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Research Article

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Expression and Genetic Variation in Neuroendocrine Signaling Pathways in Lethal and Nonlethal Prostate Cancer among Men Diagnosed with Localized Disease

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Abstract

Background: Recent data suggest that neuroendocrine signaling pathways may play a role in the progression of prostate cancer, particularly for early-stage disease. We aimed to explore whether expression of selected genes in the adrenergic, serotoninergic, glucocorticoid, and dopaminergic pathways differs in prostate tumor tissue from men with lethal disease compared with men with nonlethal disease.

Methods: On the basis of the Swedish Watchful Waiting Cohort, we included 511 men diagnosed with incidental prostate cancer through transurethral resection of the prostate during 1977–1998 with follow-up up to 30 years. For those with tumor tissue ($N = 262$), we measured mRNA expression of 223 selected genes included in neuroendocrine pathways. Using DNA from normal prostate tissue ($N = 396$), we genotyped 36 SNPs from 14 receptor genes. Lethal prostate cancer was the primary outcome in analyses with pathway gene expression and genetic variants.

Results: Differential expression of genes in the serotoninergic pathway was associated with risk of lethal prostate cancer ($P = 0.007$); similar but weaker associations were noted for the adrenergic ($P = 0.014$) and glucocorticoid ($P = 0.020$) pathways. Variants of the *HTR2A* (rs2296972; $P = 0.002$) and *NR3CI* (rs33388; $P = 0.035$) genes (within the serotoninergic and glucocorticoid pathways) were associated with lethal cancer in overdominant models. These genetic variants were correlated with expression of several genes in corresponding pathways ($P < 0.05$).

Conclusions: Our findings lend support to hypothesis that the neuroendocrine pathways, particularly serotoninergic pathway, are associated with lethal outcome in the natural course of localized prostate cancer.

Impact: This study provides evidence of the role of neuroendocrine pathways in prostate cancer progression that may have clinical utility. *Cancer Epidemiol Biomarkers Prev; 26(12); 1781–7. ©2017 AACR.*

Introduction

Prostate cancer is the most common cancer among men in Western countries (1). A majority of men diagnosed today have localized disease (2) and tend to die from causes other than prostate cancer (3). Because of both the variable and uncertain outcome for patents with early-stage disease, it is important to expand the understanding of the biological differences between men diagnosed with localized disease who with time develop lethal disease versus those whose tumors remain indolent.

Stress-related neuroendocrine signaling, involving the epinephrine, norepinephrine, serotonin, glucocorticoid, and dopamine pathways, has been suggested to play a role in prostate cancer progression (4–6). For example, experimental studies have shown that activation of the β2-adrenergic receptor (ADRB2) in the adrenergic pathway is associated with inhibited apoptosis and increased cell migration in prostate cancer (7), particularly during the early phase of cancer progression (8). Several 5-hydroxytryptamine (HTR) receptors in the serotoninergic pathway have also been associated with cell proliferation and apoptosis (9). However, evidence from clinical and epidemiologic studies is both limited and conflicting (7, 10).

Accumulating data further indicate that the link between stress and cancer progression might be more relevant in the early stages of the cancer continuum (11). Our recent work, based on a cohort of U.S. men with varying stages of prostate cancer, suggests that altered gene expression within the described pathways may influence cancer progression (6). Little is known, however, about the impact of neuroendocrine signaling in localized prostate cancer, and whether such dysregulated signaling is attributable

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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to variation in genes involved in these pathways. Genetic polymorphisms in neuroendocrine receptors that may explain variation in the perception of and response to stress (12, 13) may also play a role in carcinogenesis. Several studies have for instance suggested that genetic variants of *ADRB2* and *HTR1B* are associated with increased risk of prostate and other cancers (14–16). No study has, however, specifically examined the association with lethal prostate cancer.

Leveraging data from a cohort of Swedish men diagnosed with localized prostate cancer and managed with watchful waiting, we aimed to compare the gene expression of four selected neuroendocrine pathways, that is, adrenergic, serotoninergic, glucocorticoid, and dopaminergic pathways, in tumor tissue between men with lethal and nonlethal cancer, and to explore whether genetic variation may explain the variation in gene expression.

