miR-221 Is Down-regulated in *TMPRSS2:ERG* Fusion-positive Prostate Cancer

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Abstract. *Expression profiling studies using microarrays and other methods have shown that microRNAs (miRNAs) are dysregulated in a wide variety of human cancers. The up-regulation of miR-221 has been reported in carcinomas of the pancreas, breast, and papillary thyroid, as well as in glioblastoma and chronic lymphocytic leukaemia. In prostate cancer, however, down-regulation of miR-221 has been repeatedly confirmed in miRNA expression studies. Also unique to prostate cancer, and found in more than 50% of patients, is the aberrant expression of a known oncogene, the TMPRSS2:ERG fusion. To date, there has been no published study describing miRNA associations in prostate tumours that overexpress the ERG oncogene from the TMPRSS2:ERG fusion transcript. Herein we report that in a large and diverse cohort of prostate carcinoma samples, miR-221 is down-regulated in patients with tumours bearing TMPRSS2:ERG fusion transcripts, thus providing a link between miRNA and gene fusion expression.*

Prostate cancer exists along a biological continuum that ranges from clinically insignificant to extremely aggressive disease. Although clinically localized prostate cancer is largely manageable by surgery, with patients rarely developing clinical recurrence, recurrent disease remains essentially incurable. Because of this difference in treatment options, it is critical to establish specific biomarkers to differentiate between different stages of disease not only to determine presence of disease, but also to differentiate between indolent and aggressive cancer. The most common class of prostate cancer biomarker thus far are gene fusions resulting from chromosomal rearrangements. Numerous recurrent chromosomal rearrangements have been identified that are generally characterized by the fusion of various 5' regulatory elements to E twenty-six (*ETS*) transcription factors, leading to high expression of these oncogenic transcription factors. Transmembrane protease serine 2: *ETS*-related gene (*TMPRSS2:ERG*), present in over 50% of all prostate cancers, is the most commonly identified fusion gene (1).

TMPRSS2 is an androgen-responsive, prostate-specific serine protease of unknown function, and *ERG* is a member of the *ETS* transcription factor family and is rarely detected in normal prostate tissue (2). The consequences of *ERG* overexpression, and its correlation to the progression of prostate cancer remains unclear. What is known is that androgen stimulation induces the overexpression of an mRNA containing the *ERG* ORF and 3' UTR when *ERG* is fused to the *TMPRSS2* 5' UTR. *In vitro* studies have shown that overexpression of *ERG* stimulates cell migration and invasion, while its knockdown decreases the invasive properties of VCaP cells (3). In addition, it has been shown by our laboratory and others that *TMPRSS2:ERG* fusion mRNAs are present in prostate tumours but seldom in normal prostate cells. Thus, detection of abnormally high *ERG* expression could, at least theoretically, be a potential diagnostic and/or prognostic marker for prostate cancer. Although the chromosomal alterations seem to be important in the development of prostate neoplastic development, they alone may not be sufficient to induce cancer formation (2).

An active area of prostate cancer research is to find biomarkers that are predictive of recurrence in patients in order to aid oncologists in treatment or non-treatment decisions. *TMPRSS2:ERG* has been repeatedly, but not unanimously, associated with a poorer prognosis in prostate cancer patients and our laboratory, in collaboration with other groups, has previously demonstrated that prostate

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cancer patients with the *TMPRSS2:ERG* gene fusion have a higher risk of recurrence (4), while others have reported no association between this chromosomal rearrangement and clinical outcome (5). The reasons for this lack of congruity between findings are unclear and the value of *TMPRSS2:ERG* as an independent prognostic biomarker of prostate cancer remains contentious; however, it is possible technical discrepancies may be an important factor, such as institutional inconsistencies in disease staging, or statistical variations, such as disparate cohort sizes (1). In fact, studies that show an absence of clinical correlation between fusion and prognosis highlight the importance of finding out how the fusion may be epigenetically regulated.

MicroRNAs (miRNAs) have received considerable attention in recent years as possible biomarkers not only in prostate cancer, but also in various cancer subtypes. They are small, single-stranded, noncoding RNA molecules that regulate mRNA function by binding to the 3' UTR of mRNAs to which they are partially complementary, thereby repressing translation (6). MiRNAs are known to be involved in almost every cellular function, including early development (7), differentiation (8), apoptosis (9), and cell cycle regulation (10); as such, it is not surprising that miRNAs have also been linked to cancer, since misregulation of any of these important cellular functions can lead to cancer (11). Aberrant expression of miRNAs has been found in prostate cell lines, xenografts, and clinical tissues. Since the majority of cancer deaths are caused by complications from metastasis, miRNAs that specifically regulate cancer metastasis (metastamirs) are of particular interest. The study of miRNAs as biomarkers and their exact involvement in the formation and/or progression of prostate cancer is still at its early stages, and more research is needed to evaluate the potential use of miRNAs as diagnostic and prognostic markers of prostate cancer.

