

The identification of chromosomal translocation, t(4;6)(q22;q15), in prostate cancer

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ABSTRACT

Our previous work identified a chromosomal translocation t(4;6) in prostate cancer cell lines and primary tumors. Using probes located on 4q22 and 6q15, the breakpoints identified in LNCaP cells, we performed fluorescence *in situ* hybridization analysis to detect this translocation in a large series of clinical localized prostate cancer samples treated conservatively. We found that t(4;6)(q22;q15) occurred in 78 of 667 cases (11.7%). The t(4;6)(q22;q15) was not independently associated with patient outcome. However, it occurs more frequently in high clinical T stage, high tumor volume specimens and in those with high baseline PSA (P=0.001, 0.001 and 0.01 respectively). The t(4;6)(q22;q15) occurred more frequently in samples with two or more *TMPRSS2:ERG* fusion genes caused by internal deletion than in samples without these genomic alterations, but this correlation is not statistically significant (P=0.0628). The potential role of this translocation in the development of human prostate cancer is discussed.

Keywords: Prostate cancer, chromosome translocation, genomic instability, prognosis

Introduction

Prostate cancer is the most common male malignancy and the second most common cause of cancer death in men in the Western countries.¹ Prostate cancer is a heterogeneous disease with a highly variable natural history of disease development and progression.² Many prostate cancers diagnosed at an early stage are indolent but a proportion progress rapidly to become life threatening. However it is difficult to predict the progression potential of early stage cancers.³ Despite numerous investigations into the molecular mechanism of development of the disease,⁴ the nature and significance of genetic changes associated with prostate cancer development and progression are largely unknown. These highlight the need for genetic investigations into this malignancy.⁵

Chromosomal translocations are recognized initiating events in some hematological malignancies and soft tissue sarcomas, and are associated with tumor progression and even response to therapies.⁶ Recent identification of frequent fusion genes in prostate⁶⁻⁸ and lung^{9,10} cancers highlights the potential roles of recurrent chromosomal translocations and fusion genes in solid tumors. Fusion of the *ETS* transcription factor genes to *TMPRSS2* and other genes has been identified in a high proportion of prostate cancer cases.^{7,8} The association of *ERG* fusion with poor outcome in conservatively managed patients has been reported,¹¹ although recent studies correlating genomic alterations and prostate cancer patient outcome in large series of clinical samples demonstrated genetic instability to be critical in prostate cancer development and progression.^{12,13} In addition, recent transcriptome sequencing analysis revealed many more fusion genes and chromosomal alterations occurring in prostate cancer cells, although some of them may arise in late stage during cancer progression.¹⁴

Using multiplex fluorescence *in situ* hybridization (M-FISH) we previously identified a t(4;6) chromosomal translocation in prostate cancer cell lines and primary tumors¹⁵ and mapped the breakpoints in LNCaP cells¹⁵ using FISH analysis. In this study, according to the breakpoint locations revealed, we performed an extensive analysis of clinical prostate cancer samples by FISH on tissue microarrays (TMAs) using probes located on 4q22 and 6q15. We identified the t(4;6)(q22;q15) chromosomal translocation in a number of prostate cancer samples and correlated it with clinical features.

Materials and methods

Clinical materials and TMA construction

Four batches of TMAs were made: the first batch included one TMA containing 16 cases of non-prostate non-malignant controls from a variety of human tissue types (Table 1). The second was a TMA made from 34 benign prostate hyperplasia (BPH) samples collected from the Barts and The London Hospital transurethral resection of prostate (TURP) specimens. The third comprised two TMAs constructed from 68 archival anonymous prostate cancer samples from radical prostatectomy specimens and 6 morphologically non-malignant prostate samples obtained from the Barts and The London Hospital. These three batches of TMAs were constructed in 35x22x4 mm blocks of paraffin wax using a manual tissue microarrayer (Beecher Instruments, Sun Prairie, WI, USA). Triplicate cores of 1 mm diameter were taken from each sample. The collection of these specimens was approved by the Local Ethical Committee.

The fourth batch comprised 24 TMAs constructed from a large cohort of 808 cases of TURP prostate cancer specimens. All the patients were managed conservatively without initial treatment except early hormone management. Clinical outcome data

were available for all these cases. The median follow-up time was 121 months (8–203 months) and more than 80% of the men were diagnosed after the age of 65. The 10-year overall survival rate was 50% and 17% of the patients died of prostate cancer². TMAs were constructed using a manual tissue microarrayer into 35x22x7 mm paraffin wax blocks. Up to four tumor cores of 0.6 mm diameter were taken from each prostate sample. National approval was obtained from the Northern Multi-Research Ethics Committee for the collection of the cohort and followed by Local Research Ethics Committee approval at individual collaborating hospitals.

A pathologist (DB) examined all samples and graded each cancer specimen with Gleason scores and the samples on the fourth batch of TMAs were also centrally reviewed by pathologists (DB, CSF and VR) in the Transatlantic Prostate Group (TAPG).

