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Array Comparative Genomic Hybridization Analysis Identified The Chromosomal Aberrations and Putative Genes Involved in Prostate Tumorigenesis of Malaysian Men

(Analisis Tatasusunan Perbandingan Genom Penghibridan dalam Mengenal Pasti Aberasi Kromosom dan Gen Berkemungkinan Terlibat dalam Tumorigenesis Prostat dalam Kalangan Lelaki di Malaysia)

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ABSTRACT

The identification of chromosomal aberrations in prostate cancer has been widely studied with several known oncogenes and tumor suppressor genes have successfully been discovered. The most frequent aberrations detected in western population were losses in chromosome 5q, 6q, 8p, 13q, 16q, 17p, 18q and gains of 7p/q and 8q. The purpose of this study was to determine the chromosomal aberrations among Malaysian men of Southeast Asia population and discover those potential genes within that chromosomal aberrant region. Thirty-six formalin-fixed paraffin embedded specimens consist of eight organ-confined prostate cancer cases, five with capsular invasion, 14 showed metastasis and nine cases had no tumor stage recorded, were analyzed by array CGH technique. Chromosomal losses were frequently detected at 4q, 6q, 8p, 13q, 18q while gains at 7q, 11q, 12p, 16q and 17q. Gain of 16q24.3 was statistically significant with tumor size. Gains of 6q25.1 and Xq12 as well as losses of 3p13-p1.2 and 13q33.1-q33.3 were significantly correlated with Gleason grade whereas 12p13.31 gain was associated with bone metastasis. Several potential genes have also been found within that aberrant region which is myopodin (4q26-q27), ROBO1 (3p13-p11.2), ERCC5 (13q33.1-q33.3) and CD9 (12p13.31), suggesting that these genes may play a role in prostate cancer progression. The chromosomal aberrations identified by array CGH analysis could provide important clues to discover potential genes associated with prostate tumorigenesis of Malaysian men.

Keywords: Array CGH; chromosomal aberrations; prostate cancer; putative genes

ABSTRAK

Pengenalpastian aberasi kromosom dalam kanser prostat telah dikaji secara meluas dengan beberapa onkogen dan gen penindas tumor telah berjaya ditemui. Aberasi kromosom yang paling kerap dikesan dalam kalangan penduduk barat ialah delesi pada kromosom 5q, 6q, 8p, 13q, 16q, 17p, 18q dan amplifikasi pada kromosom 7p/q dan 8q. Tujuan kajian ini adalah untuk menentukan aberasi kromosom dalam kalangan lelaki Malaysia di Asia Tenggara dan seterusnya mengenal pasti gen berpotensi yang terkandung dalam kawasan kromosom yang mengalami aberasi. Sejumlah 36 blok tisu spesimen kanser prostat yang diawet formalin dan terbenam dalam lilin parafin, digunakan dalam kajian ini yang terdiri daripada 8 kes organ-terbatas kanser prostat, 5 kes dengan kapsular invasif, 14 kes menunjukkan metastasis manakala 9 kes tiada rekod peringkat tumor. Sampel dianalisis oleh teknik penghibridan perbandingan genomik tatasusunan. Delesi kromosom lebih kerap dikesan pada 5q, 6q, 8p, 13q, 18q manakala amplifikasi pada 7q, 11q, 12p, 16q dan 17q. Amplifikasi 16q24.3 menunjukkan hubungan yang signifikan dengan saiz tumor. Amplifikasi 6q25.1 dan Xq12 serta delesi 3p13-p1.2 dan 13q33.1-q33.3 adalah signifikan dengan gred tumor manakala amplifikasi 12p13.31 adalah signifikan dengan metastasis ke bahagian tulang. Beberapa gen yang berpotensi juga telah ditemui di dalam kawasan aberasi berkenaan termasuklah gen myopodin (4q26-S27), ROBO1 (3p13-p11.2), ERCC5 (13q33.1-q33.3) dan CD9 (12p13.31) yang berkemungkinan berperanan penting dalam perkembangan kanser prostat. Aberasi kromosom yang dikesan oleh teknik tatasusunan CGH memberi petunjuk penting terhadap penemuan gen berpotensi yang berkemungkinan terlibat dalam tumorigenesis prostat pesakit Malaysia.

Kata kunci: Aberasi kromosom; gen yang berkemungkinan; kanser prostat; tatasusunan CGH

INTRODUCTION

Prostate cancer remains one of the most common malignancies afflicting men today. A total of 914000 new cases were reported in 2008, making it the second most frequently diagnosed cancer in the world. It accounted for about 6.1% of all male cancer deaths in 2008, with close to three-quarters of the cases occur in developed countries (Ferlay et al. 2010). The incidence rates of prostate cancer vary widely across the world. The rates are relatively high in western countries such as US, Canada and Australia but lowest in Asian countries such as China and other parts of Asia. In Malaysia, prostate cancer ranked the fourth most

frequent cancer in males and accounted for 6.2% of the total cancer cases with an age standardized incidence rate (ASR) of 6.2 per 100000 population (Zainal & Nor Saleha 2011). Among the ethnic groups, Chinese recorded the highest incidence, followed by Malays and Indians (Zainal & Nor Saleha 2011).

