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PTEN loss and ERG Protein Expression are Infrequent in Prostatic Ductal Adenocarcinomas and Concurrent Acinar Carcinomas

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Abstract

Background—Prostatic ductal adenocarcinoma is an unusual and aggressive morphologic subtype of prostate cancer. *PTEN* gene deletion and *ERG* gene rearrangement are among the most common genomic changes in acinar prostate cancers. Though ductal adenocarcinoma most commonly occurs with synchronous usual-type acinar adenocarcinoma, little is known about the molecular phenotype of these mixed tumors.

Methods—We used genetically validated immunohistochemistry (IHC) assays to assess PTEN and ERG status in a group of 37 surgically treated ductal adenocarcinomas and 18 synchronous acinar adenocarcinomas where we have previously reported *ERG* gene rearrangement status by fluorescence *in situ* hybridization (FISH). A group of 34 stage and grade-matched pure acinar adenocarcinoma cases was studied as a control.

Results—ERG IHC was highly concordant with *ERG* FISH results, with 100% (36/36) concordance among ductal adenocarcinomas and 91% (31/34) concordance among 34 pure acinar carcinomas. Similar to previous FISH results, ERG expression by IHC was significantly less common among ductal adenocarcinomas (11% or 4/37) and their synchronous acinar tumors (6% or 1/18) compared to matched pure acinar adenocarcinoma cases (50% or 17/34; $p = 0.0005$ and 0.002 , respectively). PTEN loss by IHC was also less common among ductal adenocarcinomas (18% or 6/34) and their synchronous acinar tumors (22% or 4/18) compared to matched pure acinar carcinomas (50% or 17/34; $p = 0.01$ and 0.08 , respectively). As expected, PTEN loss was enriched among ERG positive compared to ERG-negative tumors in the pure acinar tumor control group (2.5-fold enrichment; $p = 0.04$) however this was not observed among the ductal adenocarcinomas (1.5 fold enrichment; $p = \text{NS}$). Of ductal adenocarcinomas with an evaluable synchronous acinar component, ERG status was concordant in 94% (17/18) and PTEN status was concordant in 94% (16/17).

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Conclusions—Based on PTEN and ERG, ductal adenocarcinomas and their concurrent acinar carcinomas may be clonally related in some cases and show important molecular differences from pure acinar carcinoma.

Keywords

Prostatic adenocarcinoma; ductal adenocarcinoma; endometrioid; PTEN; ERG; immunohistochemistry

Introduction

Ductal adenocarcinoma of the prostate is a rare morphologic subtype, accounting for less than 1% of all cases in its purest form and occurring intermixed with usual type acinar adenocarcinoma in as many as 5% of prostate cancer cases [1–4]. Classically arising in or involving the large periurethral ducts near the verumontanum, ductal adenocarcinoma frequently presents with urinary symptoms and is associated with significantly lower serum PSA levels compared to acinar prostate carcinoma cases [2, 4, 5]. Most contemporary studies have shown that ductal adenocarcinomas or mixed ductal and acinar tumors with >10% ductal component are associated with a higher stage at radical prostatectomy compared to acinar adenocarcinomas and may be less responsive to hormonal therapies [1, 3, 6–8]. Accordingly, ductal adenocarcinoma is more likely to present with distant metastasis than acinar tumors and shows a two-fold increase in risk of prostate cancer specific mortality in population-based studies [2, 4]. However, despite compelling data to suggest important clinical-pathologic differences between ductal and acinar prostatic adenocarcinoma, there have been relatively few molecular studies of the relationship between these two entities [9, 10].

With the advent of next generation sequencing, the genomic landscape of acinar prostate cancer has become increasingly clear. Although the mutation rate is relatively low compared to other epithelial tumor types, copy number alterations and rearrangements are common [11–14]. Among these, rearrangements involving the *ERG* gene or other ETS family members and deletion of the *PTEN* tumor suppressor gene are some of the most common alterations seen in primary acinar carcinomas. Though *ERG* rearrangements are not associated with altered prognosis in most surgically treated cohorts [15, 16], *PTEN* deletions are strongly associated with increased pathologic grade and stage and poor clinical outcomes [17–32]. Of note, PTEN loss likely occurs subsequent to *ERG* gene rearrangements in the majority of acinar adenocarcinomas [33–35] and is more common in *ERG*-rearranged tumors for reasons that remain unclear [13, 27–29, 33, 35–40].

