Identification of a novel germline *SPOP* mutation in a family with hereditary prostate cancer

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Abstract

Background—Family history of prostate cancer is a well-recognized risk factor. Previous linkage studies have reported a putative prostate cancer susceptibility locus at chromosome 17q21-22. *SPOP* (Speckle-type POZ protein)maps to the 17q21-22 candidate linkage region and is one of the most frequently mutated genes in sporadic prostate cancers.

Methods—We performed targeted next generation sequencing to analyze 2009 exons from 202 genes in a candidate linkage region on chromosome 17q21-22 using 94 unrelated familial prostate cancer cases from the University of Michigan Prostate Cancer Genetics Project (n= 54) and Johns Hopkins University (n=40) including the exons and UTRs of *SPOP*.

Results—We identified anovel *SPOP* missense mutation (N296I)in a man with prostate cancer diagnosed at age 43. This mutation completely segregates with prostate cancer affection status among the men in this family. The N296I mutation resides within the evolutionarily conserved Bric-a-brac, Tram track, Broad-complex (BTB) domain, involved in recruiting targets to Cul3 for degradation. Analysis of the prostate tumor from this individual verified the presence of heterozygous N296I as well as an ERG fusion.

Conclusions—We have discovered a novel mutation in *SPOP* that tracks with prostate cancer within a family and is predicted to be deleterious. Taken together, our results implicate *SPOP* as a candidate gene for hereditary prostate cancer.

Keywords

familial; gene; candidate linkage region

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Introduction

Prostate cancer is the most common non-cutaneous cancer diagnosed among American men and the second leading cause of cancer death with an estimated 233,000 new cases and 29,480 deaths expected in the United States in 2014. Known risk factors for prostate cancer are increasing age, African American race, and positive family history of the disease. Studies performed with the objective of elucidating a heritable component have succeeded in identifying a region of genetic susceptibility on chromosome 17q first reported by Lange *et al.*[1] on the basis of linkage analysis of 175 pedigrees of families with hereditary prostate cancer from the University of Michigan Prostate Cancer Genetics Project (UM-PCGP). Fine mapping of chromosome 17q, using 453 pedigrees from the UM-PCGP and Johns Hopkins University (JHU) refined this region of interest to chromosome 17q21-22[2]. Further analysis of a subset of 147 families with four or more affected men and an average age of prostate cancer diagnosis   65 years narrowed the candidate interval (1-LOD support interval) for a putative susceptibility gene to a 10cM region of 17q that contains over 75 known genes. We have previously reported the identification of a rare but recurrent G84E (rs138213197) missense mutation in *HOXB13* from the chromosome 17q-linked families described herein [3]. Carriers of G84E were reported to have a 10-20 fold increased risk of developing prostate cancer with the highest frequency in men with early-onset and familial prostate cancer. Subsequent studies have confirmed this report providing further evidence that rare genetic variants play a role in prostate cancer susceptibility [4–13]. Genome wide association studies of prostate cancer in populations of men of African and European descent have also identified risk variantswithin17q21. Specifically, rs7210100[14] andrs1165049[15] located in intron 1 and downstream of *ZNF652* respectively. *ZNF652* is located within a gene-dense region of 17q21 that harbors prostate cancer susceptibility genes *HOXB13PRAC* and *SPOP*. To date, chromosome 17q21-22 remains one of the most reproducible linkage regions for prostate cancer susceptibility loci.

The Speckle-type POZ protein or *SPOP* gene, which maps to the 17q21-22 candidate linkage region, is one of the most frequent somatically mutated genes in prostate cancer. Initially described by Kan *et al.* [16], *SPOP* mutations have been observed in (2/58) [16]and 14/111[17] prostate cancers and(2/7) tumors from men with high risk disease [18]. *SPOP* encodes the substrate-binding subunit of a Cullin-based E3 ubiquitin ligase. Studies using the MCF-7 breast cancer cell line have shown SPOP directly impacts cancer cell growth and invasion, suggesting that *SPOP* may function as a tumor suppressor gene (TSG) in breast and possibly other cancers[19]. Tissue microarray screening for SPOP expression in 18 cancer types from different organs revealed high expression of SPOP in kidney, endometrial, and germ cell cancers when compared to normal tissues [20]. Additionally, recent evidence has shown that the previously reported prostate-cancer-associated mutants of SPOP cannot promote androgen receptor (AR) ubiquitination [21], suggesting that SPOP is part of the degradation system for AR, an important driver of prostate carcinogenesis.