Given that miRNAs and fusion genes have been independently linked to prostate disease and progression, we set out to study the possible connection between miRNA regulation, prostate cancer recurrence, and *TMPRSS2:ERG* gene fusion status. We used a large cohort of men with clinically localized prostate cancer who were treated with radical prostatectomy and had long-term follow-ups. We assessed whether *miR-221*, a metastasis-promoting miRNA (12) located on the X chromosome that is differentially expressed in recurrent prostate cancer, is also associated with *TMPRSS2:ERG* fusion gene. Thereby, our study may prove applicable to future use of these regulators as surrogate biomarkers of prostate cancer.

Materials and Methods

Study participants and prostate sample collection. Tumour samples were obtained after radical prostatectomy from prostate cancer patients who had surgery at Sunnybrook Health Sciences Centre (Toronto, Canada) between 1998 and 2006. As described by Nam *et al*., following radical prostatectomy, a midsection of the specimen was snap-frozen in liquid nitrogen, and stored at −80˚C until extraction of RNA (4). Most tumours were not visible within the prostatectomy specimen, and thus, the samples obtained from the prostate were considered to be random. The banked slices of specimens were photocopied, oriented (anterior, posterior, right and left), quadrisected and cut into 5 mm sections on a cryostat. The sections were stained with haematoxylin and eosin (H&E) and then reviewed by the pathologist. The areas of tumour were marked on the stained slides and on the photocopied diagram. The marked areas were used to extract the tissue for total RNA extraction. All research was conducted with the approval of Sunnybrook Health Sciences Centre Research Ethics Board.

Patient follow-up. In 1998, a prostate tumour tissue bank was established at Sunnybrook hospital. Clinical data and follow-up information were collected prospectively. The medical records were thoroughly reviewed using standardised data entry forms by trained data abstractors and stored within a prostate cancer-specific database. Clinical follow up consisted of four assessments in the year following surgery, two assessments in the second year and one assessment every year thereafter. At each follow-up, patients had a prostate-specific antigen (PSA) test, and clinical evaluation. Biochemical recurrence was defined as a rise in blood levels of PSA in prostate cancer patients on two consecutive measurements after radical prostatectomy. Data on the following characteristics were available for each patient: Age, family history of prostate cancer, PSA score, Gleason grade, tumour stage, seminal vesicle invasion, surgical margins (categorized as positive or negative), metastasis (categorized as absence or presence), and *TMPRSS2:ERG* translocation (categorized as presence or absence). The clinical demographics of the patients used in the study are summarized in Table I.

Quantitative real-time polymerase chain reaction (PCR). Total RNA from prostate tumours was extracted with TRIzol reagent (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturers' instructions. One microgram of total RNA was reverse-transcribed using the QuantiTect Rev. Transcription Kit (Qiagen GmbH, Hilden, Germany). Quantitative real-time PCR was performed in triplicate by using QuantiTect SYBR Green PCR Kit (Qiagen GmbH, Hilden, Germany) on LightCycler Real-time PCR system (Roche Applied Science, Mannheim, Germany). The miRNA level was normalised by house keeping gene *RNU6B*. Optimized miRNA-specific primers for *miR-221,* as well as for the endogenous control *RNU6B,* are also commercially available (miScript Primer Assays; Qiagen). The relative amount of *miR-221* in each sample was calculated based on the crossing-point analysis (Relquant, version 1.01).

RT–PCR and direct DNA sequencing. Total RNA was extracted from the frozen prostate cancer tissue by homogenization in Trizol (Invitrogen Corporation) followed by ethanol precipitation. RNA pellets were dissolved in RNase-free H_2O and quality determined using 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). The presence of *TMPRSS2:ERG* was assayed using RT-PCR as previously described (4). All reactions were performed with two primer sets that yield a 125 bp (F-TAGGCGCGAGCTAAGCAGGAG, R-GTAGGC ACACTCAAACAACGACTGG) and 595 bp (F-CAGGAGGCGG AGGCGGA, R-GGCGGTTGTAGCTGGGGGTG AG) product.

Table I. *Clinical demographics of prostate cancer cases used in this study. Cohort clinical characteristics for 170 prostate cancer patients initially used in this study are summarized. Data on the following characteristics were available for each patient: Age, PSA score, Gleason grade, seminal vesicle invasion, surgical margins (categorized as positive or negative), and biochemical recurrence.*

	Total
Cohort size (n)	170
Biochemical recurrence	58
Range of follow-up (years)	1-11
Range of age (years)	38-83
Preoperative PSA (ng/ml)	
Average	9.2
Range	0.8-43.0
Gleason score	
6	31 (18.2%)
7	111 (56.3%)
8-9	14 (8.2%)
Unknown	14 (56.3%)
Pathologic stage	
Seminal vesicle invasion	17 (10%)
Positive margins	
No	86 (50.6%)
Yes	84 (49.4%)

PSA: Prostate-specific antigen.