FISH probe preparation

Bacterial artificial chromosomes (BACs) including RP11-18N21, RP11-681L8 and RP11-240J11 on distal 4q22 (probe set I, see Fig. 1A), RP11-111J1, RP11-595C20 and RP1-214H13 on 6q14.3 proximal to the 6q15 breakpoint (probe set II, see Fig. 1B) and RP1-44N23, RP1-154G14 and RP11-104N3 on distal 6q15 (probe set III, see Fig. 1C) were obtained from the Wellcome Trust Sanger Institute (Hinxton Hall, Cambridge, UK). The BAC DNA extraction, amplification and labeling were the same as previously described.¹⁶ Briefly BAC DNA was amplified using illustra GenomiPhi V2 DNA amplification kit (GE Healthcare Life Sciences, Buckinghamshire, UK) following the manufacture's instruction and then labeled with biotin or digoxigenin (DIG) using the nick translation method.

FISH analysis

The FISH analysis was performed using the method as previously described¹² with slight modifications. Briefly, 4 µm TMA sections were cut onto SuperFrostPlus glass slides (VWR International, Poole, UK) and baked at 65°C over night. TMA slides were dewaxed in xylene and fixed with boiling ethanol. The tissue sections were then brought to boiling-point in pre-treatment buffer (SPOT-light tissue pre-treatment kit, Zymed, South San Francisco, CA, USA) before cooling to room temperature and digested with pepsin solution (Digest All-3, Invitrogen, Paisley, UK). Each TMA slide was co-denatured at 95°C for 7 min with 13µl hybridization buffer containing about 100ng of each DNA probe and then hybridized at 37°C overnight in a Vysis HYBrite (Abbott Diagnostics, Berkshire, UK). For the co-localization analysis of the t(4;6)(q22;q15), the probe set I on distal 4q22 location (Fig. 1A) was labeled with biotin; the probe set II on 6q14.3 (Fig. 1B) was labeled with DIG. For the 6q15 break-apart assay, probe set III (Fig. 1C) corresponding to the 6q15 deleted region in LNCaP was labeled with biotin and it was used in combination with the DIG-labeled probe set II on proximal 6q15. Following hybridization and the post-hybridization probe wash, the biotin-labeled probes were detected using Streptavidin-Cy3 conjugate (Sigma-Aldrich, Poole, UK) and the DIG labeled probes visualized by anti-DIG-FITC (Roche, Welwyn Garden City, UK). 20 µL Vectashield anti-fade containing DAPI (Vector labs, Burlingame, CA, USA) was mounted on each slide before slides were stored or analyzed. All the TMA slides were fluorescently scanned at x40 magnification on an Ariol SL-50 system (Applied Imaging, San Jose, CA, USA) with seven 0.5 µm z-stacks, and images were stored for future analysis.

Evaluation of the FISH results in each core was performed in a double blind manner. FISH signal co-localization was defined as a red and a green signal

overlapping or separated by a distance of less than one signal-diameter. In the 6q15 probe break-apart assay, chromosome split was defined as a red and a green signal separated by a distance of more than two signal-diameters. The schematic interpretation of FISH signals associated with t(4;6)(q22;q15) was presented in Figure 2. The scanned images were reviewed manually to identify regions with apparently increased frequency of co-localization signals. We counted cells in the above regions, or in two randomly selected regions from each core with apparently equal distribution of co-localization signals. A minimum of 50 and in most cases (80%) 100 cells with both green and red signals in a continuous tissue area were counted. Cores with high background or very weak signals that affected the signal assessment were excluded from analysis. We also excluded cases without 50 scorable cells from data interpretation.

Statistical analysis

The statistical analyses were carried out as previously described.¹² Associations between t(4;6)(q22;q15) status and categorical data were examined using the χ^2 test for trend. Associations between t(4;6)(q22;q15) status and numerical variables were assessed using analysis of variance. Univariate and multivariate analyses using proportional hazard regression¹⁷ were applied to determine the impact of t(4;6)(q22;q15) on prostate cancer specific death and death for any causes. For the multivariate analyses the variables used were age at diagnosis, Gleason score, baseline PSA and tumor volume in the biopsy.

Results

Recurrence of t(4;6)(q22;q15) in primary tumor samples

As co-localization of the 4q22 and 6q14.3 probes can also be caused by co-localization of these two chromosomes by chance rather than translocation, we expected to see a low frequency of co-localized signals in normal tissue cells. Therefore we determined the frequency of signal co-localization in 16 morphologically normal tissues (Table 1). The co-localized signals in all the normal samples occurred at a low frequency, ranging from 1% to 6% with the mean of 2.7094 and standard deviation (SD) of 1.2151. A sample area without co-localized signal is shown in Figure 3A. Although it is widely accepted that the diagnostic cutoff is calculated as the mean plus three times SD of false-positive findings in at least five normal controls, we used a higher cutoff of 15% which is considered a reasonable cutoff for single co-localization analysis.¹⁸ We subsequently scored prostate tumor samples as positive for t(4;6)(q22;q15) if 15% or more co-localized signals were detected within a cancer lesion containing more than 50 cells with both green and red signals.

Applying the above criteria, we firstly screened for t(4;6)(q22;q15) on the BPH and prostate cancer samples on the Barts TMAs using FISH probe co-localization analysis. Out of the 68 prostate cancer samples on the two Barts tissue arrays, 56 were scorable and 4 out of these samples (7.2%) were considered positive for t(4;6)(q22;q15). A representative FISH image is shown in Figure 3B. A similar screening of the 34 BPH cases did not detect any positive samples. Cells with co-localization signals in these samples range from 0% to 9.7% with median of 3.1%.

