper portion of figure presents global p-value associated with each 3 SNP sliding window, unadjusted and FDR-adjusted. Lower portion of figure presents linkage disequilibrium plot with color scheme based on D' and logarithm of the odds of linkage (LOD) scores. Numbers in the squares are r² values.

Found at: doi:10.1371/journal.pone.0004895.s003 (3.22 MB TIF)

**Table S1** Results from all Growth and Differentiation, Apoptosis Polymorphisms and Renal Cell Cancer Risk.

Found at: doi:10.1371/journal.pone.0004895.s004 (2.66 MB DOC)

**Table S2** EGFR integrated haplotype and renal cancer risk.

Found at: doi:10.1371/journal.pone.0004895.s005 (0.06 MB DOC)

**Author Contributions**

Conceived and designed the experiments: PB RJH DZ VJ HK VB KS FD MN ND HM MB NC RJH. Performed the experiments: RJH IM SIB PB WHC LEM. Analyzed the data: LMD PI IARC. Contributed reagents/materials/analysis tools: SM SIB MY SJC DZ PI Russia: DZ PI Olomouc: VJ PI Czech Republic-Prague: VB PI Poland: NSD PI Romania: DM PI IARC: PB. Co PI NCI: NR WHC LEM.

**References**

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. (2008) Cancer statistics, 2008. CA Cancer J Clin 58: 71–96.
2. Kario-Kos HE, de Vries E, Sorooshmararam I, Lemmens V, Siesling S, et al. (2008) Recent trends of cancer in Europe: a combined approach of incidence, survival and mortality for 17 cancer sites since the 1990s. Eur J Cancer 44: 1345–1359.
3. Chow WH, Devesa SS (2008) Contemporary epidemiology of renal cell cancer. Cancer J 14: 280–301.
4. Mathew A, Devesa SS, Fraumeni Jr. Chow WH (2002) Global increases in kidney cancer incidence, 1973–1992. Eur J Cancer Prev 11: 171–178.
5. Levi F, Flesler J, Galeote C, Lachnich F, Negri E, et al. (2008) The changing pattern of kidney cancer incidence and mortality in Europe. BJU Int 101: 949–950.
6. McLaughlin JK, Lipworth L, Tartone RE, Blet WJ (2006) Renal Cancer. In: Schottenfeld D, Fraumeni Jr, eds. Cancer Epidemiology and Prevention. 3rd ed. New York: Oxford University Press.
7. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57–70.
8. Aaronson SA (1991) Growth factors and cancer. Science 254: 1146–1153.
9. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57–70.
10. Schottenfeld D, Fraumeni JF, eds. Cancer epidemiology and prevention. 3rd ed. New York: Oxford University Press.
11. Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, et al. (2006) Epidermal growth factor receptor (EGFR) signaling in cancer. Gene 366: 2–16.
12. Taberner J, Macarrulla T, Ramos JF, Baselga J (2005) Novel targeted therapies in the treatment of gastric and esophageal cancer. Ann Oncol 16: 1740–1748.
13. Weihua Z, Tian R, Huang WC, Wu Q, Chin CH, et al. (2008) Survival of cancer cells is maintained by EGFR independent of its kinase activity. Cancer Cell 13: 385–393.
14. Lee WS, Kanai Y, Wells RG, Hediger MA (1994) The high affinity Na+/glucose cotransporter. Re-evaluation of function and distribution of expression. J Biol Chem 269: 12032–12039.
15. Arraujo A, Ribeiro E, Arvedo I, Coelho A, Soares M, et al. (2007) Genetic polymorphisms of the epidermal growth factor and related receptor in non-small cell lung cancer—a review of the literature. Oncologist 12: 201–210.
16. Pres OA, Zhang W, Gordon MA, Yang D, Luje G, et al. (2008) Gender-related survival differences associated with EGFR polymorphisms in metastatic colon cancer. Cancer Res 68: 3037–3042.
17. Chi DD, Ding AV, Helms C, Steinbrueck T, Mishra SK, et al. (1992) Two chromosomes 7 dinucleotide repeat polymorphisms in germ line associated with breast cancer risk. Science 254: 1146–1153.
18. Thornberry NA, Lazebnik Y (1998) Caspases: enemies within. Science 281: 1312–1316.
19. Stem Cells Association Consortium. J Natl Cancer Inst 98: 1382–1396.
20. Hoggard H, Jander G, Wisniewski M, Kuritz S, et al. (2004) Allergic length of a CA dinucleotide repeat in the efg gene correlates with the frequency of amplifications of this sequence–first results of an inter-ethnic breast cancer study. J Pathol 203: 545–550.
21. Qin W, Innocenti F, Wu MH, Desai AA, Dolan ME, et al. (2005) A functional common polymorphism in a Sp1 recognition site of the epidermal growth factor receptor gene promoter. Cancer Res 65: 46–53.
22. Pollak MN, Schernhammer ES, Hankinson SE (2004) Insulin-like growth factors and neoplasia. Nat Rev Cancer 4: 503–518.
23. Renuch AG, Zwahlen M, Ginder C, O’Dwyer P, Shalea SM, et al. (2004) Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 363: 1346–1353.
24. Firth SM, Baxter RC (2009) Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 23: 824–854.
25. Rosendahl AH, Forsberg G (2006) IGF-I and IGBP-3 augment transforming growth-factor-beta actions in human renal carcinoma cells. Kidney Int 70: 1508–1515.
26. Chuang ST, Patton KT, Scherfuk KT, Papavero V, Lin F, et al. (2008) Over expression of insulin-like growth factor binding protein 3 in clear cell renal cell carcinoma. J Urol 179: 445–449.
32. Cheung CW, Vesey DA, Nicol DL, Johnson DW (2004) 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.