In the present study, we set out to analyze *SPOP* germline sequence data from 94 familial prostate cancer cases with evidence of linkage to chromosome 17 compiled at the University of Michigan and the Johns Hopkins University.

Methods

Patient Selection

University of Michigan Prostate Cancer Genetics Project (UM-PCGP)—UM-PCGP prostate cancer cases were restricted to (1) men diagnosed with prostate cancer with at least one living first- or second-degree relative also diagnosed with prostate cancer or (2) men diagnosed with prostate cancer at <56 years of age. We confirmed the diagnosis of prostate cancer by medical record review whenever possible. All subjects provided written informed consent to participate in the study. The protocol and consent documents were approved by the University of Michigan Medical School Institutional Review Board Health Insurance Portability and Accountability Act (HIPAA) regulations and all subjects gave written informed consent.

Johns Hopkins University (JHU)—Hereditary prostate cancer (HPC) families each had at least three first-degree relatives affected with prostate cancer. We verified diagnosis of prostate cancer by medical records.) The protocol and consent documents were approved by the Johns Hopkins School of Medicine Institutional Review Board and all subjects gave written informed consent.

Discordant Sibling Pairs

The details of the discordant sibling pair (DSP) project have been described elsewhere [22]. For the present study, 569 families were identified in which DNA was available from at least one pair of brothers discordant for prostate cancer.

Targeted sequencing of SPOP

We selected the youngest prostate cancer case with available DNA from 94 prostate cancer families (40 families from JHU and 54 from the UM-PCGP) as described previously [23]. Seven of the families were of African descent, 2 were of Asian descent, and the remaining 85 described themselves as being of European descent. A primer library was designed for amplification of 7053 base pairs (bp) of *SPOP* including all exons, intron/exon boundaries, and the 5' and 3' untranslated regions. We then used the Rain Dance RDT 1000 system (Rain Dance Technologies, Inc., Lexington, MA) to amplify 3 μg of sheared genomic DNA from each sample using our primer library. Purified amplicons were used as template for sequencing using the Life Technologies SOLiD™ system version 4.0 fragment library methodology (Life Technologies Corporation, Carlsbad, CA). Sequence data processing was performed using Bioscope to align the sequences to the genomic reference (Build 36, hg18). Variant detection was performed using Sam Tools 1.3[24] and Sol SNP 1.1. We confirmed and tested all variant sequences in family members using standard Sanger sequencing, capillary electrophoresis technology and BigDye® Terminator chemistry (Life Technologies, Carlsbad, CA).

Tumor Sequencing

Prostate tumor DNA from the index case was extracted from using the QIAamp DNA FFPE tissue kit (Qiagen, Valencia, CA). Sanger sequencing of N296I was performed using custom *SPOP* primers as described above.

Immunohistochemistry

Hematoxylin and Eosin (H&E) slides from the prostatectomy of the index case were reviewed and re-graded according to current ISUP guidelines. ERG immunostaining was performed essentially as described [25, 26] using pre-diluted ERG antibody (provided by Ventana Medical Systems) using the automated Discovery XT staining platform (Ventana Medical Systems).

Genotyping Assays

We genotyped N296I in the DSP cohort using a custom TaqMan SNP assay (Life Technologies). Allelic discrimination was performed on an ABI Prism 7900HT Sequence Detection System and SDS version 2.1 software (Applied Biosystems). Genotyping call rate was 99%.

Results

Molecular study result of the index case

Targeted next-generation sequencing revealed a novel heterozygous 1358 A>T mutation (Figure 1) in an individual of European descent diagnosed with Gleason 3+4=7 prostate cancer at age 43 (NM_001007226). The missense mutation is a single A to T trans version in exon 11resulting in an amino acid substitution of asparagine to isoleucine at codon 296 (N296I). This variant was confirmed by Sanger sequencing. No additional *SPOP* missense variants were observed in the remaining 93 HPC probands.