After confirming the re-occurrence of t(4;6)(q22;q15) chromosomal translocation in prostate cancer, we further investigated a large cohort of localized prostate cancers initially managed conservatively on 24 TMAs using the FISH probe co-localization assay. Out of the 808 cancers 667 cases were informative and 78 (11.7%) samples

were t(4;6)(q22;q15) positive. The chromosomal translocations were confirmed using the signal break-apart approach on two randomly selected TMAs, which were re-hybridized for FISH analysis using probes either side of the 6q15 centromeric breakpoint of LNCaP cells (Fig. 2C-E). Of the five t(4;6)(q22;q15) positive cases, all of them were confirmed with the break-apart assay: three cases with splitting signals and two cases showing loss of 6q15 probe red signals, indicating deletion of this chromosome region as shown in Figure 2E. Figure 4A and 4C show examples of cells with co-localized signals in the probe co-localization analysis; Figure 4B and 4D show the same cells but detected with the signal break-apart assay. The cell with split red and green signals is indicated in Figure 4B and the cell with missing red signal was indicated in Figure 4D.

During our FISH analysis, we also revealed that the t(4;6)(q22;q15) status was heterogeneous within each case of cancer. The t(4;6)(q22;q15) was only found in some patched areas of each section. The high frequency of signal co-localizations in one cancer area but lack of co-localizations in the other cancer areas indicates the heterogeneity of t(4;6)(q22;q15) in prostate cancer cells within individual samples.

Clinical Significance of t(4;6)(q22;q15)

As the clinical and patient outcome data are available for the large cohort of surveillance managed cancers, we studied the correlation between the t(4;6)(q22;q15) status and these clinical data. The univariate Cox model analysis showed that t(4;6)(q22;q15) was not a significant prognostic factor of either cause-specific survival (hazard ratio (HR) = 1.27, 95% CI 0.81-1.99, p=0.30) or overall survival (HR = 1.06, 95% CI 0.78-1.45, p=0.69) (Fig. 5A and B). The translocation was not significantly associated with patient age. However it had a significant positive

association with higher Gleason score, clinical stage, baseline PSA and larger tumor volume in the biopsy (p=0.04, 0.001, 0.01 and 0.001 respectively) (Table 2). Adding t(4;6)(q22;q15) to a multivariate Cox model with Gleason score, extent of disease, baseline PSA and age at diagnosis did not significantly improve the model (HR=0.98, 95% CI=0.62-1.54, p=0.93 and HR=0.90, 95% CI=0.66-1.23, p=0.50 for cause-specific survival and overall survival, respectively).

Association of t(4;6)(q22;q15) with ERG gene rearrangements

Since the translocation status of *ERG* was already defined in this large cohort of prostate cancers,¹² we assessed whether there was an association between t(4;6)(q22;q15) and *ERG* gene rearrangements. In 510 cases of the cancer samples, both t(4;6)(q22;q15) and *ERG* gene rearrangement status were available. Table 3 shows the number of t(4;6) negative and positive samples in each of the subgroups with different *ERG* genomic status as described previously.¹² In general, no correlation between t(4;6)(q22;q15) and *ERG* gene rearrangements was identified in these samples. However, t(4;6)(q22;q15) apparently occurred more frequently in 2+Edel positive than negative samples (20.9% vs 12.6%), although this correlation is not statistically significant (P=0.0628).

Discussion

Recent discovery of recurrent fusion genes in a range of carcinomas,^{6,9,10,19,20} including prostate cancer,^{7,8,14} suggests an important role for chromosomal rearrangements in solid tumors.⁶ Here we confirmed the frequent occurrence of a novel chromosomal translocation in human prostate cancer. Previously, we reported the t(4;6) translocation in prostate cancer cell lines and a small set of primary prostate

tumors, without definition of the chromosome breakpoints in these samples except in LNCaP cells.¹⁵ Using BAC clones flanking the 4q22 and 6q15 translocation breakpoints defined in LNCaP cells, we have now confirmed a similar translocation to occur in a considerable proportion of primary prostate cancers. Although FISH technology has the advantage to detect translocations occurring at a chromosome region with variant breakpoints (within a couple of Mb), it cannot define the actual breakpoints due to its relatively low resolution.

The recurrent chromosomal rearrangements previously identified mainly lead to gain of functions of genes located at the breakpoints.⁶ However the mechanism of action of the t(4;6)(q22;q15) translocation is less clear. In LNCaP cells, a small deletion adjacent to the translocation breakpoints occurred at both chromosomes 4 and 6 leading to four breakpoints instead of two.¹⁵ *UNC5C* located at the centromeric breakpoint on 4q22 was the only known gene interrupted by these four breakpoints. No currently known genes were located at the other three breakpoints. We failed to identify *UNC5C* fusion transcripts by RT-PCR in LNCaP cells (unpublished data) and no fusion transcripts involved *UNC5C* or correspond to this chromosomal rearrangement have been identified in this cell line by deep sequencing using the next generation sequencing technology.¹⁴ One possibility is that the t(4;6)(q22;q15) may contribute to prostate cancer development through inactivation of tumor suppressor genes rather than gain of function by forming fusion genes. Inactivation of genes through chromosomal translocations has been reported previously.^{6,21-26} Inactivation of *UNC5C*, a putative tumor suppressor gene occurs in various human tumors, including prostate cancer²⁷⁻³⁰ and we detected under-expression of *UNC5C* in LNCaP and other prostate cancer samples (data to be published separately). 4q and 6q are among the most frequently deleted chromosome regions in human tumors and 6q15