It has been shown that chromosomal alteration is a common event in cancer (Lengauer et al. 1998). Chromosomal alterations include translocation, amplification, rearrangements, deletion and mutation usually affect many important genes involved in cell cycle regulation, apoptosis, signal transduction and DNA repair (Diamandis 1997; Fearon & Vogelstein 1990; Gelmann 2008). These affected genes are classified as oncogenes and tumor suppressor genes. Like other solid tumors, the prostate tumorigenesis involves multiple genetic alterations across the genome.

The identification of chromosomal alterations is important in discovering genes which may play a role in prostate tumorigenesis. The emergence of molecular technique such as comparative genomic hybridization (CGH) since the mid-1990s followed by the more recent technology; array comparative genomic hybridization (array CGH) and DNA microarray in the past few years, have helped to identify some critical genetic aberrations in prostate cancer. Array CGH was first introduced by Solinas-Toldo et al. (1997) and it demonstrated higher sensitivity for detection of DNA copy number changes that are either gain or loss in specific chromosomal region of tumor samples.

Previous studies had applied these platforms to screen for the chromosomal changes in prostate cancer cases. The most common chromosomal alterations identified using CGH and array CGH in early stage clinical prostate cancer are losses in 5q, 6q, 8p, 13q, 16q, 17p, and 18q and gains of 7p/q and 8q (Paris et al. 2004; van Dekken et al. 2004; Visakorpi et al. 1997). Furthermore, there were multiple frequent altered regions of losses detected at 2q21-22, 5q13-21, 6q14-21, 8p21-23, 10q23-25, 13q14-22, 16q13-24, 18q12-23 and 21q22 as well as gain of 3q23-33, 7q21-33, 8q12-23, 17q24-25, and Xq11-23 (Fu et al. 2000; Kasahara et al. 2002; Nupponen et al. 1998). In addition to that, previous array CGH studies had identified several novel recurrent copy number aberrations of chromosomal gains at 2p25, 9p13-21, 11p15.4, 16p13.3 and 16p12.2-p11.2 (Paris et al. 2004; Ribeiro et al. 2006; Saramaki et al. 2006). The chromosomal aberrations study of prostate cancer has led to the identification of several important tumor suppressor genes and oncogenes. Some examples include *NKX3-1* (8p loss), *PTEN* and *MXI1* (10q loss), *FOXO1A* (13q loss), *EZH2* and *MCM7* (7q gain), *MYC* (8q gain) and the *androgen receptor* (*AR*) gene at Xq12.

The chromosomal alterations involved in the development and progression of prostate cancer among Malaysian men of Southeast Asia population is yet unknown. In order to gain new insights into the genetic changes involved in prostate cancer of Malaysian male, we therefore attempt to determine the chromosomal aberrations and characterize those potential genes involved in prostate cancer progression in our patients. We applied array CGH to formalin-fixed paraffin embedded (FFPE) tumor blocks of patients at various stages of prostatic tumor development. The array CGH profiles were then correlated with clinicopathological parameters (age, tumor size, Gleason grade and tumor stage).

Materials & Methods

SPECIMEN

Tumor specimens consisted of 36 formalin-fixed paraffin-embedded (FFPE) prostate carcinomas obtained between year 1998 and 2008. Of 36 cases, eight were organ-confined prostate cancer, five were prostate cancer with capsular invasion), 14 showed metastasis while nine cases had no tumor stage recorded. All specimens were adenocarcinomas, taken from patients who underwent transurethral resections or orchidectomies at Universiti Kebangsaan Malaysia Medical Centre (UKMMC). The patients comprised of 29 (81%) Malays, 6 (17%) Chinese and 1 (3%) were of other races. The age of these patients ranged between 60 and 88, with the mean age at diagnosis was 70.5 years (median 72). The tumors were graded according to the Gleason grading system (Gleason, 1992) and staged according to the TNM classification. The mean tumor Gleason score was G7 (range G5 to G10). The presence of tumor area on hematoxylin and eosin (H&E) was verified by a pathologist.