Molecular study result of other family members

Sanger sequencing of additional family members revealed complete segregation of N296I and prostate cancer affection status amongst the men in this family (Figure 2). Sequence data from five additional male relatives revealed that the father and a brother with prostate cancer carry the mutation, as does another brother affected with both prostate and kidney cancer. Two unaffected brothers, ages 46 and 56, do not carry the N296I allele. Female relatives with DNA were also genotyped, the mother of the pro band did not carry the N296I, yet the sister of the pro band was positive for the variant. To our knowledge, neither of the female relatives has been diagnosed with cancer.

Molecular and histopathologic characterization of the prostate tumor

Sanger sequencing of DNA extracted from archival tissue confirmed the presence of the heterozygous N296I variant in the prostate tumor from the pro band. Additionally, ERG expression was observed in a Gleason 3+4=7 focus of prostate cancer from the index case consistent with the presence of an ERG rearrangement[27] (Figure 3). SPOP expression was also observed in the prostate tissue (data not shown).

Molecular study result of DSPs

No additional N296I carriers were detected amongst 786 affected and 654 unaffected men from 569 families.

Discussion

SPOP encodes a 374 amino acid protein that contains three domains: an N-terminal meprin and TRAF-homology (MATH) domain (amino acids 31-164), a Bric-a-brac, Tramtrack, Broad-complex (BTB) substrate binding, otherwise known as a pox virus and zinc finger (POZ), domain (amino acids 184-297) and a C-terminal nuclear localization sequence (amino acids 365-374)[28, 29]. *SPOP* missense mutations have been described in up to 15% of prostate tumors; classifying *SPOP* as one of the genes most commonly affected by somatic missense mutations in prostate cancer [16, 17, 30, 31]. Additionally, targeted sequencing of exons 6 (amino acids 80-106) and 7 (amino acids 120-140) of SPOP in prostate tumors from a cohort of demographically diverse patients showed an overall mutation rate of 8.1% [32]. In the present study, we describe the identification of a novel germline *SPOP* mutation (N296I) in a family with early-onset and hereditary prostate cancer that showed evidence of linkage to chromosome 17. The N296I mutation was found to completely co-segregate with prostate cancer disease status amongst the men in this family. Furthermore, a family member with both prostate and kidney cancer was also shown to carry N296I. Although tumor tissue from the kidney cancer was unavailable for analysis, it should be noted that SPOP has been previously implicated in renal cell carcinoma (RCC). Specifically, SPOP was shown to be over expressed in 77% (199/258) of RCCs, where as all normal kidney samples were negative [20]. In addition, staining for SPOP also indicated its utility as a highly specific and sensitive biomarker capable of distinguishing histological subtypes of RCCs.

Molecular characterization of prostate tumors has generated evidence of distinct molecular subtypes defined by the presence or absence of TMPRSS2-ERG fusions, SPINK1, and somatic *SPOP* non-synonymous point mutations [25, 33–39]. Previous studies have indicated that TMPRSS2-ERG fusions are mutually exclusive to SPINK1 over expression and somatic *SPOP* mutations. Immunohistochemistry for ERG expression revealed diffuse moderate to strong nuclear staining associated with ERG+ carcinoma in the tumor of our index case, which also harbored an N296I mutation. Although N296I is exceedingly rare, this report expands the knowledge of variation in *SPOP* and provides evidence in opposition of a mutually exclusive relationship between *SPOP* point mutations, specifically those in the BTB domain, and TMPRSS2-ERG fusions.

Recent studies of localized and advanced prostate tumors have identified a cluster of SPOP mutations exclusive to the MATH domain (Figure 1). Studies conducted to gain insight on the effect of these mutations have concluded SPOP mutants lack the ability to interact with or degrade SRC-3, thus attenuating the tumor suppressor gene function of wild-type SPOP [31]. SRC-3 is known to bind directly to the SPOP MATH domain prior to ubiquitination and degradation via the Cul3 based ubiquitin ligase complex [19]. Over-expression of SRC-3 has been noted in several human cancers including prostate, breast, and ovarian [40–43] and is often associated with poor prognosis [44, 45]. In prostate cancer, SRC-3 over expression is thought to promote tumorigenesis and more rapid progression to castrate resistant prostate cancer.