Materials and Methods

Study population

This study is based on the Swedish Watchful Waiting Cohort, including 1,367 men with localized prostate cancer diagnosed incidentally by transurethral resection of the prostate (TURP) for symptomatic benign prostatic hyperplasia in the Örebro and South East Health Care Regions in Sweden between 1977 and 1998 (17). In accordance with prevailing guidelines, all participants were followed expectantly for up to 30 years. No initial treatment was introduced at the time of diagnosis. The specimens of formalin-fixed paraffin embedded (FFPE) tumor tissue were available for 1,256 (92%) participants and were rereviewed for cancer confirmation.

On the basis of the cohort, we sampled twice to profile gene expression and genotypes separately using an extreme-case design (17), due to the availability of tumor and normal tissue of prostate (Supplementary Table S1). For the pathway analyses, we sampled 262 men with tumor tissue of high quality, which includes 141 men who died of prostate cancer during follow-up (lethal cases) and 121 men who lived at least seven years after cancer diagnosis (nonlethal cases). For the single nucleotide polymorphisms (SNP) analyses, we sampled 396 men with available normal tissue, including 126 lethal and 270 nonlethal cases. Eighty-two lethal and 72 nonlethal cases were present in both samples. In total, we included 186 lethal and 325 nonlethal cases for this study.

Pathway selection and construction

We focused on expression of genes in four neuroendocrine pathways with a suspected or confirmed link to psychologic stress: the adrenergic, serotoninergic, glucocorticoid, and dopaminergic pathways. The majority of candidate genes in the pathways were selected using KEGG (http://www.genome.jp/kegg/pathway.html), Pathway Maps (http://pathwaymaps.com/maps/), and literature search as described previously (6). Focusing on tumor-specific impact, genes in signaling branches leading to cardiovascular and neuronal functions were excluded from the adrenergic pathway. In total, we included 223 genes for the pathway analysis (Supplementary Table S2). In addition, to conservatively measure the pathway effect without crosstalk, we further defined 116 exclusive genes that were specific to each pathway and were not shared with the other pathways (Supplementary Table S2).

RNA extraction and profiling

To conduct the profiling in FFPE tissue, whole transcriptome amplification was paired with microarray technologies, as reported previously (17). Briefly, 0.6-mm biopsy cores were taken from tumor-enriched areas (>90% tumor) of FFPE tissue blocks. RNA was extracted from these cores in a 96-well format using the CyBi-Well liquid handling system (CyBio AG). The cores were first deparaffinized and the RNA was extracted using TRIzol LS reagent. The RNA was quantified using a NanoDrop spectrophotometer (NanoDrop Technologies). An array of 6,100 genes was profiled thereafter using the four complementary DNA (cDNA)-mediated annealing, selection, ligation, and extension (DASL) assay panels (Illumina). Quality assessment was performed assuming a log-normal distribution. Poor quality samples, defined as those with <55% of gene expression measurements over the 95th percentile of expression level of 27 control probes, were excluded (18). The remaining raw data were normalized using a cubic spline algorithm. The dataset is available at GEO (accession number: GSE16560).

Genotyping

With a specific interest in germline genetic polymorphisms of neuroendocrine receptors, we selected 36 SNPs across the main receptor genes ($n = 14$) in the aforementioned pathways according to previous literature reporting a link to prostate or other cancers (Supplementary Table S3). Genotyping was performed on DNA extracted from adjacent normal prostate tissue using the Sequenom iPLEX platform assay at the Genotyping Core Facility at Children's Hospital (Boston, MA; ref. 19). On each 96-well plate, we included 4% quality control specimens. A total of 30 SNPs had high genotyping success; SNPs ($n = 4$: rs6276, rs6295, rs6311, and rs2770292) with a call rate <95% were excluded as were those with a minor allele frequency < 5% (rs6294) or displaying significant deviation from Hardy–Weinberg equilibrium (i.e., $P < 10^{-5}$; rs1042711). In addition, we excluded data from 8 men because of low genotyping quality (genotyped <85% quality SNPs).

Biomarkers and histopathologic features

As secondary outcomes, we employed multiple biomarkers and histopathologic features to explore potential mechanisms underlying the link between neuroendocrine pathways and lethal prostate cancer. The sample size differs by individual biomarker and histopathologic features given the data availability.