DNA EXTRACTION

DNA from FFPE samples was isolated according to manufacturer's recommended procedure and is based on the method previously described (van Beers et al. 2006). Briefly, the FFPE tumor blocks were sliced into 4-6 slices of 20 μm thickness and placed in 1.5 mL eppendorf tube. Sections were heat deparaffinated in 480 μL PBS and 20 μL 10% Tween 20 at 90°C for 10 min and immediately placed on ice for 2 min. Wax disc were remove with a tweezer. Tissues were incubated overnight in 1 M NaSCN to remove crosslink, followed by proteinase-K (20 mg/mL stock) treatment at 55°C at 450 rpm (Eppendorf® Thermomixer) to digest the tissues. Two more aliquots of 40 μL proteinase-K were added at 6 and 10 h. Following two days of proteinase-K treatment, DNA extraction was continued using the DNeasy tissue extraction kit (Qiagen). The genomic DNA (gDNA) concentration was quantitated with Nanodrop UV-VIS Spectrophotometer (Agilent, US) and gel electrophoresis. Normal commercial genomic DNA (Human Male Genomic DNA, Promega) was used as reference sample. Reference sample were heat fragmented at 95°C until its median fragment length was similar to that of the test samples.

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (ARRAY CGH)

Array CGH was performed according to manufacturer's instructions (Agilents Technologies, USA). Briefly, tumor DNA (0.5 µg) and normal male reference DNA (0.5 µg) were labeled with ULS-Cy5 and ULS-Cy3 respectively before incubated at 85°C for 30 min. Labeled gDNA was then purified using Agilent-KREApure^{TM} columns (Agilent Technologies, USA) for unbound dye removal. The Cy5- and Cy3-labeled gDNA were combined and mixed with 5 µL of Human Cot-1 DNA (Invitrogen), 11 µL of Agilent 10X CGH Blocking Agent and 55 µL of Agilent 2X CGH hybridization buffer before denatured at 95°C for 3 min and incubated at 37°C for 30 min. Agilent CGHblock was then added and the hybridization mixtures were hybridized to the Agilent Human Genome CGH 4 X 44K Microarray slide. The 4X 44K slide contains four identical arrays consisting of ~43,000 *in situ* synthesized 60-mer oligonucleotide probes that represented the coding and non-coding sequences. The hybridization was carried out in an Agilent SureHyb chamber in a rotator oven at 65°C for 40 h. Three washing steps were done; room temperature with Agilent's Oligo CGH Wash buffer 1 for 5 min, a 37°C wash with Agilent's Oligo CGH Wash buffer 2 for 1 min and an acetonitrile rinsed at room temperature for 1 min.

ARRAY CGH IMAGING AND DATA ANALYSIS

The slides were scanned on an Agilent DNA Microarray Scanner. Scanned images were analyzed using Feature Extraction software (version 9.5) and then imported into Agilent Genomic DNA Workbench software (version 5.0) for analysis. Chromosomal aberrations were considered as gains when the log2 ratio was higher than 0.25 and as loss when the ratio was below -0.25. The aberration detection module-1 (ADM-1), an aberration detection algorithm from Agilent's DNA Genomic Workbench software was used to identify regions of copy number gains or losses.

STATISTICAL ANALYZES

The correlation between chromosomal aberrant regions and the clinicopathological parameters (tumor size, Gleason grade, TNM cancer stage and metastasis status) were performed using *Chi-square* test whereas the *Fisher's Exact* test was used when appropriate. The *p*-value of <0.05 was considered to be significant.

RESULTS

GENETIC CHANGES IN PROSTATE CARCINOMA CASES

Chromosomal aberrations were detected in all 36 prostate cancer cases with multiple aberrations including both gains and losses of chromosomal materials (Figure 1). The mean aberrations were 43.2 per tumor (range, 1 to 85). There were 54.2% cases showed chromosomal losses whereas 45.8% cases displayed gains. Chromosomal losses tend to be more frequent than gains in the majority of cases. The mean number of losses was 23.4 per tumor (range, 1 to 59) and of gains were 20.7 per tumor (range, 1 to 50).

The most frequent chromosomal aberrations in all 36 cases were losses on chromosome 4q (75%), 6q (74%), 8p (61.1), 13q (69.4%) and 18q (52.8%) while gains were encountered most frequently at 7q (52.8%), 11q (72.2%), 12p (75%), 16q (52.8%) and 17q (86.1%). Losses of chromosome arms 4p, 8p, 18q, 19q and gains of 8q, 11q, 17q and Xq were commonly shared between organ-confined prostate cancer cases, prostate cancer with capsular invasion and metastatic cases. We also detected the gain of 8q at four independent regions at lower frequency which were 8q23.3-q24.1 (27.8%), 8q24.2 (25%), 8q21.1 (22.2%) and 8q24.3 (22.2%). The clinicopathological data obtained from 36 prostate cancer patients and genetic aberrations are summarized in Table 1. Table 2 depicts the chromosomal aberrations detected in each ethnic group. Loss at 4q25 and gains at 17q12 and Xq12 were the frequent aberrant regions shared by each group. All regions of gains and losses in these 3 ethnic groups were correlated with clinicopathological data, however no statistical differences were found (data not shown).