Table II. *miR-221 is associated with TMPRSS2:ERG fusion gene status. Cohort clinical characteristics for 153 prostate cancer patients are listed for TMPRSS2:ERG fusion positive and negative patients. Age, years of follow-up, Gleason score, surgical margins, treatments, and number of patients with metastasis in both fusion-positive and -negative groups, along with miR-221 expression levels are listed.*

	TMPRSS2:ERG		
	Total	Positive	Negative
Cohort size (n)	153	83	70
Age at diagnosis, range (years)	31-75	44-75	31-73
Average follow-up (years)		4.69	5.4
Gleason score			
6	33 (21.6%)	19 (22.9%)	14 (20%)
7	106 (69.3%)	58 (69.9%)	48 (68.6%)
8-9	14 (9.1)	6 (7.2%)	8 (11.4%)
Positive margins			
No	83 (54.2%)	45 (54.2%)	38 (54.3%)
Yes	70 (45.8%)	38 (45.8%)	32 (45.7%)
Initial treatment			
Surgery only	111 (72.5%)	60 (72.3%)	51 (72.9%)
Surgery + adjuvant treatment	42 (27.5%)	23 (27.7%)	19 (27.1%)
Metastasis		7	2
Average miR-221 level		4.52	7.70

Statistical analysis. Results were statistically analysed using Prism v4.0 software (GraphPad Software Inc., La Jolla, CA, USA). Scatter plots were analysed using Student's *t*-tests which were two-tailed and unpaired. *P*-values less than 0.01 were considered statistically significant.

Results

Patient demographics. RNA was extracted from and *TMPRSS2:ERG* status analyzed in 170 radical prostatectomy samples. The distribution of clinical characteristics such as PSA, Gleason grade, pathological stage, and surgical margin status are described in Table I. Of these 170 patients, we opted to eliminate 17 from our study because certain clinical information was missing. Table II summarizes the fusion gene status and complete clinical information of the 153 remaining patients.

The age range at diagnosis of the 153 patients was 31-75 years. The average follow-up lasted 5.4 years in the fusion-negative group, and 4.69 years in the fusion-positive group. Among the cohort, 54.2% of the patients had tumours confined to the prostate gland and 69.3% of tumours were of Gleason score 7.

miR-221 is down-regulated in TMPRSS2:ERG fusion gene-positive prostate tumours. Due to the high prevalence of *TMPRSS2:ERG* in prostate cancer and its association with higher chances of recurrence and poorer prognosis, we hypothesized that *miR-221*, an miRNA previously linked to metastasis and recurrence in prostate cancer, may be associated with the fusion gene. To test our hypothesis, *TMPRSS2:ERG* status was determined for 153 radical prostatectomy samples by using RT–PCR and sequencing using random and oligo-dT primers. Prostate samples from 83 out of 153 (54.2%) patients were found to be positive for transcripts of *TMPRSS2:ERG*, while 70 (45.8%) lacked the fusion gene (Table II). *TMPRSS2:ERG* fusion-positive samples produced the expected 125 or 595 bp bands depending upon which primer set was used, as previously described (4). To analyse whether *miR-221* expression was associated with the presence of the fusion gene, quantitative RT-PCR was performed, which showed that the mean expression level of *miR-221* was significantly lower ($p<0.01$) in fusion-positive (4.52±0.34) compared with fusion-negative (7.70±1.09) tumours (Figure 1). Gleason grade and surgical margin status were not different between fusion-positive and fusion-negative populations (Table II).

Low miR-221 expression is associated with metastasis and biochemical recurrence of prostate tumour. To further analyse the association of *miR-221* levels with the aggressiveness of prostate cancer, we categorized the tumour samples into two subgroups: those from patients who had metastasis at the time of radical prostatectomy and/or had biochemical recurrence

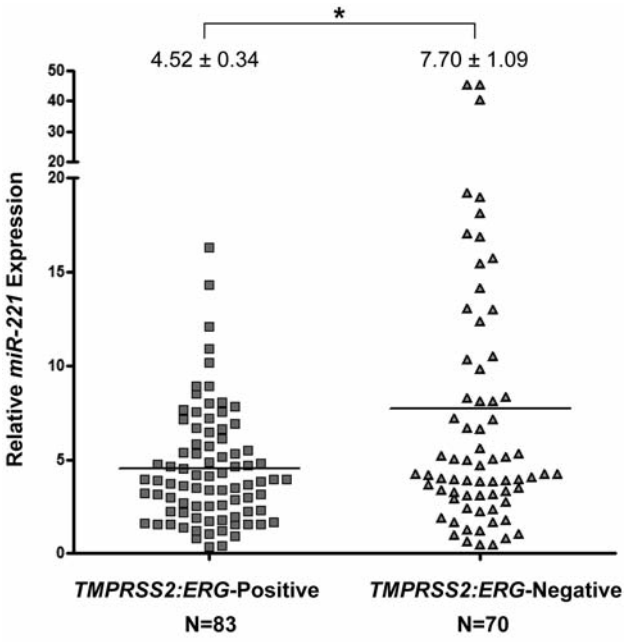

Figure 1. *miR-221 is down-regulated in prostate tumours with TMPRSS2:ERG fusion gene. Relative miR-221 expression was analysed in 153 prostate carcinoma samples using qRT-PCR. Specifics of the patient samples are summarized in Table II. *Significant reductions in the median expression levels (black lines) between subgroups (p<0.01). p-Values were calculated with two-tailed t-test.*