Cell proliferation. The expression of Ki-67 was assessed on 4-μm sections of tumor tissue to evaluate the cell proliferation, using polyclonal anti-Ki67 antibody (Vector Laboratories) for IHC staining as described previously (20). The Ariol instrument SL-50 (Applied Imaging) was used thereafter to generate the percentage of Ki-67–positive nuclei among all tumor nuclei.

Apoptosis. With the Apoptag Peroxidase In situ kit (Chemicon International), the terminal deoxynucleotide transferase–mediated dUTP nick end labeling (TUNEL) assay was used on 4-μm sections to estimate the percentage of tumor cells undergoing apoptosis (i.e., the percentage of positively stained cells among all tumor cells), as described previously (21).

TMPRSS2–ERG fusion. As an indicator of poor prognosis, we employed an ERG break-apart FISH assay to determine *ERG*

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rearrangement status (22). If cases were not assessable by FISH, qPCR was performed instead with an aliquot of the RNA extraction used for DASL. cDNA was synthesized as above using the Illumina kit (Illumina Inc). The *TMPRSS2–ERG* fusion product was detected using SYBR green assay (Qiagen Inc) with *TMPRSS2–ERG_f* and *TMPRSS2–ERG_r* primers (GenBank accession code NM_ DQ204772.1; ref. 23). Relative quantification was carried out using the comparative $\Delta\Delta C_t$ method (24).

Other histopathologic features. Hematoxylin and eosin slides were rereviewed by a pathologist, blinded to the clinical outcome, to assess the Gleason score, tumor stage, as well as the presence of perineural invasion and prostatic intraepithelial neoplasia (PIN) in the adjacent area.

Statistical analyses

Our primary outcome was the risk of lethal prostate cancer, with biomarkers and histopathologic features as secondary outcomes. We performed two analyses for both the primary and secondary outcomes: first the expression of four candidate pathways and then the genetic variants of candidate receptor genes.

Pathway analyses

Only gene expression data from the 223 candidate genes in the selected pathways were used for analyses. For each of the four candidate pathways, we assessed the overall association between the genes in the pathway and the risk of lethal prostate cancer using the global test (25), adjusting for age at diagnosis. Because our aim was to describe the differences observed in these pathways between lethal and nonlethal disease rather than to develop prognostic models, our primary model does not control for Gleason score (4–6, 7, or 8–10) or stage (T1a or T1b). In our secondary models, we both controlled for these variables and conducted subgroup analyses by these variables. In a sensitivity analysis, to reduce overlap between pathways, we assessed the associations of 116 genes that were exclusively present within a single pathway with lethal cancer risk using the global test.

To further understand potential underlying mechanisms for the observed associations, we tested pathway associations with cell proliferation, apoptosis, inflammation, and other histopathologic features. All quantitative biomarkers were treated as continuous outcomes in the model, while histopathologic features were classified as binary.

Although our primary aim was to examine the effect at the pathway level, we also summarized mean expression levels for each candidate gene in lethal and nonlethal prostate cancers and provided the ORs at the gene level for risk of lethal cancer using logistic regression with adjustment for age at diagnosis (Supplementary Table S2).

SNP analyses

We tested the associations of SNPs with lethal cancer risk using logistic regression without any adjustment and explored different effect models (i.e., additive, recessive, dominant, and overdominant models). The overdominant model compared the heterozygous genotype with two homozygotes. For statistically significant associations, we further calculated ORs and 95% confidence intervals (CI). To provide information for potential mechanisms, we subsequently examined the significant SNPs detected for lethal cancer in association with the aforementioned secondary outcomes, using the same effect model as for the primary outcome.

To understand the impact of genetic variation on the pathway expression, we further examined the associations of significant SNPs with expression of the genes in the same pathway using expression Quantitative Trait Loci (eQTL) analysis, based on linear regression (26). For this analysis, we adjusted for age at diagnosis, calendar period at diagnosis, tumor stage, Gleason score, and lethal cancer (which may have differential molecular signatures compared with nonlethal cancer) within the group of 154 men that was profiled for gene expression and had genotype data (82 lethal and 72 nonlethal cancers).

For the *ADRB2* gene, we further explored haplotypes but did not find any to be associated with lethal prostate cancer. The results and methods for this analysis are described in Supplementary Methods.