Loss at chromosomal region 4q26-q27 (75.0%) was the most frequent aberration found in this study. Genes identified at these aberrant chromosomal regions were *SYNPO2* (4q26-q27). Loss of 4q26-q27 were found in 29.6% of organ-confined prostate cancer cases, 7.4% in capsular invasion and 37.0% in metastasis. Table 3 highlighted the most common chromosomal aberrant regions of losses detected in all 36 cases together together with the genes involved within the aberrant regions. Amplification of chromosomal region 17q12 (86.1%) were the most frequent aberration found in this study. One of the genes identified within this aberrant chromosomal region was *HER2* (17q12). *HER2* amplified region was found in 25.8% of organ-confined prostate cancer, 16.1% of capsular invasion and 35.5% in metastasis. The most common chromosomal aberrant regions of gains in all 36 cases and the genes located within the aberrant regions are summarized in Table 4.

Array CGH analysis detected several genes known to be established in prostate cancer as well as other potential genes which have not been described. Chromosomal losses contain genes such as *ROBO1* (3p13-p11.2), *SYNPO2* (4q26-q27), *MAP3K7* (6q15-q16.1), *MSR1* (8p22), *NKX3-1* (8p21.2), *PTEN* (10q23.2-q23.31), *CCND2* (12p13.33-p13.31), *ERCC5* (13q33.1), *bcl2* (18q11.2-q23) and *ELAC2* (13q14.2) while gains contain genes such as *EZH2* (7q36.1), *TRPS1* (8q23.3-q24.1), *MYC* (8q24.2), *PSCA* (8q21.1), *TCEB1* (8q24.3), *CCND1* (11q13.1), *CD9* (12p13.31), *CDK10* (16q24.3), *HER2* (17q12) and *AR* (Xq12).

The correlation between chromosomal aberrations and clinicopathological parameter (tumor size, Gleason grade and metastasis) were evaluated and summarized in Table 5. Gain of 16q24.3 showed correlation with tumor