To begin to molecularly subclassify prostate ductal adenocarcinoma, we used genetically validated immunohistochemistry assays to examine ERG and PTEN status in ductal tumors and their concurrent acinar components, as well as in a matched control group of pure acinar tumors. Previously, we used fluorescence *in situ* hybridization (FISH) in the same cohorts to demonstrate that *ERG* gene rearrangements occur significantly less frequently in prostatic ductal adenocarcinomas compared to pure acinar carcinomas [9]. Though ERG protein expression is highly correlated with *ERG* gene rearrangement in untreated acinar

carcinomas, this concordance is less robust in subtypes of prostatic carcinoma with decreased androgen signaling and relatively low PSA levels, including small cell carcinoma [41, 42] and prostatic adenocarcinomas exposed to androgen deprivation therapy [43]. Because ductal carcinoma typically presents with lower levels of PSA, we hypothesized that androgen signaling might be decreased in this tumor subtype and thus there might be a greater discordance between ERG protein expression and gene rearrangement status than seen in acinar carcinoma.

Here, we show that ERG expression is, in fact, highly correlated with underlying *ERG* gene rearrangement status in ductal adenocarcinomas. Further, we demonstrate that PTEN loss occurs significantly less frequently in ductal adenocarcinoma and associated acinar carcinoma compared to pure acinar carcinoma and is not significantly enriched among ERG-positive ductal adenocarcinomas as it is in ERG-positive acinar carcinomas. Finally, using combined PTEN and ERG status, we provide additional evidence of a likely clonal relationship between concurrent ductal and acinar adenocarcinomas in some cases.

Materials and Methods

Tissue Selection

The tumor cohorts utilized in this study have been described in detail previously [9, 44] and the study was approved by the Johns Hopkins IRB. Briefly, two tissue microarrays (TMAs) were manually constructed from 39 radical prostatectomy specimens with ductal adenocarcinoma retrieved from the surgical pathology and consultation files of the Johns Hopkins Hospitals from 1984–2005. Overall, 95% (37/39) had evaluable tumor tissue present on the TMAs for ERG and/or PTEN immunohistochemistry. In 49% (18/37) of these cases, a concurrent acinar component was present and included and evaluable in the TMA. The ductal and acinar adenocarcinoma components were considered geographically separate primaries when the different morphologic components were separated by at least 5 mm in all dimensions. This was the case in 33% of tumors with a concurrent acinar component (6/18, Table 1). In 66% (12/18), the ductal and acinar tumor components were intermingled, or closer than 5 mm from one another (Table 1). In each case, quadruplicate 0.6 mm cores were punched from the ductal adenocarcinoma component, the acinar component (when present) and the surrounding benign prostatic tissue, with up to 16 total cores from each patient represented on the array.

As an internal control group, 14 “pure” acinar adenocarcinoma cases (Gleason pattern 4) were included and evaluable on the ductal adenocarcinoma tissue microarray. To provide additional pure acinar adenocarcinoma controls, an additional 20 acinar adenocarcinoma cases were selected for matched pathologic stage from a second tissue microarray constructed from a PSA-era prostatectomy cohort (cases dating from 1993–2000) [15]. As a group, these 34 pure acinar adenocarcinoma controls were matched with the ductal adenocarcinoma group for pathological stage and grade.

Immunohistochemistry

PTEN immunohistochemistry (IHC) was performed on the Ventana platform (Ventana Discovery Ultra, Ventana Medical Systems, Tucson, AZ) using a rabbit anti-human PTEN antibody (Clone D4.3 XP; Cell Signaling Technologies, Danvers, MA). We previously validated a manual version of this assay using the same primary antibody [30]. PTEN protein status was visually scored by a urologic pathologist (TLL). A tissue core was considered to have PTEN protein loss if the intensity of cytoplasmic and nuclear staining was markedly decreased or entirely negative across >10% of tumor cells compared to surrounding benign glands and/or stroma, which provide internal positive controls [30]. If PTEN was lost in >10% and <100% of the tumor cells sampled in a given core, the core was annotated as showing heterogeneous PTEN loss. Alternatively, if the core showed PTEN loss in 100% of sampled tumor glands, the core was annotated as showing homogeneous PTEN loss. Cores were scored as having ambiguous PTEN IHC results when the intensity of the tumor cell staining was light or absent in the absence of evaluable internal benign glands or stromal staining. For statistical analysis, each patient's tumor sample was scored for the presence or absence of PTEN loss by summarizing the scores of the sampled cores. Based on a comparison with fluorescence *in situ* hybridization (FISH) data from 550 prostate cancer cases in an independent multi-institutional radical prostatectomy cohort, any loss of PTEN immunostaining (homogeneous or heterogeneous) using this assay was 98% sensitive for detecting homozygous *PTEN* gene deletion, 62% sensitive for detecting hemizygous *PTEN* gene deletion and 91% specific for intact *PTEN* gene [45].