deletion has been associated with a subtype of prostate cancer.³¹⁻³³ The 6q15 chromosome region has been proposed to harbour candidate tumor suppressor genes in prostate cancer, such as the *MAP3K7*.³¹ The subsequent effect of t(4;6)(q22;q15) may be a consequence of the inactivation of candidate tumor suppressor genes located either at the breakpoints or within the adjacent deleted regions. The high resolution provided by recently developed microarray technology allows detection of sub-microscopic deletions associated with cytogenetically balanced translocations.^{34,35} In a wide range of leukaemias, including CML, AML and ALL, these microdeletions coupled with chromosomal translocations are associated with poor prognosis.³⁶

A second possibility is that the translocation is a reflection of genomic instability. Genomic instability is an important mechanism in carcinogenesis^{37,38} and there is evidence that genomic instability, particularly chromosomal instability, involves in prostate cancer development and progression and it occurs as early as at the precursor stage.³⁹⁻⁴¹ Prostate cancer frequently presented as multiple foci lesions and different genomic alterations, including multiple forms of *TMPRSS2:ETS* fusions, frequently occur in different foci within a same case of prostate cancer, which indicated that these multiple foci arise independently.⁴¹⁻⁴⁴ This independent generation of multiple foci within a same prostate, together with the identification of genetic alterations in the normal prostate epithelia cells adjacent to cancer lesions, suggests that there is underlying mechanism leading to genomic instability in the prostate cells.⁴¹ This genomic instability may induce multiple genomic alterations and chromosomal rearrangements, among them the t(4;6) translocation. This genomic instability caused t(4;6) translocation may explain why the t(4;6)(q22;q15) was identified in only a proportion of cancer cells in each specimen. The occurrence of genetic alterations in a proportion of cancer cells has been observed previously in prostate cancer, including

the commonly deleted *PTEN* genes.^{45,46} Heterogeneity of *PTEN* deletions has also been observed in our FISH analysis of prostate cancer samples from prostatectomy (unpublished data). While the t(4;6) translocation may represent genomic instability in the translocation positive samples, the frequent detection of t(4;6)(q22;q15) may also indicate that 6q15 and 4q22 are unstable genomic regions in prostate cancer cells, which correlates with the frequent genomic copy number changes of these regions as discussed above. Further investigations are now required to understand the biological mechanism of the t(4;6)(q22;15) translocation.

In the current study, t(4;6)(q22;q15) translocation was not independently associated with prognostic potential, specifically poor patient outcome. However it was significantly associated with tumors of high tumor volume and relatively late clinical T stage ($p = 0.001$). In our previous analysis of factors affecting patient outcome in this cohort, Gleason score was shown as the strongest predictor while the clinical T stage had little impact.² Therefore, t(4;6)(q22;q15) may affect cancer cell proliferation locally in the prostate, but not contribute much to tumor metastasis or other features associated with poor patient outcome. In this study, we determined the correlation between *ERG* gene rearrangements and t(4;6)(q22;q15) and found that t(4;6)(q22;q15) has slightly increased frequency in 2+Edel positive prostate cancer samples. In our previous study, we have demonstrated that prostate cancers with 2+Edel are associated with bad prognosis.¹² There might be a subtype of t(4;6) which is associated with 2+Edel positive and bad prognosis cancers. Due to the relatively low frequency of both t(4;6) and 2+Edel in prostate cancer, the number of double positive cases in this study ($n=9$) is small for statistical analysis. Further study in a larger sample series is required to determine the real correlation between t(4;6) and 2+Edel genomic alterations.

Due to the complexity and heterogeneity of the genomic changes in epithelial originated cancer, chromosome translocations and fusion genes were only revealed in recent years as common genetic alterations in carcinomas, particular in prostate cancer, with the development of new genomic technology.⁶⁻¹⁰ The *TMPRSS2:ERG* fusion, the most common fusion gene and occurring in about half of prostate cancer, has been well studied, but its association with the disease progression is still debatable and its actual contribution to prostate carcinogenesis has to be further investigated. Although initial studies linked *TMPRSS2:ERG* fusion to high clinical stage and more aggressive cancers,⁴⁷⁻⁴⁹ additional studies showed that this fusion gene is not generally associated with prostate cancer patient outcome, except the amplified fusion genes with deletion between *TMPRSS2* and *ERG*.^{12,13} There are other ETS transcription factor family genes fused to either *TMPRSS2* or other genes at low frequency (<10%).⁸ The recent transcriptome sequencing analysis, using the currently most advanced technology - the next generation sequencing, revealed many more fusion genes and chromosomal alterations occurring in prostate cancer cells.¹⁴ However, all the above abnormalities in prostate cancer were only detected using approaches focused on identifying chromosome rearrangements producing expressed fusion products (proteins or transcripts). In human cancers, many genomic translocations or fusions do not lead to fusion RNA or protein product. While it is known that some chromosome translocations can lead to inactivation of genes located at or close to the breakpoints,⁶ the significance of these translocations without fusion transcripts should be extensively investigated when the capacity of the next generation is further increased to sequence the entire human genome. The t(4;6)(q22;q15) is the first recurrent chromosome translocation potentially without a

fusion transcript identified in prostate cancer and its contribution to prostate cancer development and progression is to be further clarified.