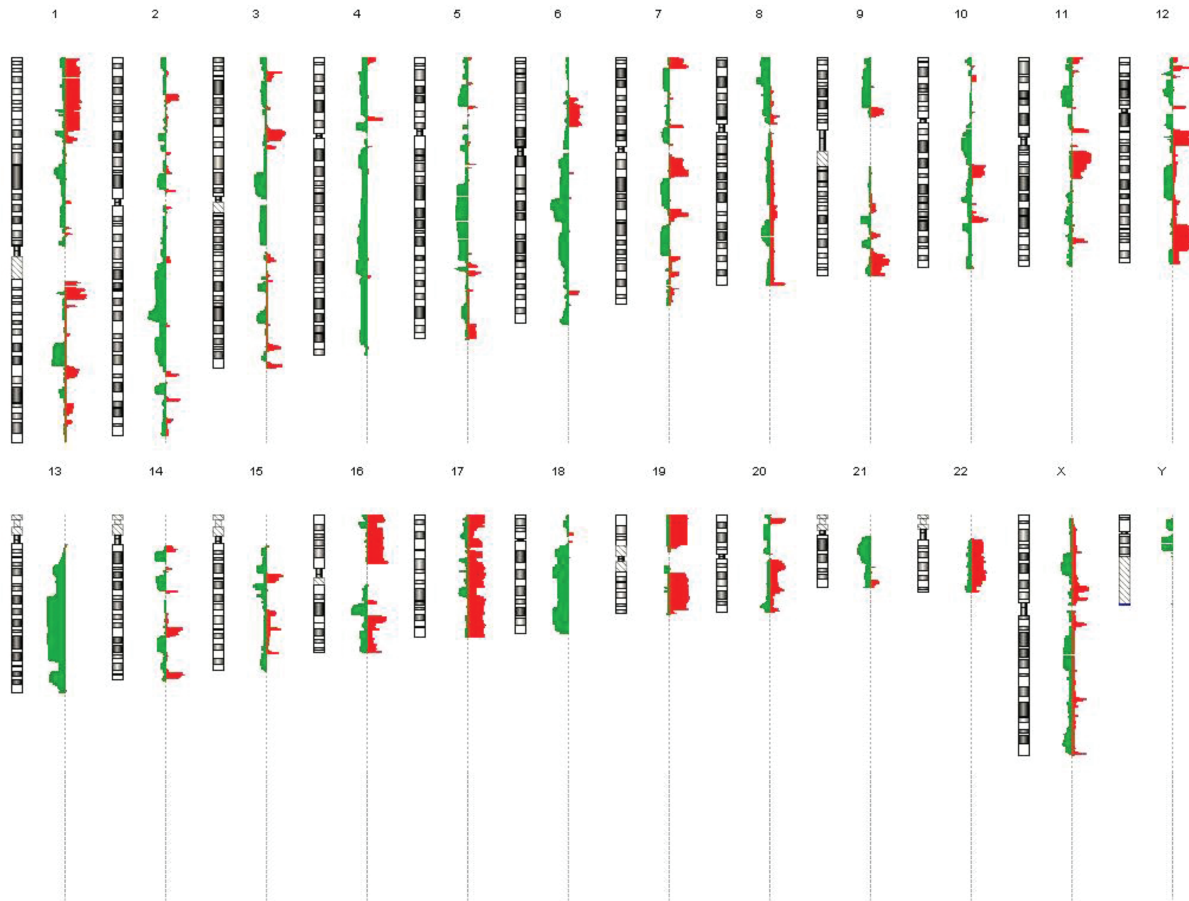

FIGURE 1. Chromosomal aberrations of 36 prostate tumors. Gains and losses are shown as red and green bars, respectively, representing the overall chromosomal changes observed in all cases

size >4.5 cm (p=0.023). Losses of 3p13-p11.2 (p=0.044) and 13q33.1 (0.017) and gains at 6q25.1 (0.019) and Xq12 (0.044) were correlated significantly with high Gleason grade (>Gleason 7). The 12p13.31 gain correlated significantly with bone metastases (p=0.043). 12p13.31 gain was also found in 25.9% of organ-confined prostate cancer and 11.1% cases with capsular invasion.

DISCUSSION

This array CGH study represents the first genome-wide screening of chromosomal aberrations in prostate cancer of Malaysian men. Losses of 4q, 6q 8p, 13q and 18q as well as gains of 7q, 11q, 12p, 16q and 17q appeared to be the most common aberrations found in our cases. The overall genomic profile and the chromosomal aberrant regions identified in prostate cancer of Malaysian men seem in accordance with previously reported in western populations. However, in our study the gain of 8q was found at lower frequency than that of previously reported. We have also found minimal overlapping regions at several chromosomal sites, containing several established tumor suppressor genes and oncogenes such as *HER2*, *AR*, *MYC*, *BIN1*, *MAP3K7* as well as potential genes such as *myopodin*, *ERCC5*, *CD9*, *ROBO1* and *CDK10*. Furthermore, we detected several aberrant regions which showed significant correlation with tumor size, high tumor grade and bone metastasis. Such aberrations include losses of 3p13-p11.2, 4q26-q27 and 13q33.1-q33.3 as well as gains of 6q25.1, 12p13.31, 16q24.3, 17q12 and Xq12.

CHROMOSOMAL LOSSES

Chromosomal region 4q26-27 was the most frequent losses found in our study. This region includes *SYNPO2* gene, that also known as *myopodin*. *Myopodin* is an actin-bundling protein, the second member of the synaptopodin protein family (Weins et al. 2001). *Myopodin* is normally expressed in neurons and podocytes however its expression has also been detected in colon, uterus, stomach, lung, small intestine and kidney (Lin et al. 2001; Weins et al. 2001). Loss of *myopodin* expression has been previously observed in prostate cancer cases, and it has been shown to suppress tumor growth and metastasis in several prostate cancer cell lines *in vitro* (Jing et al. 2004). Previous analysis also demonstrated the tumor suppressor activity of *myopodin* in bladder cancer, and suggested that loss of nuclear *myopodin* expression can be a predictive value of clinical outcome for patients with progressive bladder cancer (Sanchez-Carbayo et al. 2003). The

TABLE 1. Clinicopathological data of 36 prostate adenocarcinoma and the most frequent genetic aberrations in all cases