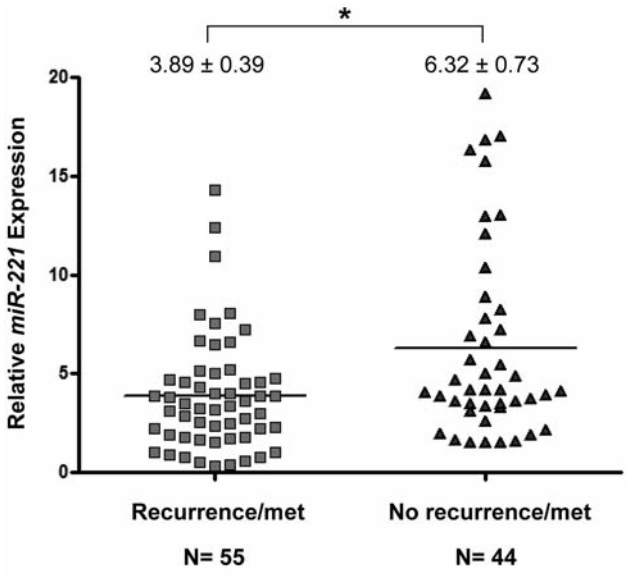

Figure 2. *Low miR-221 expression is significantly associated with metastasis and biochemical recurrence of prostate tumours. Relative miR-221 expression was analysed in 99 prostate carcinoma samples using qRT-PCR. Subsequently, the patients were divided into subgroups of tumour aggressiveness based on the presence of metastasis or biochemical recurrence (left plot), or its absence (right plot). Specifics of the patient samples are summarized in Table III. *Significant reductions in the median expression levels (black lines) between subgroups (p<0.01). p-Values were calculated with two-tailed t-test.*

in follow-up years and those from patients with non-metastatic and non-recurrent disease. Long-term follow-up information on all 153 patients was not available, as some had moved to other hospitals or did not follow-up with their appointments. Of the 153 tumours, 99 were from patients with sufficient follow-up, more than 5 years on average (Table III). These 99 patients were divided into two groups: those with recurrent and/or metastatic tumours, and those with non-recurrent and non metastatic tumours.

Quantitative RT-PCR was carried out in order to analyse the expression levels of *miR-221* in prostate tumours. The qRT-PCR analysis using the 55 recurrent and/or metastatic samples and 44 non-recurrent and non-metastatic samples confirmed that the mean expression level of *miR-221* was down-regulated ($p<0.01$) in the tumours with metastasis and/or recurrence (3.89±0.39) compared to tumours with no metastasis or recurrence (6.32±0.73) (Figure 2).

miR-221 levels are lower in recurrent/metastatic TMPRSS2:ERG fusion-negative tumours. We wanted to further analyse the differential expression of *miR-221* in relation to both genetic aberrations and clinical parameters: specifically, *TMPRSS2:ERG* fusion status, metastasis and recurrence. All 99 tumour samples from patients with long-term follow-ups were studied. We found that tumours positive for the oncogenic *TMPRSS2:ERG* had down-regulated *miR-221* levels with or without metastasis and biochemical recurrence (4.20 and 4.22, respectively) (Figure 3). In *TMPRSS2:ERG* fusion-negative tumours, on the other hand, *miR-221* expression differed with clinical status: *miR-221* levels were significantly lower in patients with recurrence and/or metastasis (3.52) than in those with no recurrence or metastasis (8.62) (Figure 3).

Discussion

The potential utility of the *TMPRSS2:ERG* fusion product as an independent prognostic marker for patients with clinically localised prostate cancer is becoming clearer every year. Initial studies comparing clinico-pathological parameters (4, 13-16) and prognostic significance (4, 13, 14, 17-19) of this fusion gene showed conflicting results. Some studies showed no correlation between histological grade (Gleason score) (13), while others found positive associations (16, 17), and yet others demonstrated correlations between fusion status and tumour stage (14, 15). Wang *et al*. examined 119 patients for fusion status from a case-control approach and found significant correlations with tumour stage, but no associations were found with early recurrence (20).