In summary, we have confirmed in a large series of prostate cancer clinical samples that t(4;6)(q22;q15) is a frequent chromosomal translocation in prostate cancer. While it does not affect patient outcome independently, it does not occur in non-malignant prostatic epithelium and hence it may define the development of a specific cohort of prostate cancers. The consequence of this genomic alteration and affected genes should be further investigated.

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Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Maps showing the position of the BACs used as probes in FISH assays. (A) Probe set I includes three BACs, RP11-18N21, RP11-681L8 and RP11-240J11, corresponding to a 0.54Mb region of distal 4q22 breakpoint. (B) Probe set II includes three BAC clones, RP11-111J1, RP11-595C20 and RP1-214H13, corresponding to a 0.39Mb region of 6q14.3 (proximal to 6q15 breakpoint). (C) Probe set III includes three BACs, RP1-44N23, RP1-154G14 and RP11-104N3, corresponding to a 0.80Mb region of 6q15 deleted in LNCaP cells.

Figure 2. Schematic representation of FISH detection of t(4;6)(q22;q15). In the signal co-localisation study (A-B), probe set I and II were used and in the signal split apart analysis (C-E), probe set II and III were used. (A) In a normal cell, there are two pairs of normal chromosome 4 and 6 carrying hybridized red (probe set I) and green (probe set II) signals respectively. In an interphase nucleus, it shows two pairs of separated green and red signals. (B) In a cancer cell with t(4;6)(q22;q15), there are one of each normal chromosome 4 and 6 carrying hybridized red and green signals. The translocation brings probe set I on chromosome 4 and probe set II on chromosome 6 together. In an interphase nucleus, it shows one pair of separated red and green signals and one pair of co-localized red and green signals. (C) In a normal cell, one pair of chromosome 6 carry hybridized green (probe set II) and red (probe set III) signals. In an interphase nucleus, it shows two pairs of co-localized green and red signals. Bp1: centromeric breakpoint; bp2: telemetric breakpoint. (D) In a cancer cell with t(4;6)(q22;q15), one chromosome 6 carries hybridized green and red signals, but on the chromosome 6 with translocation, the signals are split apart. In an interphase nucleus, it shows one pair of co-localized green and red signals and another pair of

separated red and green signals. (E) In the case if the telemeric part of 6q is lost or two breakpoints (bp1 and bp2) occurs due to the t(4;6) rearrangement, besides the normal chromosome 6 carrying hybridized red and green signals, only one green signal is left on the abnormal chromosome 6 fragment(s). In an interphase nucleus, it shows one pair of co-localized green and red signals and a single green signal.

Figure 3. Examples of FISH signals using the probe co-localization analysis as illustrated in Figure 2A and 2B. (A) A co-localization negative area in a normal control prostate sample where red signals were seen separated from the green signals. (B) In a prostate cancer sample, co-localization of red (4q22 probes) and green (6q14.3 probes) signals (arrow) were found in many cells.

Figure 4. Confirmation of the t(4;6) positive cells using split signal approach. (A) and (C) The co-localized signals generated by probes on 4q22 (probe set I, red) and 6q14.3 (probe set II, green) are indicated by yellow arrows. (B) and (D) The nuclei from A and B, respectively, were re-hybridized with probes on 6q14.3 (green) and 6q15 (probe set III, red). (B) The signals generated by probes on 6q14.3 and 6q15 showed split green and red signal (green and red arrows respectively), indicating translocation break point at 6q15 region as illustrated in Figure 2D. (D) A single green signal generated by the probe on 6q14.3 was detected (green arrow), whilst the red signal (probe on 6q15) was lost, indicating deletion at this region as illustrated in Figure 2E.

Figure 5. Kaplan-Meier analysis comparing prostate cancer patient outcomes with the t(4;6)(q22;q15) status. (A) and (B) Cause-specific survival and overall survival respectively.