Case	Tumour size	Gleason score	TNM	Most frequent aberrant regions
Organ-confined prostate cancer				
S1	0.0	5 (3+2)	$T_2N_0M_0$	**Gains:** 1q32.1, 3p21.31-p21.1, 7p22.3-p22.1, **8q24.3**, 9q33.3-q34.3, 11p11.2, 11q12.2-q13.3, **11q23.3**, 12p13.31, 14q11.2-q12, 16p13.3-p11.2, 16q24.3, **17q12**, 17q21.2-q21.31, 19p13.3-p12, 19q13.11-q13.42, 20p13-p12.3, **Xq28**.
S2	4.0	8(3+5)	$T_2N_0M_1$	
S3	6.0	7(3+4)	$T_1N_XM_0$	
S4	4.5	8 (4+4)	$T_2N_0M_0$	
S5	3.0	8 (4+4)	$T_2N_0M_0$	**Losses:** 1q21.3, 4p13-p12, 4q26-q27, 8p22, 6p21.2-p21.1, 13q33.1-q33.3, 14q12, 16p13.3-p13.2, 17q11.2-q12, 18q11.2-q23, **19p13.3**.
S6	2.5	9 (5+4)	$T_2N_0M_0$	
S7	4.0	6 (3+3)	$T_1N_0M_0$	
S8	6.0	5 (3+2)	$T_2N_0M_0$	
Capsular invasion				
S9	3.0	N/A	$T_3N_0M_0$	**Gains:** 1q23.3, 2q35, 3q29, 4p14, 6p25.2, **8q24.3**, 9q34.11, 11p15.4, **11q23.3**, 12p13.31, 16p11.2, 16p13.3, 16q24.3, **17q12**, 17q21.2-q21.21, 19p13.3, 20p13-p12.3, **Xq28**.
S10	6.5	9 (5+4)	$T_3N_0M_0$	
S11	2.0	6 (3+3)	$T_3N_0M_X$	
S12	4.0	4 (2+2)	$T_3N_0M_0$	**Losses:** 3q26.31, 5q31.1, 12q13.13, 14q24.2, 16p13.3, 17q12, **19p13.3**, 22q13.1.
S13	10.0	5 (3+2)	$T_4N_0M_1$	
Metastasis				
S14	10.0	7 (3+4)	$T_4N_0M_1$	**Gains:** 1p36.11-p35.3, 1q32.1, 2p11.2-2q35, 3q29, 4p14, 5q31.1, 6q25.1, 7q22.1, **8q24.3**, 9p22.1, 10q21.3-q22.2, **11q23.3**, 12p13.31, 14q24.2-q24.3, 15q15.1, 16p11.2, **17q12**, 20p13-p12.3, 22q13.1, **Xq28**.
S15	4.0	8 (3+5)	$T_4N_0M_2$	
S16	6.0	7 (3+4)	$T_4N_0M_1$	
S17	7.5	9 (5+4)	$T_4N_0M_1$	
S18	6.0	7 (4+3)	$T_4N_0M_1$	**Losses:** 1q21.3, 3p13-p11.2, 3q26, 4p13-p12, 4q26-q27, 5q31.1-q31.2, 6p21.2-p21.1, 7q21.1-q21.3, 8p21.2, 11p15.4, 12q13.13, 13q33.1-q33.3, 14q12, 16p13.3-p13.2, 17q11.2-q12, 18q11.2-q23, **19p13.3**.
S19	4.5	9 (4+5)	$T_4N_0M_1$	
S20	2.0	9 (5+4)	$T_4N_0M_1$	
S21	3.5	8 (4+4)	$T_4N_0M_2$	
S22	6.0	9 (4+5)	$T_4N_0M_3$	
S23	3.0	8 (5+3)	$T_4N_0M_4$	
S24	6.0	7 (3+4)	$T_4N_0M_5$	
S25	4.0	8 (4+4)	$T_3N_0M_1$	
S26	4.0	9 (5+4)	$T_4N_0M_1$	
S27	4.5	9 (5+4)	$T_1N_1M_1$	
Cases with non-recorded tumour stage				
S28	4.0	9 (4+5)	-	**Gains:** 2q35, 4p14, 6q25.1, 7p22.3-p22.1, 8q24.3, 11q23.3, 12p13.31, 14q24.2-q24.3, 16q22.1, 22q13.1, Xq28.
S29	7.0	8 (5+3)	-	
S30	6.0	8 (5+3)	-	
S31	6.0	8 (4+4)	-	**Losses:** 1q21.3, 12q13.13, 17q21.33-q22, 18q11.2-q23.
S32	7.5	8 (4+4)	-	
S33	3.5	7 (3+4)	-	
S34	3.0	10 (5+5)	-	
S35	4.0	9 (4+5)	-	
S36	5.0	7 (3+4)	-	

* Aberration's were defined by log2 ratio of 0.25 for chromosomal gains and -0.25 for chromosomal losses. The bold chromosome arms showed the aberrant chromosomal regions shared by each group

frequent deletion of *myopodin* gene was also reported and this was shown to correlate with prostate cancer aggressiveness (Sanchez-Carbayo et al. 2003; Yu & Luo 2006; Yu et al. 2006). In our study, 4q26-q27 minimal deleted region containing *myopodin gene*, was detected in 27 (75%) out of 36 cases. Of the 27 cases, deletion of *myopodin* was found in all organ-confined prostate cancer (29.6%), 7.4% in prostate cancer with capsular invasion while 37% of cases showed distant metastases. We postulated that the deletion of *myopodin* might be involved in the progression of prostate cancers from early to advanced stages. However, no significant correlation between *myopodin* deletion and clinicopathological parameters was found.