33. Ibanez de Caceres I, Dulaimi E, Hoffman AM, Al-Saleem T, Uzzo RG, et al. (2006) Identification of novel target genes by an epigenetic reactivation screen of renal cancer. Cancer Res 66: 5021–5028.

34. Hughes TA (2006) Regulation of gene expression by alternative untranslated regions. Trends Genet 22: 119–122.

35. Cheng I, Penney KL, Straw DO, Le Marchand L, Giorgi E, et al. (2006) Haplotype-based association studies of IGFBP1 and IGFBP3 with prostate and breast cancer risk: the multiethnic cohort. Cancer Epidemiol Biomarkers Prev 15: 1993–1997.

36. Patel AV, Cheng I, Canzian F, Le Marchand L, Thun MJ, et al. (2008) IGF-1, IGFBP-1, and IGFBP-3 polymorphisms predict circulating IGF levels but not breast cancer risk: findings from the Breast and Prostate Cancer Cohort Consortium (BPC3). PLoS ONE 3: e2578.

37. Cheng I, DeLellis Henderson K, Haiman CA, Kolonel LN, Henderson BE, et al. (2007) Genetic determinants of circulating insulin-like growth factor (IGF)-I, IGF binding protein (BP)-1, and IGFBP-3 levels in a multiethnic population. J Clin Endocrinol Metab 92: 3660–3666.

38. Martinou F, Tschopp J (2007) Inflammatory caspases and inflammasomes: master switches of inflammation. Cell Death Differ 14: 10–22.

39. Nicholson DW (1999) Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 6: 1028–1042.

40. Offman J, Gascoigne K, Britov F, Macpherson P, Bignami M, et al. (2005) Repeated sequences in CASPASE-5 and FANCD2 but not NF1 are targets for mutation in microsatellite-unstable acute leukemia/myelodysplastic syndrome. Mol Cancer Res 3: 251–260.

41. Hosomi Y, Gemma A, Hosoya Y, Nara M, Okano T, et al. (2003) Somatic mutation of the Caspase-5 gene in human lung cancer. Int J Mol Med 12: 443–446.

42. Yamanouchi T, Iijima T, Mori T, Takahashi K, Matsumoto H, et al. (2006) Accumulation profile of frameshift mutations during development and progression of colorectal cancer from patients with hereditary nonpolyposis colorectal cancer. Dis Colon Rectum 49: 399–406.

43. Song YH, Jeong EG, Ahn CH, Kim SS, Song SY, et al. (2008) Mutational analysis of caspase 1, 4, and 5 genes in common human cancers. Hum Pathol 39: 895–900.

44. Lin J, Pu X, Wang W, Matin S, Tannir NM, et al. (2008) Case-Control Analysis of Nucleotide Excision Repair Pathway and the Risk of Renal Cell Carcinoma. Carcinogenesis.

45. Maher ER (1996) Inherited renal cell carcinoma. Br J Urol 78: 542–543.

46. Smith WM, Zhou XP, Kurose K, Gao X, Latif F, et al. (2001) Opposite association of two PPARG variants with cancer: overrepresentation of H449H in endometrial carcinoma cases and underrepresentation of P12A in renal cell carcinoma cases. Hum Genet 109: 146–151.

47. Kawai Y, Sakano S, Koronen Y, Eguchi S, Naito K (2007) Associations of single nucleotide polymorphisms in the vascular endothelial growth factor gene with the characteristics and prognosis of renal cell carcinomas. Eur Urol 52: 1147–1155.

48. Abe A, Sato K, Habuchi T, Wang L, Li Z, et al. (2002) Single nucleotide polymorphisms in the 3′ untranslated region of vascular endothelial growth factor gene in Japanese population with or without renal cell carcinoma. Tohoku J Exp Med 198: 181–190.

49. Wacholder S, Rothman N, Caporaso N (2002) Counterpoint: bias from population stratification is not a major threat to the validity of conclusions from epidemiological studies of common polymorphisms and cancer. Cancer Epidemiol Biomarkers Prev 11: 513–520.