Table 1. Scoring of non-malignant control samples on the TMAs for co-localization of probes on 4q22 and 6q14.3

Sample	Total cells	Co-localization positive	Percentage
Appendix	98	5	5.10
Breast	60	2	3.30
Cervix 1	104	4	3.80
Cervix 2	67	1	1.49
Colon	59	1	1.69
Foreskin	57	2	3.51
Inflamed gall bladder	54	1	1.90
Kidney	53	1	1.89
Lung	50	1	2.00
Placenta	60	2	3.30
Prostate	65	1	1.50
Small Intestine	59	3	5.10
Stomach	55	1	1.80
Testis	50	1	2.00
Tonsil	63	2	3.17
Uterus	55	1	1.80

Table 2. Relationship of t(4;6)(q22;q15) (as a binary variable) with demographics and tumor characteristics using a 15% cut-off to define negative and positive cases

Variable	t(4;6) % of positive cells		p-value ^a
	<15% (n=589)	≥15% (n=78)	
<i>Mean age ± SD (years)</i>	69 ± 5	70 ± 5	0.93
<i>Classes of age (years)</i>			0.93
≤ 65	113 (90%)	13 (10%)	
> 65 - 70	159 (86%)	25 (14%)	
> 70 - 73	152 (89%)	19 (11%)	
> 73 - 76	165 (89%)	21 (11%)	
<i>Gleason score ^b</i>			0.04
<7	311 (91%)	29 (9%)	
=7	140 (83%)	28 (17%)	
>7	136 (87%)	21 (13%)	
<i>Clinical stage ^c</i>			0.001
T1	163 (93%)	12 (7%)	
T2	132 (90%)	15 (10%)	
T3	56 (78%)	16 (22%)	
<i>Baseline PSA</i>			0.01

≤ 10	221 (92%)	18 (8%)	
> 4 - 10	123 (88%)	17 (12%)	
> 10 - 25	112 (86%)	18 (14%)	
> 25 - 50	85 (84%)	16 (16%)	
> 50 - 100	48 (84%)	9 (16%)	
<i>Cancer in biopsy (%)^d</i>			0.001
≤ 6	180 (93%)	14 (7%)	
> 6 - 20	143 (89%)	18 (11%)	
> 20 - 40	86 (91%)	8 (9%)	
> 40 - 75	70 (82%)	15 (18%)	
> 75 - 100	99 (81%)	23 (19%)	

^a Test for trend in “≥15%” group (except for mean age).

^b Restricted to patients for which Gleason score was available.

^c Restricted to patients for which clinical T stage was available.

^d Restricted to patients for which extent of disease was available.

Abbreviations: SD, standard deviation; PSA, prostate specific antigen.

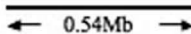
Table 3. Relationship of t(4;6) with *ERG* status (n=510 patients)

ERG status	t(4;6) status	
	Negative (n=442)	Positive (n=68)
Normal	295 (88%)	40 (12%)
1Esplit	36 (84%)	7 (16%)
2+Esplit	17 (89%)	2 (11%)
1Edel	60 (86%)	10 (14%)
2+Edel	34 (79%)	9 (21%)

1Esplit: one *ERG* split signal; 2+Esplit: two or more *ERG* split signals; 1Edel: one *ERG* deletion signal; 2+Edel: two or more *ERG* deletion signals.