Because our hypothesis included four independent pathways, we considered a $P < 0.0125$ to indicate statistical significance in pathway analyses to correct for multiple testing (27). As we undertook a pathway-based approach to test specific *a priori* hypotheses, we did not control for multiple comparisons in the subsequent analyses of SNPs. All analyses were performed in R (Version 3.1.0). This study was approved by the Regional Ethical Review Board in Uppsala, Sweden.

Results

Men with lethal prostate cancer were older at diagnosis and showed more advanced Gleason score and tumor stage than men with nonlethal prostate cancer (Table 1). A similar pattern was observed for men included either in the pathway or SNP analysis (Supplementary Table S1).

Pathway analyses

Differential gene expression in the serotoninergic pathway was statistically significantly associated with the risk of lethal prostate cancer ($P = 0.007$; Table 2). In the adrenergic and glucocorticoid pathways, differential gene expression was associated with lethal cancer, although not statistically significant ($P = 0.014$ and $P = 0.020$, respectively). None of these associations were statistically significant after adjusting for Gleason score and stage, suggesting that gene expression in these pathways might mediate or entail tumor differentiation. The association of the serotoninergic pathway with lethal prostate cancer tended to be stronger among men with stage T1a, although analyses in subgroups of tumor stage and Gleason score were underpowered. When we restricted

Table 1. Clinical characteristics of prostate cancer patients in the Swedish Watchful Waiting Cohort

	Lethal prostate cancer	Nonlethal prostate cancer
Number	186	325
Age at diagnosis, y	74.1 ± 6.6	71.5 ± 6.6
Gleason score		
4–6	39 (21.0)	179 (55.1)
7	64 (34.4)	95 (29.2)
8–10	62 (33.3)	26 (8.0)
Unknown	21 (11.3)	25 (7.7)
Tumor stage		
T1a	43 (23.1)	168 (51.7)
T1b	143 (76.9)	157 (48.3)

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Table 2. Pathway tests for lethal prostate cancer (*P* values) in the Swedish Watchful Waiting Cohort[a]

	No. of patients	Adrenergic pathway	Serotoninergic pathway	Glucocorticoid pathway	Dopaminergic pathway
All genes in pathways					
No. of genes		95	48	49	31
Primary model	262	0.014	0.007	0.020	0.092
Adjusting for stage	262	0.013	0.008	0.029	0.063
Adjusting for stage and Gleason score	236	0.093	0.211	0.346	0.161
Stratified by stage					
Among stage T1a	79	0.119	0.047	0.445	0.958
Among stage T1b	183	0.112	0.089	0.067	0.059
Stratified by Gleason score					
Among Gleason 4–6	89	0.797	0.528	0.623	0.791
Among Gleason 7	95	0.462	0.795	0.151	0.335
Among Gleason 8–10	52	0.585	0.994	0.880	0.673
Exclusive genes in pathways					
No. of genes		56	18	34	8
Primary model	262	0.017	0.026	0.006	0.203

[a]All primary models were adjusted for age at diagnosis, whereas the secondary models were additionally adjusted for stage and Gleason score when indicated.

the analyses to the genes exclusive to each pathway, differential expression in the serotoninergic, adrenergic, and glucocorticoid pathways was still suggestive for lethal prostate cancer.

We found that both the adrenergic and serotoninergic pathways were statistically significantly associated with cell proliferation and presence of the *TMPRSS2–ERG* fusion (Table 3). Genes in the glucocorticoid pathway were differentially expressed in tumors with the *TMPRSS2–ERG* fusion versus without fusion or in tumors with a Gleason score of 8–10 versus 4–7, whereas the dopaminergic pathway was significantly related to cell proliferation. Moreover, there was a suggestive association of the serotoninergic and dopaminergic pathways with apoptosis, and of the dopaminergic pathway with the *TMPRSS2–ERG* fusion. None of the pathways was differentially expressed in terms of perineural invasion or PIN.

SNP analyses

Only three out of the 30 candidate SNPs were associated with risk of lethal prostate cancer (Table 4). On the basis of the overdominant model, rs2296972 in *HTR2A* (of the serotoninergic pathway) conferred a decreased risk of lethal cancer ($P = 0.002$), whereas the risk of lethal cancer tended to be higher for rs33388 in *NR3C1* (of the glucocorticoid pathway) and rs6277 in *DRD2* (of the dopaminergic pathway) ($P = 0.035$ and $P = 0.022$, respectively). However, none of the variants were associated with biomarkers or histopathologic features, except for a suggestive association between rs6277 and risk of T1b stage ($P = 0.027$). The results of other candidate SNP associations with lethal cancer are presented in Supplementary Table S4.