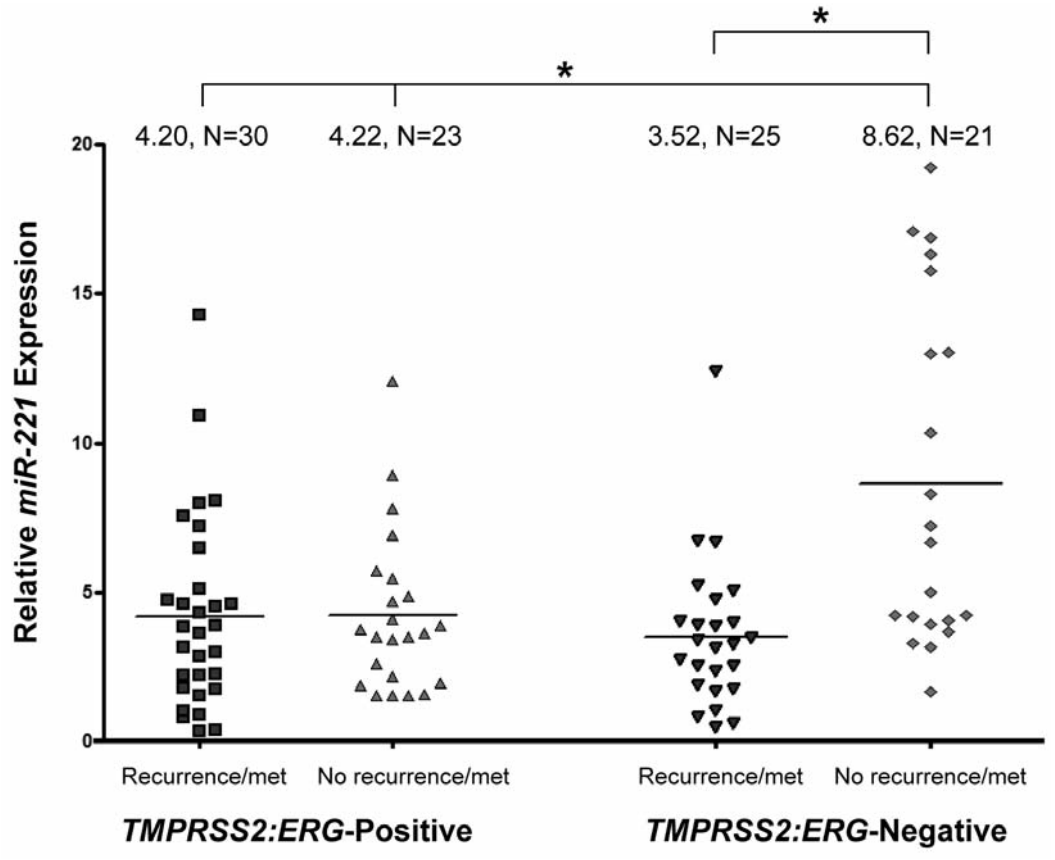

Figure 3. *miR-221 levels are lower in recurrent/metastatic TMPRSS2:ERG fusion-negative tumours. Relative miR-221 expression was analysed in 99 prostate carcinoma samples using qRT-PCR in relation to both TMPRSS2:ERG and metastatic potential. *Significant reductions in the median expression levels (black lines) between subgroups (p<0.01). p-Values were calculated with two-tailed t-test.*

Table III. *miR-221 is predictive of prostate cancer recurrence. Cohort clinical characteristics for 99 prostate cancer patients are listed for patients with recurrent and/or metastatic disease and those with non-recurrent and non-metastatic disease. Age, years of follow-up, PSA, Gleason score, surgical margins, seminal vesicle invasion, and miR-221 expression levels are summarized.*

	Total	Recurrentand/or metastatic	Non recurrent and Non-metastatic
Cohort size (n)	99	54	45
Age at diagnosis, range (years)	44-75	50-75	44-70
Average follow-up (years)	5.33	5.23	5.45
Preoperative PSA (ng/ml)			
Average	9.24	10.36	7.73
Range	0.8-42.99	3-42.99	0.8-27
Gleason score			
6	17	2	15
7	73	43	30
8-9	9	9	0
Positive margins			
Yes	61	47	14
No	38	7	31
Seminal vesicle invasion T3b	16	16	0
Average *miR-221* level		3.89	6.32

Furthermore, Lapointe *et al*. in another case-control study found no correlations with any clinico-pathological parameter and recurrence-free survival (13). However, two cohort studies of men with clinically localised prostate cancer who did not undergo treatment (*i.e*. watchful waiting) showed that men who had *TMPRSS2:ERG* fusion had lower prostate cancer-specific survival compared to men without fusion expression (17, 18). Patients managed and selected for watchful waiting from these cohorts have different baseline distributions in grade, stage and PSA level to patients treated with surgery and may not be comparable. Initially, it was unclear whether the *TMPRSS2:ERG* gene fusion is only a surrogate marker for established prognostic factors of grade and stage, or whether it is an independent molecular-based marker for disease recurrence with no association with grade or stage, particularly for patients who are candidates for surgery for clinically localised prostate cancer.

Since that time, other groups, including our own, have found robust associations. More than two dozen high-impact reports have found clinically significant links to fusions in greater than 50% of over 1500 samples of clinically localized prostate cancer (1). Furthermore, a recent publication by Carver *et al*. showed that prostatic intraepithelial neoplasia is induced in host prostates by transgenic overexpression of ERG (21), suggesting that high frequency of ETS genetic rearrangements and subsequent overexpression of *ETS* factors may represent a crucial event in prostate tumourigenesis (22).