Recently, similar to SRC-3, SPOP has been shown to interact with AR in prostate cancer cells and target AR for degradation [21]. Interestingly, mutated alleles of *SPOP* found in prostate cancers were unable to bind or target AR for degradation, suggesting that the accumulation of AR due to this mechanism may play an important role in prostate carcinogenesis and perhaps disease progression. It will be of interest to determine if the N296I mutant observed in this study is similarly deficient in targeting AR, SRC-3, or other protein substrates for degradation.

The N296I mutation is located in a highly conserved region of the SPOP BTB domain which binds directly to Cul3, a scaffold protein required for substrate ubiquitination. Overall, this mutation appears to be rare, as it was not observed in our familial and early-onset prostate cancer DSP population nor was it seen in large public databases including the 1000 Genomes [46] project and the Exome Sequencing Project (ESP) which includes 4300 individuals of European ancestry and 2203 individuals of African American ancestry [47]. Despite its low frequency and its position outside of the structurally defined substrate-binding MATH domain, data exists in support of the notion that N296I may be involved in carcinogenesis. Geng et al. have recently demonstrated that expression vectors lacking the BTB domain cannot bind Cul3 and subsequently cannot promote the degradation of SRC-3 in prostate cancer PC-3 cells [31].

In summary, we have identified a novel germline SPOP mutation in a hereditary prostate cancer family which exhibits complete co-segregation with disease status. The early-on set nature of prostate cancer diagnoses and the co-occurrence of kidney cancer in one of the mutation carriers suggest that germline mutations in *SPOP* may be increasing cancer risk in this family. As additional hereditary prostate cancer families are studied, *SPOP* should be considered a candidate prostate cancer susceptibility gene worthy of additional focus.

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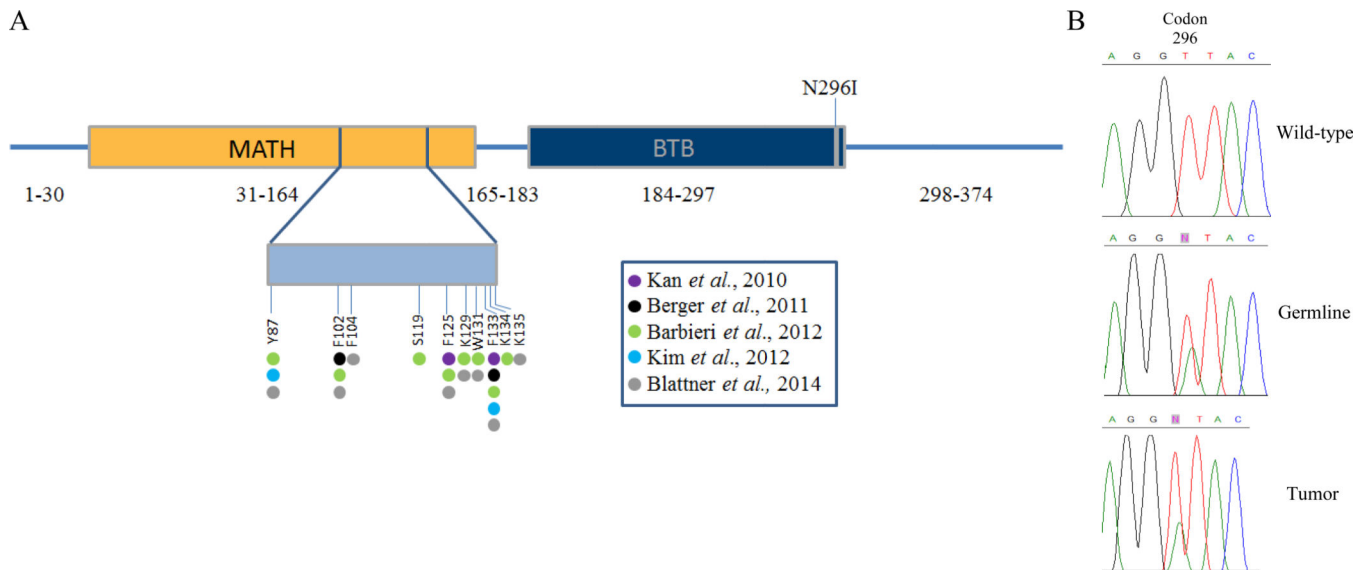
Figure 1. Location of the *SPOP* N296I mutation