A

Probe set I



RP11-18N21 RP11-240J11

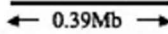
RP11-681L8

4q22.3

C4orf37

B

Probe set II



RP11-111J1 RP1-214H13

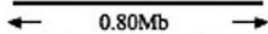
RP11-595C20

6q14.3

LOC643962

C

Probe set III



RP1-44N23

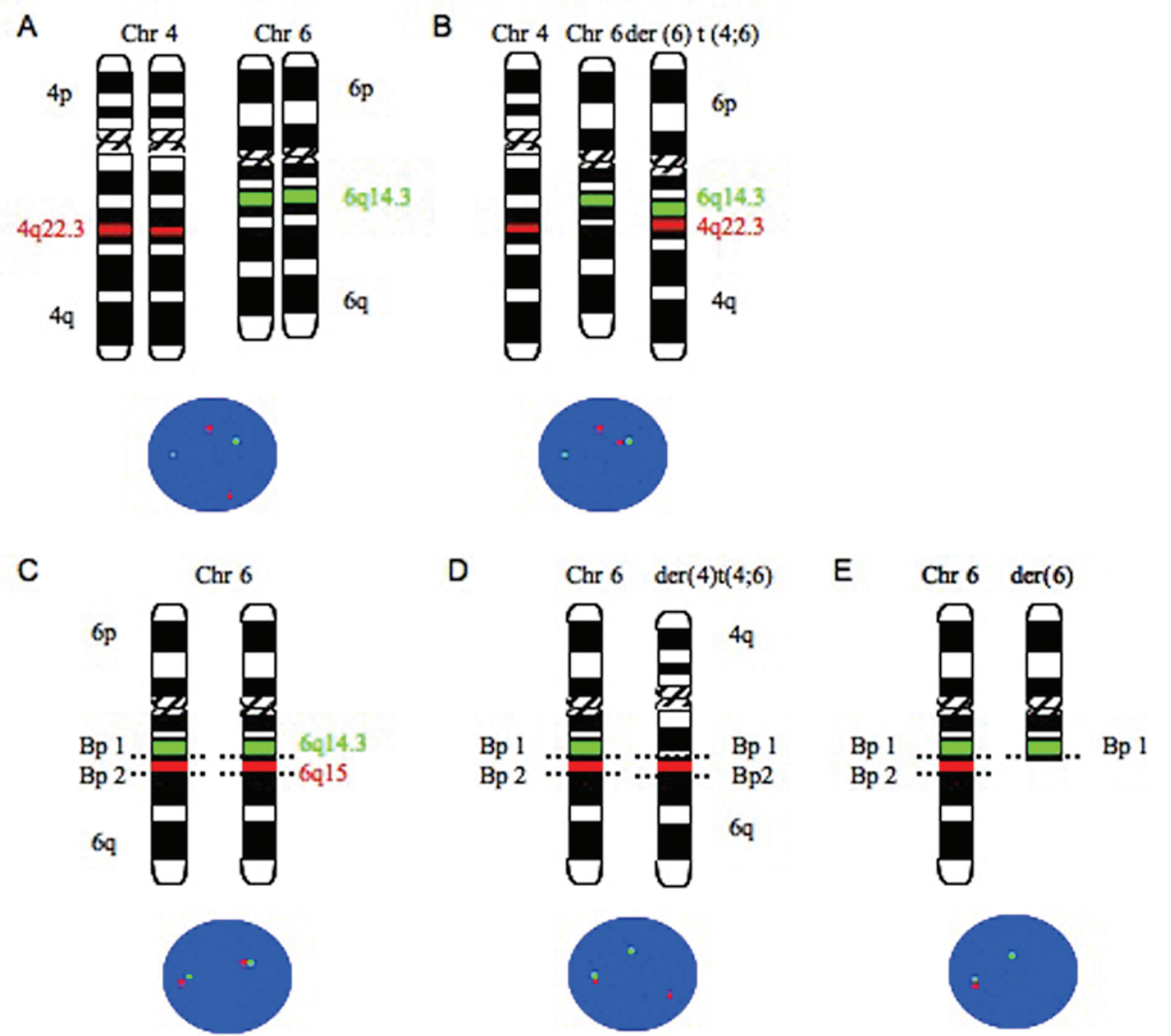