Further analyses showed that rs2296972 in *HTR2A* was associated with *GNAI1*/*GNAI2* expression in the serotoninergic pathway, while the variation of rs6277 in *DRD2* was correlated with the expression of genes *FOS* and *SRC* in the dopaminergic pathway. The genetic variation in rs33388 in *NR3C1* was associated with expression of genes *SMAD4*, *MAPK14*, *SLC22A1*, *POU2F1*, *MMP13*, and *NFKB1* in the glucocorticoid pathway (all $P < 0.05$; Table 5).

Discussion

In line with our previous findings in U.S. men, this study demonstrates that altered gene expression in neuroendocrine pathways might be involved in prostate cancer progression. Compared with our earlier study, this study includes a larger proportion of early prostate cancer. The current findings, therefore, add new knowledge indicating that the serotoninergic pathway, rather than the adrenergic and glucocorticoid pathways, might play a more important role in the progression of early prostate cancer. Analyses of secondary outcomes further suggest that the mechanisms involved, in addition to cell proliferation and tumor differentiation, might include the presence of the *TMPRSS2–ERG* fusion. Also, our data indicate that variants in receptor genes, particularly of HTRs, may help explain differential expression in tumors of men who develop lethal prostate cancer from localized disease.

Serotoninergic pathway

Genes in the serotoninergic pathway have previously been associated with prostate cancer differentiation. It has been reported that HTR1 and HTR4 are overexpressed in high-grade prostate cancer cells while HTR2 is upregulated in both high- and low-grade cells (28, 29). Although conflicting results exist regarding the impact of serotoninergic agonists or antagonists on cell lines (28–31), the downstream regulatory pathway through MAPK and PI3K/Akt signaling has been proposed for prostate cancer progression (32). In contrast with our previous work (6), we observed a more clear and consistent association between the serotoninergic pathway and the risk of lethal prostate cancer. The absence of an association after adjusting for Gleason score suggests that this pathway may function through tumor differentiation to impact cancer progression. Moreover, the serotoninergic pathway seemed to be linked both to increased cell proliferation and inhibited apoptosis. This finding is consistent with experimental studies demonstrating that activation of serotonergic receptors in prostate cancer cells can facilitate cell proliferation, whereas the antagonist induces apoptosis (9).

Our data further showed that the genetic polymorphism of HTR2A (rs2296972) may confer a decreased risk of lethal prostate cancer and was associated with expression of GNAI1/2, G protein subunits coupling with serotoninergic receptors in the serotoninergic pathway. The interpretation of the results from the overdominance model is not clear, but it is not inconceivable that the heterozygote carries a more influential effect on the phenotype than the homozygote (i.e. heterosis; ref. 33). Heterozygote

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Table 3. Pathway tests for secondary outcomes (*P* values) in the Swedish Watchful Waiting Cohort[a]

Secondary outcomes	No. of patients	Adrenergic pathway (95 genes)	Serotoninergic pathway (48 genes)	Glucocorticoid pathway (49 genes)	Dopaminergic pathway (31 genes)
Cell proliferation (Ki-67)	217	0.0001	<0.0001	0.090	0.001
Apoptosis (TUNEL)	250	0.161	0.016	0.222	0.047
TMPRSS2–ERG fusion	178	0.005	0.001	0.012	0.043
Gleason 8–10	236	0.182	0.152	0.0001	0.138
Tumor stage T1b	262	0.224	0.443	0.211	0.846
Perineural invasion	257	0.107	0.539	0.144	0.231
PIN	257	0.502	0.379	0.164	0.127

[a]All models were adjusted for age at diagnosis in linear regression (for continuous outcomes, i.e. cell proliferation and apoptosis), or logistic regression (for dichotomous outcomes).

advantage in various health outcomes has been documented for multiple neuroendocrine genes, including *HTR2A* (33). It is plausible that a third factor, psychologic stress in our case, may also interact with the molecular expression independent of the expected additive effect of genetic polymorphism (33). Although further studies are required to replicate our findings, together with the transcriptional data, the genetic variants of *HTR2A* may thus modulate the risk of lethal prostate cancer through serotoninergic signaling. However, while offering first possible evidence, this study cannot completely rule out alternative driving forces such as somatic mutations in tumor cells or posttranslational modifications.