In addition to *TMPRSS2:ERG*, there are increasing reports of miRNAs or specific miRNA signatures that correlate with a wide range of clinico-pathological features in prostate cancer. Many miRNAs are found to be predictive of patient clinical outcome and/or response to treatment, suggesting that miRNAs can be used as diagnostic or prognostic/predictive biomarkers (23). Investigations of miRNA dysregulation in prostate cancer typically compare miRNA expression profiles in prostate cancer *versus* normal/benign tissues. Two studies have included prostate cancer among other human cancer types, finding overall down-regulation of miRNAs in solid tumours compared with normal tissues (24, 25). A study comparing prostate tumours with benign prostate tissue found 37 down- and 14 up-regulated miRNAs (26). Others have found either overall down-regulation of miRNAs (27) or general up-regulation of miRNAs (28). Most significantly for prostate cancer, Spahn *et al*. assessed miRNA expression profiles in lymph node metastasis of prostate carcinoma and found that *miR-221* down-regulation was a hallmark of metastasis (29). In a larger patient group, they found that the expression of *miR-221* was associated with prostate cancer progression and clinical recurrence.

miR-221 is known to be overexpressed in tumours of the breast (30), pancreas (31), glioblastoma (32), papillary thyroid carcinoma (33), and chronic lymphocytic leukaemia (34). Up-regulation of *miR-221* in chronic lymphocytic leukaemia was found to be associated with a poor prognosis. Functional studies have found that *miR-221* induces down-regulation of *p27* and that inhibition of *miR-221* impairs tumour formation in xenografted mice (35). Indeed, high levels of *miR-221* are required in many different cancer types to inhibit the expression of p27, and stimulate proliferation.

However, none of these studies have looked for miRNA associations in prostate tumours that overexpress the *ERG* oncogene from the *TMPRSS2:ERG* fusion transcript. To that end, we investigated *miR-221* expression in tumours bearing the *TMPRSS2:ERG* fusion transcript. Using a large and diverse cohort of prostate carcinoma samples, including patients with or without the fusion gene, as well as patients with different clinical progressions, we found that *miR-221* is down-regulated in both patients with *TMPRSS2:ERG* fusion gene and in patients with more aggressive tumours.

As a major centre for prostate cancer, we assembled a large database and tissue bank from patients undergoing a prostate biopsy to determine the presence of prostate cancer and surgery for clinically local prostate cancer. This resource has been used for study of multiple genetic and serological markers for prostate cancer diagnosis and prognosis (4, 14, 36-40). The resource includes men who underwent a prostate biopsy because of an abnormal PSA or digital rectal examination and preserves outcome data, along with DNA, plasma, and paraffin-embedded tumour samples. *TMPRSS2:ERG* status was determined as presence or absence by RT-PCR of RNA for samples used in this study and Table I shows the range of clinical and molecular characteristics.

Almost half of the samples (54.2%) bear *TMPRSS2:ERG* fusion transcripts (Table II). We found that *miR-221* expression in fusion-positive tumours is 1.7-fold lower than in fusion-negative samples, with a tighter distribution (Figure 1, Table II). Overexpression of the *ERG* transcription factor is a powerful inducer of prostate tumourigenesis, as we and others have reported. Similarly, one would also expect to see down-regulation of tumour suppressors, such as *miR-221* in at least some prostate tumours. It is therefore possible that the *ERG* transcription factor or one of its target genes could directly down-regulate *miR-221*.

A subset of 99 cases from the full cohort had enough clinical follow-up with which to examine the long-term consequences of *miR-221* dysregulation in prostate cancer patients (Table III). Average *miR-221* expression was 1.6-fold lower in samples from tumours that recurred or metastasized after surgery (Figure 2). Previously we found that the *TMPRSS2:ERG* fusion is also strongly associated with recurrence. That report and our finding here that *miR-221* is reduced in fusion-positive tumours suggests that they have a cumulative effect on tumour aggressiveness. It may be that *TMPRSS2:ERG* fusion has the stronger effect as we found that tumours positive for the oncogenic *TMPRSS2:ERG* have lower levels of *miR-221* regardless of recurrence status (Figure 3).

In numerous other cancer types, *miR-221* is up-regulated and acts to induce proliferation and tumour formation by down-regulating the *p27* tumour suppressor. The role of *miR-221* in prostate cancer appears to be different than in other cancer types, as reported here and by others (29). Although the Spahn group did not examine the mechanisms, they found that miR-221 is down-regulated in metastatic tumours (29). We also found down-regulation of *miR-221* in prostate tumours and that such down-regulation can occur in the absence of *TMPRSS2:ERG*. Although results shown in Figure 1 suggested a link between fusion status and *miR-221* status, direct dependence of *miR-221* expression on *TMPRSS2:ERG* in every tumour seems unlikely given the independence of *miR-221* down-regulation in fusion-negative tumours.