A. The 374 amino acid SPOP protein consists of an N-terminal meprin and TRAF-homology (MATH) domain, a BTB substrate binding, otherwise known as a pox virus and zinc finger (POZ), domain, and a C-terminal nuclear localization sequence. The *SPOP* N296I mutation resides in the Bric-a-brac, Tramtrack, Broad-complex (BTB) binding domain. The somatic mutations identified in prostate tumors are located in the MATH domain. B) Chromatograms showing the presence of the N296I missense mutation in both germline and tumor DNA from our index case.
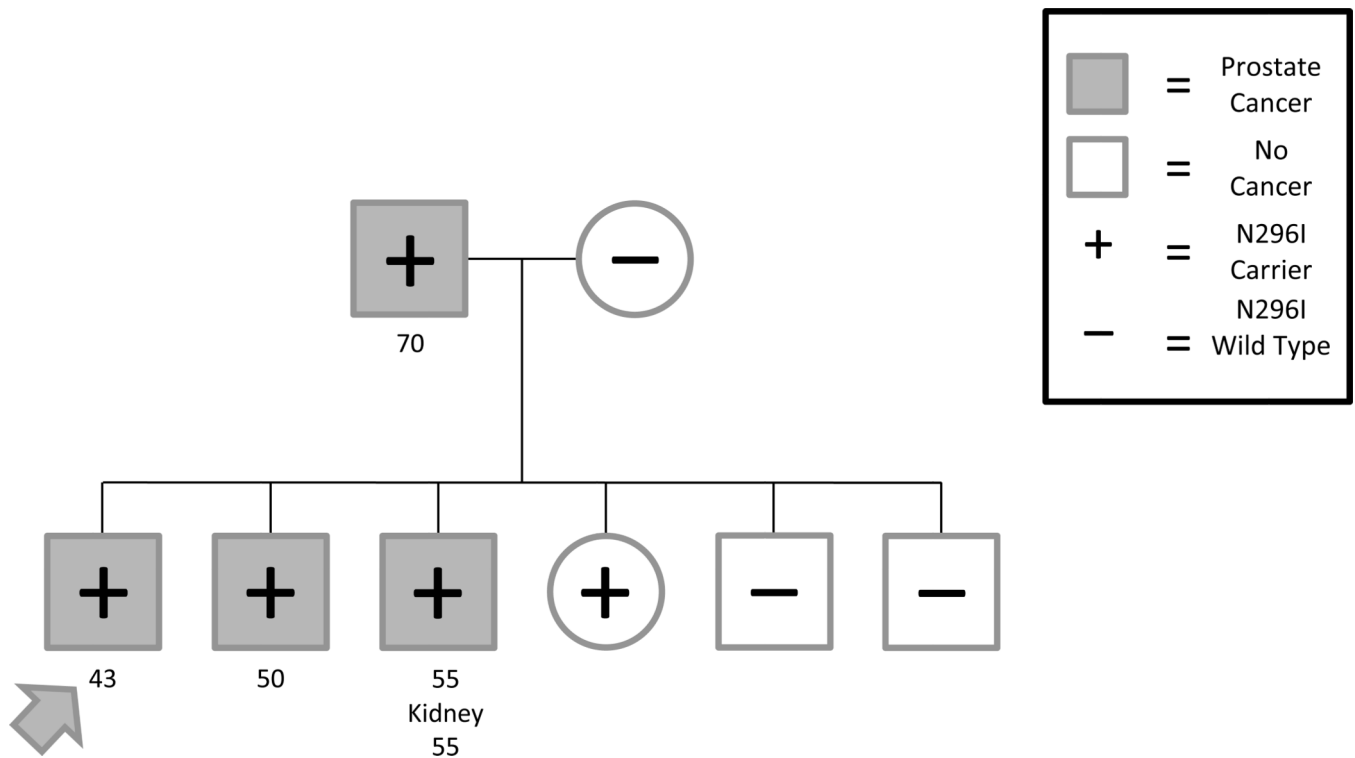
Figure 2.
Pedigree of the family harboring the *SPOP* N296I mutation. The pro band initially selected for sequencing is indicated