ERG IHC was performed on the Ventana platform (Ventana Discovery Ultra, Ventana Medical Systems, Tucson, AZ) using a rabbit anti-human ERG antibody (Clone EPR3864; Ventana Medical Systems, Tucson, AZ). ERG protein status was visually scored by a urologic pathologist (TLL) blinded to the *ERG* FISH results. Cases were scored as positive if any tumor cells demonstrated nuclear ERG staining. Based on a comparison with FISH data from 408 prostate cancer cases at radical prostatectomy from Johns Hopkins, positive ERG immunostaining using this assay was 88% sensitive for detecting *ERG* gene rearrangement and negative ERG immunostaining was 87% specific for absence of *ERG* gene rearrangement ([15] and GJ Netto, AM De Marzo and TL Lotan, unpublished data). Further, the results of the automated ERG assay were highly concordant with results using a manual staining protocol and an independent ERG antibody clone (9FY, Biocare Medical), showing agreement in 96.2% of 289 cases (kappa = 0.923 or "very good agreement") ([46] and GJ Netto, AM De Marzo and TL Lotan, unpublished data).

Statistical Analysis

Fisher's exact test was used to compare frequency of PTEN loss and ERG expression fusion in ductal adenocarcinoma cases versus acinar adenocarcinoma controls.

Results

ERG expression

ERG protein was expressed in 11% (4/37) of evaluable ductal adenocarcinomas. Overall, ERG expression was highly concordant with presence of underlying *ERG* gene

rearrangement by FISH, with 100% (36/36) of ductal adenocarcinomas with IHC and FISH results showing concordance. Three cases with *ERG* rearrangement via deletion showed *ERG* protein expression, one case with *ERG* gene rearrangement via translocation showed *ERG* protein expression and 32 cases lacking *ERG* gene rearrangements by FISH were all negative for *ERG* protein (Table 1, Figure 1).

ERG IHC results were evaluable in 18 acinar carcinomas occurring concurrently with ductal adenocarcinoma. Among these, 6% (1/18) showed *ERG* protein expression. This acinar tumor showed *ERG* rearrangement via translocation on FISH and occurred with a ductal adenocarcinoma with an identical FISH and IHC result (Figure 1). The remaining 17 cases lacking *ERG* protein expression also lacked *ERG* gene rearrangement, such that there was 100% concordance (18/18) overall for *ERG* IHC and FISH among concurrent acinar adenocarcinomas.

Finally, *ERG* IHC was evaluable in 34 pure acinar adenocarcinomas. These tumors were roughly matched by stage and grade with the ductal adenocarcinomas as described previously [9]. Among these cases, 50% (17/34) showed *ERG* protein expression. Overall, there was 91% (31/34) concordance for *ERG* FISH and *ERG* IHC results in the pure acinar adenocarcinomas, including 9 cases rearranged by deletion, 6 cases rearranged by translocation and 16 cases without *ERG* gene rearrangement. Of the 3 discordant cases, one showed *ERG* gene rearrangement by deletion without *ERG* protein expression and two cases showing *ERG* protein expression without detection of underlying *ERG* gene rearrangement by FISH. Confirming the results of our prior FISH study, the lower rate of *ERG* protein expression among ductal adenocarcinomas (11%) and their associated acinar counterparts (6%) compared to pure acinar adenocarcinomas (50%) was statistically significant ($p=0.0005$ and 0.002 , respectively by Fisher's exact test).