This is the first study to reveal that *miR-221* down-regulation in prostate cancer is associated with the presence of the oncogenic *TMPRSS2:ERG* fusion transcript. Understanding how this association leads to greater metastasis and biochemical recurrence will facilitate understanding of prostate cancer biology. More practically, a deeper molecular understanding of the mechanisms of prostate cancer genesis and maintenance will eventually provide therapeutic interventions designed to affect the unique characteristics of individual prostate tumours.

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References

1 Kumar-Sinha C, Tomlins SA and Chinnaiyan AM: Recurrent gene fusions in prostate cancer. Nat Rev Cancer *8*: 497-511, 2008.

2 Clark J, Merson S, Jhavar S, Flohr P, Edwards S, Foster CS, Eeles R, Martin FL, Phillips DH, Crundwell M, Christmas T, Thompson A, Fisher C, Kovacs G and Cooper CS: Diversity of *TMPRSS2-ERG* fusion transcripts in the human prostate. Oncogene *26*: 2667-2673, 2007.

3 Tomlins SA, Laxman B, Varambally S, Cao X, Yu J, Helgeson BE, Cao Q, Prensner JR, Rubin MA, Shah RB, Mehra R and Chinnaiyan AM: Role of the *TMPRSS2-ERG* gene fusion in prostate cancer. Neoplasia *10*: 177-188, 2008.

4 Nam RK, Sugar L, Yang W, Srivastava S, Klotz LH, Yang LY, Stanimirovic A, Encioiu E, Neill M, Loblaw DA, Trachtenberg J, Narod SA and Seth A: Expression of the *TMPRSS2:ERG* fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer *97*: 1690-1695, 2007.

5 Gopalan A, Leversha MA, Satagopan JM, Zhou Q, Al-Ahmadie HA, Fine SW, Eastham JA, Scardino PT, Scher HI, Tickoo SK, Reuter VE and Gerald WL: *TMPRSS2-ERG* gene fusion is not associated with outcome in patients treated by prostatectomy. Cancer Res *69*: 1400-1406, 2009.

6 He L and Hannon GJ: MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet *5*: 522-531, 2004.

7 Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, and Bartel DP: MicroRNAs in plants. Genes Dev *16*: 1616-1626, 2002.

8 Chen CZ, Li L, Lodish HF and Bartel DP: MicroRNAs modulate hematopoietic lineage differentiation. Science *303*: 83-86, 2004.

9 Xu P, Vernooy SY, Guo M and Hay BA: The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. Curr Biol *13*: 790-795, 2003.

10 Bueno MJ, Perez de Castro I and Malumbres M: Control of cell proliferation pathways by microRNAs. Cell Cycle *7*: 3143-3148, 2008.

11 Calin GA and Croce CM: MicroRNA–cancer connection: the beginning of a new tale. Cancer Res *66*: 7390-7394, 2006.

12 Hurst DR, Edmonds MD and Welch DR: Metastamir: the field of metastasis-regulatory microRNA is spreading. Cancer Res *69*: 7495-7498, 2009.

13 Lapointe J, Kim YH, Miller MA, Li C, Kaygusuz G, van de Rijn M, Huntsman DG, Brooks JD and Pollack JR: A variant *TMPRSS2* isoform and *ERG* fusion product in prostate cancer with implications for molecular diagnosis. Mod Pathol *20*: 467-473, 2007.

14 Nam RK, Sugar L, Wang Z, Yang W, Kitching R, Klotz LH, Venkateswaran V, Narod SA and Seth A: Expression of *TMPRSS2:ERG* gene fusion in prostate cancer cells is an important prognostic factor for cancer progression. Cancer Biol Ther *6*: 40-45, 2007.

15 Perner S, Demichelis F, Beroukhim R, Schmidt FH, Mosquera JM, Setlur S, Tchinda J, Tomlins SA, Hofer MD, Pienta KG, Kuefer R, Vessella R, Sun XW, Meyerson M, Lee C, Sellers WR, Chinnaiyan AM and Rubin MA: *TMPRSS2:ERG* fusion-associated deletions provide insight into the heterogeneity of prostate cancer. Cancer Res *66*: 8337-8341, 2006.

16 Rajput AB, Miller MA, De LA, Boyd N, Leung S, Hurtado-Coll A, Fazli L, Jones EC, Palmer JB, Gleave ME, Cox ME and Huntsman DG: Frequency of the *TMPRSS2:ERG* gene fusion is increased in moderate to poorly differentiated prostate cancers. J Clin Pathol *60*: 1238-1243, 2007.

17 Attard G, Clark J, Ambroisine L, Fisher G, Kovacs G, Flohr P, Berney D, Foster CS, Fletcher A, Gerald WL, Moller H, Reuter V, De Bono JS, Scardino P, Cuzick J and Cooper CS: Duplication of the fusion of *TMPRSS2* to *ERG* sequences identifies fatal human prostate cancer. Oncogene *27*: 253-263, 2008.