We noted a loss of 13q33.1-q33.3 at a relatively high frequency, observed in 25 (69.4%) out of 36 cases. This region includes the *ERCC5* gene. *ERCC5* is the DNA repair gene, which is one of the vital components of DNA repair mechanisms in nucleotide excision repair (NER) pathway. Previous analyses have suggested that several polymorphisms in the coding sequence of the ERCC5 gene were associated with an increased risk of some

TABLE 2. Most frequent regions of gains and losses detected by array CGH in 36 prostate adenocarcinomas by ethnicity

Ethnic group	Total (n=36)	(%)	Chromosomal aberrations	
			Gains	Losses
Malay	29	81%	1q32.1, 2p11.2, 3p14.3, 4q31.1, 5q35, 6p21.3, 7q36.1, 8q24.2, 8q21.1, 8q24.3, 9q34.1, 10q21.3, 10q22.2, 11q13.3, 12p13.31, 13q12.11, 14q11.2-q12, 15q24, 16q24.3, **17q12**, 18q21.3, 19q13.33, 20p13-p12, 21q22.3, **Xq12**.	1q21.3, 2p13.1 - p11.2, 3p13 - p11.2, **4q25**, 5q31.1-q31.2, 5q31.1-q31.2, 6q15-q16.1, 17q21.2-q22, 8p22, 9p22.1-p21.2, 10q23, 10q25.1-q25.2, 13q33.1, 14q12, 15q21, 17p12, 18q11.2.
Chinese	6	17%	1q32.1, 2q11.2, 3q29, 4p14, 5q31.1, 6q25.1, 7q36.1, 8q24.2, 9p22.1, 10q22.2, 11q13.3, 12p13.31, 14q11.2-q12, 16q24.3, **17q12**, 19q13.33, **Xq12**.	1q21.3, 2q24, 3q11.2-q13.3, **4q25**, 5p14.1-p13.3, 6q15-q16.1, 7q21.11 - q21.3, 8p22, 9p22.1-p21.2, 10q23.2-q23.3, 12q13.31, 13q33.1, 14q12, 16p13, 17q11, 17q11.2-q12, 18q11.2-q23.
Others	1	3%	2q24.3-q35, 3q22.2-q27.1, 6q11.1-q24.1, 7q11.23-q31.11, 12q15-q23.2, 14q12-q23.1, **17q12**, **Xq12**.	1p36.3-p35.3, 1q32.1, 3p21.1, 4p16.3-p15.3, **4q25**, 5p15.33, 6q27, 8q24.22-q24.3, 9q34, 10q11.2-q26.3, 11p15, 11q12-q13, 12q13.1, 13q34, 15q23-q26, 16p13.3, 16q23-q24, 17q25, 19p13.3-p12, 19q13.2, 20q13.33, 21q22. 22q13.33.

* The bold chromosome arms showed the aberrant chromosomal regions shared by each ethnic group

TABLE 3. Most frequent regions of losses detected by array CGH in 36 prostate adenocarcinomas and the list of genes contained in the affected area

Aberrant regions	Genes contained in the regions	Number of cases (%)
2q13.2-q14.3	*GLI2, TFCP2L1, CLASP1, RNU4ATAC, MKI67IP, TSN, CNTNAP5, GYPC,* ***BIN1...***	7 (19.4)
3p13-p11.2	*CNTN3, FAM86D, FRG2C, ZNF717, ROBO2,* ***ROBO1****, GBE1, CADM2, CHMP2B, POU1F1...*	8 (22.2)
4q26-q27	*TRAM1L1, NDST3, SNHG8, PRSS12, CEP170L, METTL14, SEC24D,* ***SYNPO2****, MYOZ2, FABP2..*	27 (75.0)
6q15-q16.1	***MAP3K7****, EPHA7, TSG1, MANEA, FUT9...*	27 (75.0)
8p22	*SGCZ, TUSC3,* ***MSR1****, FGF20, EFHA2..*	22 (61.1)
8p21.2	*SLC25A37,* ***NKX3-1****, NKX2-6, STC1..*	13 (36.1)
10q23.2-q23.31	***PTEN****, LIPM, ANKRD22, STAMBPL1, ACTA2, FAS..*	17 (47.2)
12p13.33-p13.31	*PRMT8, PARP11,* ***CCND2****, AKAP3, NDUFA9, GALNT8, KCNA6, NTF3..*	5 (13.9)
13q33.1	*FGF14, TPP2, KDELC1, BIVM,* ***ERCC5****, SLC10A2, DAOA, EFNB2, ARGLU1, LIG4..*	25 (69.4)
18q11.2-q23	*MC4R, CDH20, RNF152, PIGN, ZCCHC2, PHLPP,* ***BCL2****, KDSR..*	19 (52.8)
17p12	*RICH2,* ***ELAC2****, HS3ST3A1, CDRT15P, COX10, CDRT15, PMP22, TEKT..*	8 (22.2)

*Several known oncogenes, tumour suppressor genes as well as potential genes were shown in bold

cancers, such as lung (Kiyohara & Yoshimasu 2007) and bladder cancer (Sanyal et al. 2004). Other study found no significant association of *ERCC5* gene with the risk of prostate cancer (Hooker et al. 2007) whereas recent study found a significant association of this gene with prostate cancer risk due to the combined effect with other NER genes (Berhane et al. 2012). Despite that, *ERCC5* gene was reported for having loss of heterozygosity (LOH) in prostate cancer, concluded that this gene is important in the development of prostate cancer. In our study, the *ERCC5* gene was found to be deleted and showed significant correlation with high Gleason grade (p=0.017). To the best of our knowledge, this is the first study showing deletion of *ERCC5* gene in a series of prostate cancer.