PTEN loss

PTEN loss by IHC was evaluable in 34 ductal adenocarcinomas, of which 18% (6/34) showed loss (Figures 1 and 2). Though PTEN loss is frequently focal or heterogeneous among acinar adenocarcinomas [30, 33–35], the loss was homogeneous in all sampled tumor cells in all 6 ductal adenocarcinomas (Figure 1). Among acinar carcinomas occurring concurrently with ductal adenocarcinoma, 22% (4/18) showed PTEN loss, and the loss was homogeneous in all cases (Figures 1 and 2). In contrast, 50% (17/34) of pure acinar adenocarcinomas unassociated with a ductal component showed PTEN loss, with heterogeneous loss in 12% (2/17). The lower rate of PTEN loss among ductal adenocarcinomas and their associated acinar components compared to pure acinar adenocarcinomas was statistically significant or fell just short of conventional statistical significance ($p = 0.01$ and 0.08 , respectively by Fisher's exact test). As seen in prior FISH studies [13, 27–29, 33, 35–40], PTEN loss was about 2.5-fold enriched among *ERG*-positive compared to *ERG*-negative tumors in the pure acinar tumor control group (71% or 12/17 vs 29% or 5/17, respectively; $p=0.04$). However significant enrichment was not observed among the ductal adenocarcinomas (25% or 1/4 vs 17% or 5/30; $p=0.55$), a result that could potentially be due to the low numbers of cases with *ERG* expression among the ductal tumors.

Comparison of PTEN/ERG status in ductal adenocarcinoma and concurrent acinar tumors

Finally, of ductal adenocarcinomas with an evaluable concurrent acinar component, ERG status was concordant between the two components in 94% (17/18), including 16 ERG-negative cases and one ERG positive case with underlying ERG translocation in both components (Figure 1). The single discordant case showed ERG expression and *ERG* gene rearrangement by deletion in the ductal component and absence of ERG expression and gene rearrangement in the acinar component (Figure 1, case 30). PTEN expression was intact in both components. As previously reported, these components were spatially separated and may have represented synchronous but clonally distinct tumors. Similarly, PTEN status was concordant in 94% (16/17) of ductal adenocarcinomas with an evaluable concurrent acinar component, including 3 cases with PTEN loss and 13 cases with intact PTEN immunostaining. The single discordant case showed intact PTEN expression in the ductal component and loss of PTEN expression in the comingled acinar component, and lack of ERG protein expression or *ERG* gene rearrangement in both components (Figure 2).

Discussion

ERG gene rearrangements have emerged as one of the earliest and most common genomic changes in acinar prostate cancer, occurring in roughly 50% of Caucasian cases in most surgical cohorts [16]. Of interest, *ERG* gene rearrangements are less common in acinar carcinomas occurring in African-American patients [47–49], slightly less common among older patients in low grade tumors [50] and infrequent among some unusual morphologic subtypes of prostate cancer such as p63-expressing acinar carcinoma [51]. In addition, the frequency of *ERG*-rearranged acinar tumors appears to depend on tumor location. Both anterior and transition zone carcinomas have been reported to have lower rates of *ERG* gene rearrangement compared to posterior peripheral zone tumors [52, 53], and lower rates have also been seen in studies where tumor tissue was sampled on transurethral resection of the prostate [54, 55]. Given that the occurrence of *ERG* gene rearrangements has been shown to be mediated in part by androgen receptor activity [56,57], these findings raise the important question of whether lower rates of *ERG* rearrangement may be due to lower basal levels of androgen signaling in some of these settings.

Perhaps more similar to centrally located acinar tumors occurring in the transition zone, prostatic ductal adenocarcinomas classically occur in or at least involve the periurethral region in a large fraction of cases [58] and show low rates of *ERG* rearrangement by both FISH and IHC compared to peripheral zone acinar carcinomas in the current study. Interestingly, ductal adenocarcinomas classically present with lower PSA levels than acinar tumors [2], suggesting that they may also have lower levels of basal androgen signaling, another potential explanation for their low rate of *ERG* gene rearrangement. Despite this possibility that they may have lower basal androgen signaling, all ductal tumors with *ERG* gene rearrangement by FISH did show ERG protein expression, confirming that some AR signaling is occurring in the rearranged tumors, where *ERG* oncogene expression is driven by an androgen regulated gene, *TMPRSS2*. In contrast, in prostate tumors with absent androgen receptor signaling due to androgen deprivation therapy or absence of the androgen receptor itself (such as small cell carcinoma), ERG IHC is relatively insensitive for detecting

underlying *ERG* gene rearrangement [41–43]. Of interest, the only other study to investigate *ERG* protein expression in ductal adenocarcinomas did not find a lower rate of expression in these tumors versus acinar carcinomas [59]. Though this study did not do confirmatory FISH, this discrepancy with our current results raises the interesting question of whether variable criteria for diagnosing ductal adenocarcinoma (vs acinar carcinoma) may in part explain these differences [60]. Indeed, ductal adenocarcinoma and intraductal spread of acinar carcinoma of the prostate have overlapping morphologic features [3] and previous work by our group has suggested a relatively high rate of *ERG* expression among the latter entity [61].