18 Demichelis F, Fall K, Perner S, Andren O, Schmidt F, Setlur SR, Hoshida Y, Mosquera JM, Pawitan Y, Lee C, Adami HO, Mucci LA, Kantoff PW, Andersson SO, Chinnaiyan AM, Johansson JE and Rubin MA: *TMPRSS2:ERG* gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene *26*: 4596-4599, 2007.

19 Narod SA, Seth A and Nam R: Fusion in the *ETS* gene family and prostate cancer. Br J Cancer *99*: 847-851, 2008.

20 Wang J, Cai Y, Ren C and Ittmann M: Expression of variant *TMPRSS2/ERG* fusion messenger RNAs is associated with aggressive prostate cancer. Cancer Res *66*: 8347-8351, 2006.

21 Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, Alimonti A, Nardella C, Varmeh S, Scardino PT, Cordon-Cardo C, Gerald W and Pandolfi PP: Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nat Genet *41*: 619-624, 2009.

22 Squire JA: *TMPRSS2-ERG* and PTEN loss in prostate cancer. Nat Genet *41*: 509-510, 2009.

23 Garzon R, Calin GA and Croce CM: MicroRNAs in cancer. Annu Rev Med *60*: 167-179, 2009.
24 Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR and Golub TR: MicroRNA expression profiles classify human cancers. Nature *435*: 834-838, 2005.
25 Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC and Croce CM: A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA *103*: 2257-2261, 2006.
26 Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL and Visakorpi T: MicroRNA expression profiling in prostate cancer. Cancer Res *67*: 6130-6135, 2007.
27 Ozen M, Creighton CJ, Ozdemir M and Ittmann M: Widespread deregulation of microRNA expression in human prostate cancer. Oncogene *27*: 1788-1793, 2008.
28 Ambs S, Prueitt RL, Yi M, Hudson RS, Howe TM, Petrocca F, Wallace TA, Liu CG, Volinia S, Calin GA, Yfantis HG, Stephens RM and Croce CM: Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. Cancer Res *68*: 6162-6170, 2008.
29 Spahn M, Kneitz S, Scholz CJ, Stenger N, Rudiger T, Strobel P, Riedmiller H and Kneitz B: Expression of microRNA-221 is progressively reduced in aggressive prostate cancer and metastasis and predicts clinical recurrence. Int J Cancer *127*: 394-403, 2010.
30 Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M and Croce CM: MicroRNA gene expression deregulation in human breast cancer. Cancer Res *65*: 7065-7070, 2005.
31 Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postier RG, Brackett DJ and Schmittgen TD: Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer *120*: 1046-1054, 2007.
32 Ciafre SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, Negrini M, Maira G, Croce CM and Farace MG: Extensive modulation of a set of microRNAs in primary glioblastoma. Biochem Biophys Res Commun *334*: 1351-1358, 2005.
33 He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM and de la Chapelle A: The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci USA *102*: 19075-19080, 2005.
34 Calin GA, Ferracin M, Cimmino A, Di LG, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M and Croce CM: A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med *353*: 1793-1801, 2005.
35 Mercatelli N, Coppola V, Bonci D, Miele F, Costantini A, Guadagnoli M, Bonanno E, Muto G, Frajese GV, De MR, Spagnoli LG, Farace MG and Ciafre SA: The inhibition of the highly expressed miR-221 and miR-222 impairs the growth of prostate carcinoma xenografts in mice. PLoS One *3*: e4029, 2008.
36 Nam RK, Jewett MA, Krahn MD, Robinette MA, Tsihlias J, Toi A, Ho M, Evans A, Sweet J and Trachtenberg J: Delay in surgical therapy for clinically localized prostate cancer and biochemical recurrence after radical prostatectomy. Can J Urol *10*: 1891-1898, 2003.
37 Nam RK, Trachtenberg J, Jewett MA, Toi A, Evans A, Emami M, Narod SA and Pollak M: Serum insulin-like growth factor-I levels and prostatic intraepithelial neoplasia: a clue to the relationship between IGF-I physiology and prostate cancer risk. Cancer Epidemiol Biomarkers Prev *14*: 1270-1273, 2005.
38 Nam RK, Zhang WW, Jewett MA, Trachtenberg J, Klotz LH, Emami M, Sugar L, Sweet J, Toi A and Narod SA: The use of genetic markers to determine risk for prostate cancer at prostate biopsy. Clin Cancer Res *11*: 8391-8397, 2005.
39 Nam RK, Toi A, Klotz LH, Trachtenberg J, Jewett MA, Loblaw A, Pond GR, Emami M, Sugar L, Sweet J and Narod SA: Nomogram prediction for prostate cancer and aggressive prostate cancer at time of biopsy: utilizing all risk factors and tumor markers for prostate cancer. Can J Urol *13(Suppl 2)*: 2-10, 2006.
40 Vesprini D, Nam RK, Trachtenberg J, Jewett MA, Tavtigian SV, Emami M, Ho M, Toi A and Narod SA: HPC2 variants and screen-detected prostate cancer. Am J Hum Genet *68*: 912-917, 2001.

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