Glucocorticoid pathway

In line with our prior report (6), we observed that the differential expression of glucocorticoid pathway is associated with the risk of lethal prostate cancer. Although the primary analysis including all genes did not reach the predefined significance level, the analysis of the smaller number of exclusive genes revealed strong associations and reduced our concerns for a false-positive finding due to multiple comparisons. It is further possible that, in early prostate cancer (as in this study), the downstream glucocorticoid pathway after SMAD4 is bypassed by the TGFβ/SMAD4 signaling axis, well-known to orchestrate prostate cancer development (34, 35). That said, the downstream genes included in the glucocorticoid pathway might be less relevant to cancer progression in localized disease. This could explain the stronger and statistically significant association observed when these downstream genes were excluded in our sensitivity analysis. The association of the glucocorticoid pathway with lethal prostate cancer was diminished when adjusting for Gleason score, suggesting that glucocorticoid pathway might influence cancer progression through tumor differentiation as well. It is in line with the noted association of glucocorticoid pathway with higher-grade tumors. Our results further suggest that the increased risk of lethal cancer observed with a genetic variant of *NR3C1* (rs33388) may to some extent be explained by its influence on the expression of a number of genes (including SMAD4) in the glucocorticoid pathway. More studies are needed to confirm the heterosis effect as well.

Table 4. Associations of rs2296972 (*HTR2A*), rs33388 (*NR3C1*), and rs6277 (*DRD2*) with lethal prostate cancer and secondary outcomes (*P* values) in the Swedish Watchful Waiting Cohort[a]

SNPs Genes	rs2296972 *HTR2A*	rs33388 *NR3C1*	rs6277 *DRD2*
Lethal outcome			
Additive model	0.490	0.761	0.198
Recessive model	0.075	0.099	0.014
Dominant model	0.043	0.361	0.802
Overdominant model	0.002	0.035	0.022
OR (95% CI)	0.49 (0.31–0.76)	1.59 (1.03–2.44)	1.66 (1.08–2.55)
Secondary outcomes[b]			
Cell proliferation (Ki-67)	0.817	0.190	0.528
Apoptosis (TUNEL)	0.930	0.413	0.388
TMPRSS2–ERG fusion	0.211	0.256	0.933
Gleason 8–10	0.421	0.918	0.403
Tumor stage T1b	0.986	0.654	0.027
Perineural invasion	0.477	0.493	0.267
PIN	0.141	0.920	0.262

[a]Only three SNPs associated with lethal prostate cancer were further assessed in associations with secondary outcomes.

[b]Because the overdominant model had the best fitting in analyses of the SNPs and lethal prostate cancer, the analyses of secondary outcomes were therefore all based on the overdominant model.

Adrenergic pathway

The adrenergic pathway has been reported to be more relevant to the early phase of prostate cancer development than for disease dissemination in animal models (8). In this study, a link has been suggested between lethal prostate cancer among men with localized disease and the adrenergic pathway, either including all or only pathway exclusive genes. The suggested (although not statistically significant) association between adrenergic pathway and cell proliferation in localized disease was also consistent with our previous finding (6). However, our results did not support any prominent role of genetic polymorphisms of adrenergic receptor genes in cancer progression in localized disease.

We observed strong associations between differential expression in all neuroendocrine pathways and presence of the *TMPRSS2-ERG* fusion. It lends support to the earlier findings that

Table 5. Associations of rs2296972(*HTR2A*), rs33388 (*NR3C1*), and rs6277 (*DRD2*) with mRNA expression in the corresponding pathways in the Swedish Watchful Waiting Cohort[a]

mRNAs	β	*P*
In association with rs2296972 (*HTR2A*)		
GNAI2	−0.156	0.023
GNAI1	0.223	0.035
In association with rs33388 (*NR3C1*)		
SMAD4	−0.222	0.010
MAPK14	−0.133	0.018
SLC22A1	0.161	0.028
POU2F1	−0.201	0.030
MMP13	0.068	0.040
NFKB1	0.122	0.049
In association with rs6277 (*DRD2*)		
FOS	0.302	0.021
SRC	0.151	0.032

[a]All models were adjusted for age at diagnosis, calendar period at diagnosis, tumor stage, Gleason score, and lethal cancer. Only mRNAs associated with the index SNP ($P < 0.05$) were presented in the table.