Another interesting finding in current study is that *PTEN* loss occurs relatively uncommonly in ductal adenocarcinoma and acinar carcinomas associated with ductal carcinoma compared to acinar carcinomas occurring without a ductal component. In acinar carcinoma, we and others have shown that *PTEN* loss is more frequent among tumors with higher Gleason grade, occurring in more than 40% of Gleason score 8–10 tumors [30]. There is a similar association of *PTEN* loss with higher pathologic stage [30]. Given that ductal differentiation is considered roughly equivalent to Gleason score 8 acinar disease and typically presents at higher stage than acinar tumors [1–4], it is surprising that *PTEN* loss occurs in ductal adenocarcinomas at roughly half the rate of grade and stage-matched acinar carcinomas in the current series. This low rate of *PTEN* loss is also seen in acinar carcinomas occurring concurrently with ductal carcinomas, suggesting they may belong to a common, and molecularly distinct subclass from usual-type acinar carcinomas. Additional evidence that ductal adenocarcinomas are molecularly distinct from acinar tumors comes from the fact that *PTEN* loss does not appear to be enriched among *ERG* positive ductal tumors as is seen in acinar carcinoma. Although it remains unclear why this enrichment of *PTEN* loss occurs among *ERG*-positive acinar tumors in humans, genetically engineered mouse models have suggested these two genetic alterations may have synergistic effects on tumor cell growth, migration and androgen signaling, leading to selection for *ERG*-positive *PTEN*-negative tumor clones [37, 38, 62]. However, human studies examining the interaction between *PTEN* loss and *ERG* expression for disease outcomes have been mixed [29, 36, 50, 63, 64]. This raises the possibility that *ERG* rearrangement, which precedes *PTEN* deletion in most cases, may directly or indirectly predispose tumor cells to *PTEN* loss. In either case, the lack of enrichment of *PTEN* loss among *ERG*-positive ductal tumors may imply important biological and molecular differences between ductal and acinar tumors. However, one limitation of the current study is that since the rates of *ERG* expression and *PTEN* loss are both quite low among ductal tumors, we had relatively low power to detect an interaction in the ductal data. Thus larger studies of ductal carcinoma are required to substantiate this finding.

Finally, comparison of both *PTEN* and *ERG* status between ductal carcinomas and their concurrent acinar tumors adds additional evidence that when they co-exist, these two tumor types may be clonally related. Consistent with our prior study where we examined only *ERG* status on the two tumor components [9], the frequently concordant *PTEN* status between the two components also suggests a likely clonal relationship in some cases. Importantly, our *PTEN* IHC assay is highly genetically validated, enabling us to use it as a proxy for

underlying PTEN gene status to infer a likely clonal relationship between two tumors. In a recent comparison with 4-color FISH for *PTEN* gene deletion, intact PTEN immunostaining was 91% specific for the absence of *PTEN* gene deletion by FISH and 98% and 62% sensitive for the detection of homozygous and hemizygous gene deletion, respectively, by FISH [45] (TL Lotan and Jeremy A. Squire et al, in preparation). Thus, in cases where there is concordant PTEN protein loss between the ductal and acinar tumor components, it is highly likely that this signifies a similar *PTEN* gene deletion in both components and, accordingly, a clonal relationship between the two. Clearly, however, the relatively rare occurrence of PTEN loss and ERG expression in the ductal cases means that it is impossible to assess the likely clonality of the majority of ductal tumors co-occurring with acinar carcinoma and thus additional whole genome or whole exome sequencing studies will be required to confirm these data.

In summary, we have found that ERG protein expression and *ERG* FISH are highly concordant in ductal adenocarcinomas of the prostate, just as seen in acinar carcinomas. Using ERG IHC, we have confirmed that ductal adenocarcinomas have relatively lower rates of *ERG* gene rearrangement compared to peripherally-located acinar tumors, and more consistent with rates seen in centrally-located acinar tumors. In addition, we find that PTEN loss is relatively less common in ductal adenocarcinoma than in stage- and grade-matched acinar tumors, and PTEN status is highly concordant between concurrently occurring ductal and acinar tumors in the same patient, additional evidence for a clonal relationship between the two in at least some cases.