RP11-104N3

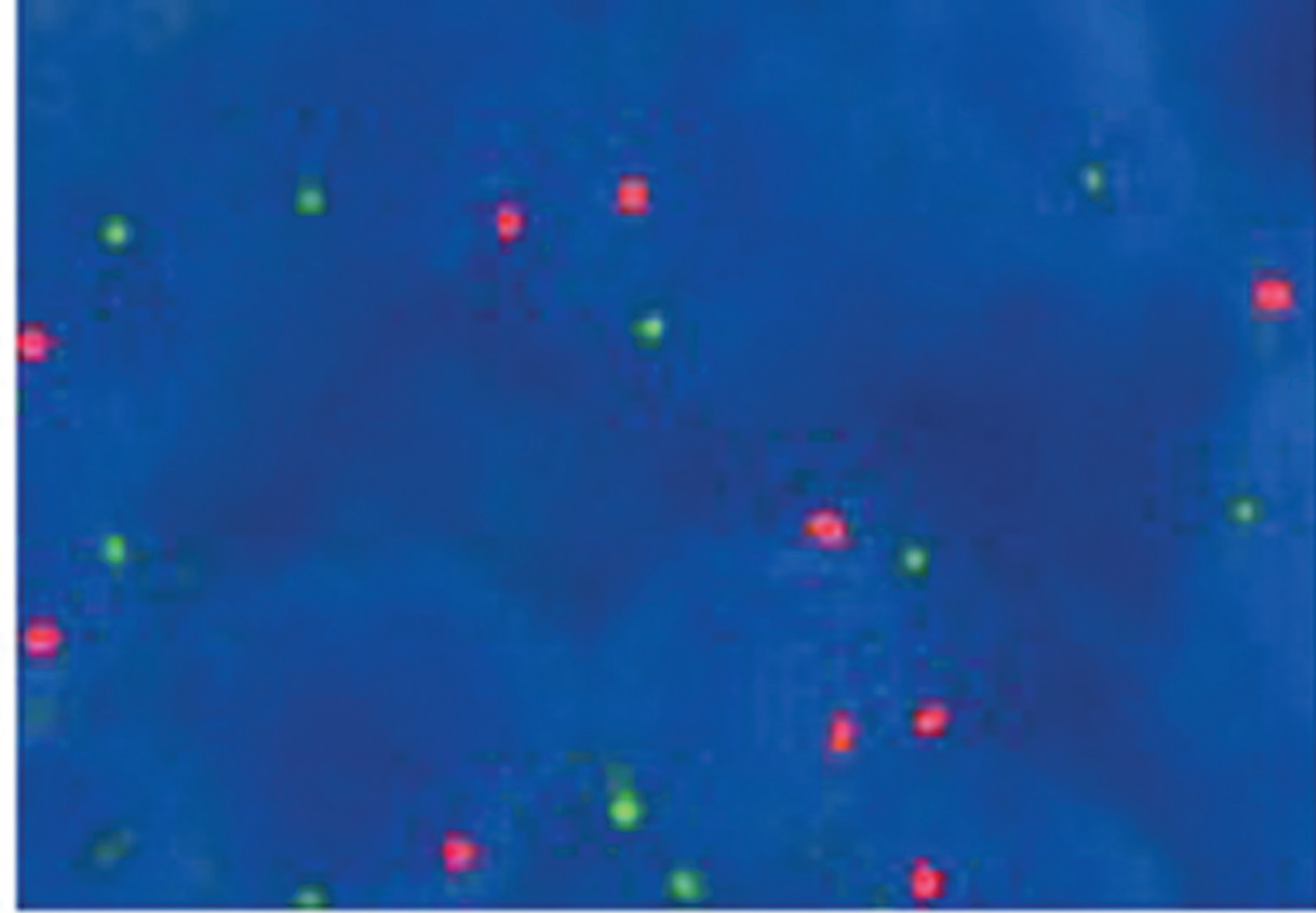
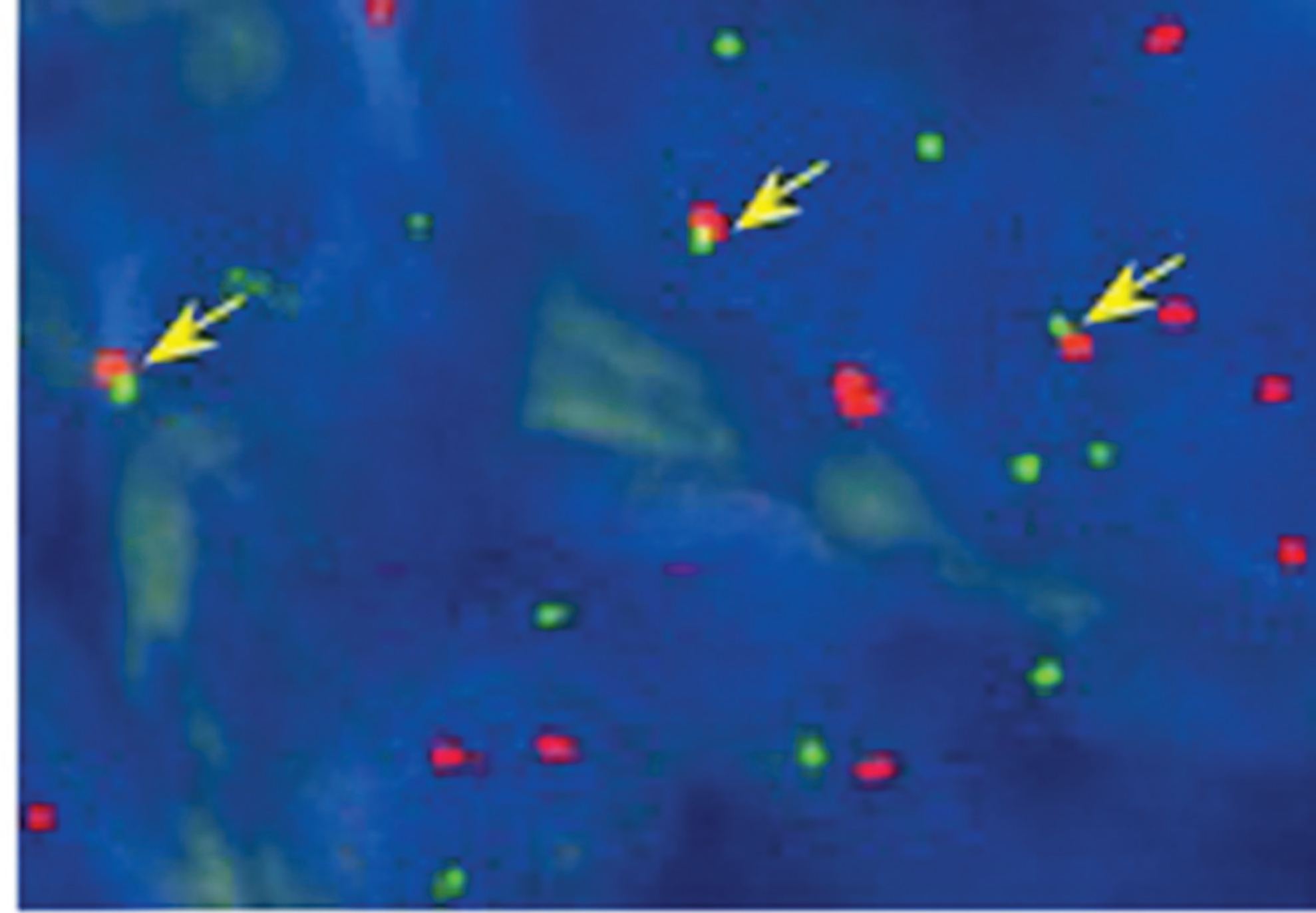
RP1-154G14

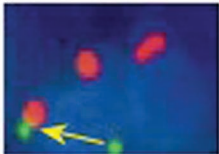
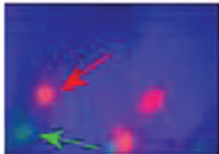
6q15

MAP3K7

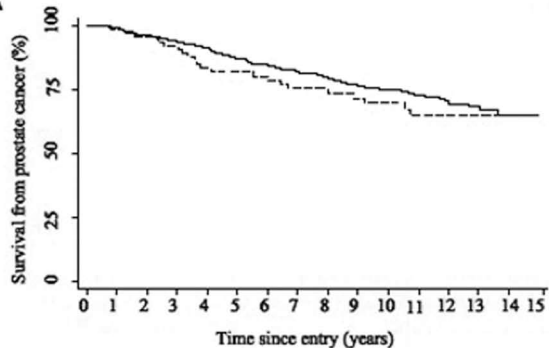
BACH2



A**B**

A**B****C****D**

A



B