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TMPRSS2-ERG predisposes tumor cells to androgen signaling in early prostate cancer and blocks neuroendocrine transformation by downregulating neuroendocrine signaling (36). Finally, the current data are in line with our earlier study (6) reporting that the dopaminergic pathway is not associated with risk of lethal prostate cancer. Although experimental studies have shown that dopamine inhibits tumor growth and angiogenesis (37) and our data support an impact of dopaminergic pathway on cell proliferation, future studies are needed to determine its role in prostate cancer progression.

Strengths and limitations

Major merits of our study include the population-based design, the long and complete follow-up of the cohort, and the rich information spanning from genotype, gene and protein expression to clinical characteristics, all facilitating a comprehensive investigation. Also, because all cancers were detected incidentally and the men were followed without primary treatment, the profile of gene expression is not influenced by cancer treatment and the experience of the study population virtually reflects the natural course of the disease.

Several potential weaknesses need to be considered. First, the shared downstream genes may compromise the independence of the studied pathways. To this end, we present associations between lethal prostate cancer and expression in subgroups of exclusive genes in the selected pathways, which should specifically represent the neuroendocrine pathway of interest. On the basis of the consistent results between the primary and sensitivity analyses, shared genes between pathways seemed less likely to entirely explain our findings. However, the sensitivity analysis cannot completely rule out potential influence, if any, of some well-known oncogenic pathways. We, for example, kept the *EGFR* gene in the sensitivity analysis of the adrenergic pathway, but reassuringly additional analyses where EGFR was excluded showed essentially unchanged results ($P = 0.015$). Second, the measured mRNA expression may partially be derived from adjacent peripheral neurons or stromal cells. However, gene expression between tumor and adjacent cells are highly correlated in prostate cancer due to a field effect where tumor and adjacent cells tend to be alike in many biological aspects (38). Third, although the four neuroendocrine pathways are part of the stress axis, other players such as tumor-intrinsic or microenvironmental factors may also have influenced the expression of selected pathways. In the analyses of different genotypes, we made no further adjustments for multiple statistical comparisons and it is possible that some of the associations may be false-positive findings. We, however, emphasize the importance of consistent results (39) noted between pathway expression and genetic variants in association with individual neuroendocrine, particularly serotoninergic, pathways, largely refutes the possibility that our findings are completely explained by chance. Another potential weakness is that, compared with the Affymetrix microarray used in our previous study (6), fewer genes were profiled in the present microarray with 18%–37% of genes unmeasured across pathways. Nevertheless, the global test that we applied restricted every gene to have a small contributory effect on the overall pathway effect. Selection effects should therefore only have limited (if any) influence on our results. It should be noted, however, that the general null findings in pathway analyses adjusted or stratified for tumor stage and Gleason score may largely reflect limitations in statistical power rather than any biological mechanism. Finally, genes in a candidate pathway could either be overexpressed or underexpressed, and the pathway analysis does not inform about the direction of association at the pathway level. While providing a biological snapshot to describe the molecular differences in these pathways in terms of clinical and histopathologic features, we did not aim to predict clinical outcomes or to make functional claims. The novelty of this study is to add knowledge about the potential role of neuroendocrine pathways in the progression of localized prostate cancer, and to provide the insight that genetic predisposition to the neuroendocrine gene expression might be worth pursuing in the future.

In conclusion, our findings lend support to the hypothesis that dysregulated gene expression of stress-related molecular pathways, particularly the serotoninergic pathway, is involved in the progression of early-stage prostate cancer. We also present data suggesting that neuroendocrine gene expression to some extent may be explained by genetic predisposition, although further studies are needed for verification. If the altered signaling of neuroendocrine pathways in lethal prostate cancer is confirmed by functional studies, these findings could open up new opportunities for research targeted toward reducing risk of prostate cancer progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D. Lu, L.A. Mucci, U. Valdimarsdóttir, O. Andrén, F. Fang, K. Fall

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