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Figure 1.

Top panels: ERG protein is expressed consistent with previously reported *ERG* gene rearrangement by translocation in the ductal and acinar components of Case 16. Similarly, PTEN protein is lost in both components, with positive internal control staining in the prostatic stroma and endothelial cells (all micrographs at 100× magnification). Bottom panels: ERG protein is expressed by the ductal component of Case 30 and absent from the spatially separate (>3mm away) acinar component, consistent with previously reported *ERG* gene rearrangement by deletion in the ductal component and absence of the rearrangement in the acinar component. PTEN expression is intact in both components (all micrographs at 100× magnification).

Figure 2.

Case 21 shows intact PTEN immunostaining in the ductal component, with loss of PTEN expression in the acinar component, while ERG is not expressed in either component. Adjacent benign glands provide an internal positive control for PTEN immunostaining.

Table 1

Case #:	Pathologic stage	Overall Gleason score	Spatial relationship	ERG FISH ductal component*	ERG FISH acinar component*	ERG IHC ductal component	ERG IHC acinar component	PTEN IHC ductal component	PTEN IHC acinar component
1	TX NX MX	U		D		1		1	
2	TX NX MX	U		N		0		1	
3	TX NX MX	U		N		0		1	
4	T3A N0 MX	7		N		0		1	
5	T3A N0 MX	7		N		0		0	
6	T2 N0 MX	7	Comingled	N	N	0	0	1	1
7	TX NX MX	U	Unknown	N	N	0	0	1	1
8	T3B N0 MX	7	Comingled	N	N	0	0	0	0
9	T2 N0 MX	7	Comingled	N	N	0	0	1	1
10	T3A N0 MX	7		D		1		1	
11	T3B N0 MX	8		N		0		A	
12	T3A N0 MX	8	Unknown	N	N	0	0	0	0
13	T3A N0 MX	8		N		0		1	
14	T3B N0 MX	7		N		0		1	
15	T3A N0 MX	7	Separate	N	N	0	0	A	1
16	T3A N0 MX	7	Comingled	T	T	1	1	0	0
17	T2 N0 MX	7	Comingled	N	N	0	0	A	A
18	TX N0 MX	7	Comingled	N	N	0	0	1	1
19	T3A N0 MX	7		N		0		1	
20	T2 N0 MX	7		N		0		1	
21	T2 N0 MX	8	Comingled	N	N	0	no tumor	1	0
22	T2 N0 MX	8	Comingled	N	N	0	0	1	1
23	T2 N0 MX	7		N		no tumor		no tumor	
24	T2 N0 MX	8	Unknown	N	N	0	0	1	1
25	T3A N0 MX	7		N		0		1	
26	T3A N0 MX	8		N		0		1	

Case #:	Pathologic stage	Overall Gleason score	Spatial relationship	ERG FISH ductal component *	ERG FISH acinar component *	ERG IHC ductal component	ERG IHC acinar component	PTEN IHC ductal component	PTEN IHC acinar component
27	T3B N1 MX	9	Comingled	N	N	0	0	1	1
28	TX N0 MX	7		N		no tumor		no tumor	
29	T3A N0 MX	7	Separate	N	N	0	0	1	1
30	T3A N0 MX	8	Separate	D	N	1	0	1	1
31	T2 N0 MX	7	Comingled	N	N	0	no tumor	1	no tumor
32	T2 N0 MX	7	Separate	N	N	0	no tumor	1	no tumor
33	T2 N0 MX	7		N		0		1	
34	T2 N0 MX	8	Comingled	N	N	0	0	1	1
35	T2 N0 MX	8	Comingled	N	N	0	0	1	1
36	T3B N1 MX	8	Comingled	N	N	0	0	1	1
37	T3B N0 MX	9	Comingled	N	N	0	0	1	1
38	T3A N0 MX	7		N		0		0	
39	T3A N0 MX	7		NA		0		0	

* FISH results presented for comparison purposes and previously published in Reference 9.

N= Normal ERG FISH; D=ERG gene rearrangement via deletion; T=ERG gene rearrangement via translocation; 0=absence of staining by IHC; 1=staining present by IHC; NA=not assessed; U=unknown.