by the arrow. With the exception of the pro band, the ages of diagnosis have been rounded to the nearest 5-year interval and are shown under the subject.
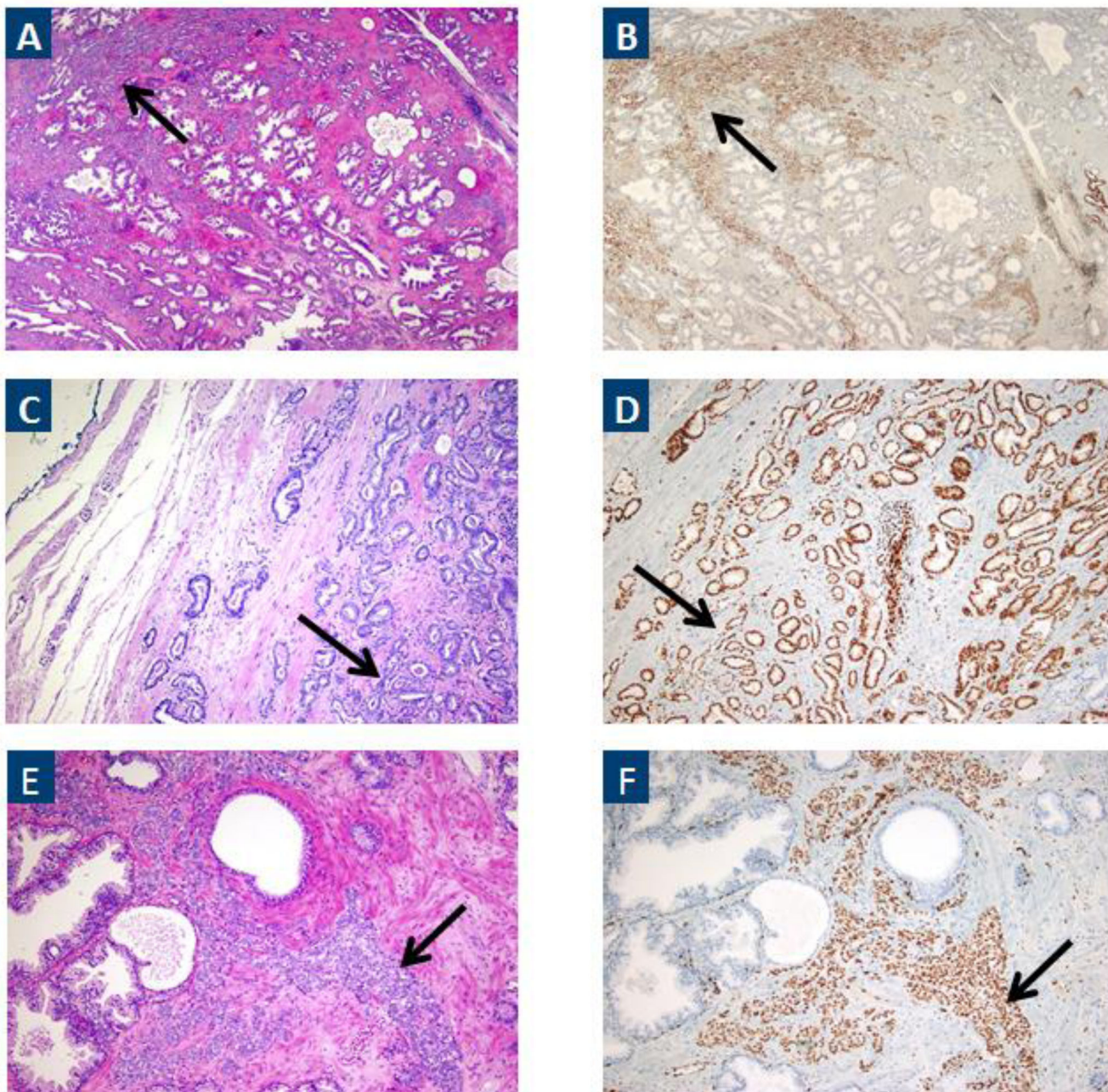
Figure 3.
A) H&E staining of the index focus (Gleason score 3+4=7) on prostatectomy. Prostatic adenocarcinoma is indicated by the black arrow. B) Consecutive sections were stained using a monoclonal antibody against ERG (EPR3864). Specific, diffuse moderate to strong nuclear staining was observed in cancer (black arrow). Original magnification 4X. C&D) As in A&B except higher power (10X) of an area of Gleason pattern 3 carcinoma. E&F) As in A & B, except higher power (10X) of an area of Gleason pattern 4 carcinoma.