Loss of 3p was also noted, with the minimal deleted region detected at 3p13-p11.2. We have found that deletion of 3p13-p11.2 region showed significant correlation with high Gleason grade. One of the genes affected in this region is *ROBO1*, a gene which encodes a transmembrane receptor. *ROBO1* was found to interact with *SLIT1* protein during development of the nervous system (Andrews et al. 2007). Previous study showed that *ROBO1* expression was significantly decreased in prostate tumors compared to normal prostate (Latil et al. 2003). High *ROBO1* expression was also detected in endometrial

TABLE 4. Most frequent regions of gains detected by array CGH in 36 prostate adenocarcinomas and the list of genes contained in the affected area

Aberrant regions	Genes contained in the regions	Number of cases (%)
7q36.1	***EZH2***, *PDIA4, ZNF786, ZNF425, ZNF398, ZNF398, ZNF282, ZNF212…*	19 (52.8)
8q23.3-q24.1	*PKHD1L1, EBAG9, GOLSYN, KCNV1, CSMD3,* ***TRPS1****, EIF3H..*	10 (27.8)
8q24.2	*SQLE, KIAA0196, NSMCE2, TRIB1, FAM84B, POU5F1P1, POU5F1B, LOC727677,* ***MYC****, PVT1…*	9 (25.0)
8q21.1	*TSNARE1, BAI1, ARC, JRK,* ***PSCA****, LY6K, C8orf55, SLURP1, LYPD2..*	8 (22.2)
8q24.3	*RPL7, RDH10, STAU2, UBE2W,* ***TCEB1****, TMEM70, LY96, JPH1..*	8 (22.2)
11q13.3	*MYEOV,* ***CCND1****, ORAOV1, ANO1, FADD, PPFIA1, CTTN, SHANK2, DHCR7, NADSYN1..*	26 (72.2)
12p13.31	***CD9****, PLEKHG6, TNFRSF1A, SCNN1A, LTBR, SRP14P1, LOC678655, CD27, TAPBPL, VAMP1, MRPL51, NCAPD2, SCARNA10, GAPDH..*	27 (75.0)
16q24.3	*ANKRD11, SPG7, RPL13, CPNE7, DPEP1, CHMP1A, C16orf55,* ***CDK10****, SPATA2L, FANCA..*	19 (52.8)
17q12	*PPP1R1B, STARD3, TCAP, PNMT, PERLD1,* ***HER2****, GRB7..*	31 (86.1)
Xq12	***AR****, OPHN1..*	7 (19.4)

*Several known oncogenes, tumour suppressor genes as well as potential genes were shown in bold

TABLE 5. Specific regions that significantly correlated with clinicopathological parameters

Clinicopathological parameter	Aberrant regions		*p*-value
	16q24.3 gain		
Tumor size (cm)	+	-	
<4.5	7 (36.8)	13 (76.5)	
≥4.5	12 (63.2)	4 (23.5)	0.023
	19/36 (52.8)	17/36 (47.2)	
	6q25.1 gain		
Gleason grade	+	-	
< Gleason 7	0 (0.0)	5 (29.4)	
≥ Gleason 7	18 (100.0)	12 (70.6)	0.019
	18/36 (50.0)	17/36 (47.2)	
	Xq12 gain		
Gleason grade	+	-	
< Gleason 7	3 (42.9)	2 (7.1)	
≥ Gleason 7	4 (57.1)	26 (92.9)	0.044*
	7/36 (19.4)	28/36 (77.8)	
	3p13-p11.2 loss		
Gleason grade	+	-	
< Gleason 7	3 (42.9)	2 (7.1)	
≥ Gleason 7	4 (57.1)	26 (92.9)	0.044*
	7/36 (19.4)	28/36 (77.8)	
	13q33.1 loss		
Gleason grade	+	-	
< Gleason 7	1 (4.0)	4 (40.0)	
≥ Gleason 7	24 (96.0)	6 (60.0)	0.017
	25/36 (69.4)	10/36 (27.8)	
	12p13.31 gain		
Metastasis	+	-	
Bone	11 (68.8)	1 (25.0)	
Other site	0 (0.0)	2 (50.0)	0.043
No metastasis	5 (31.2)	1 (25.0)	
	16/36 (44.4)	4/36 (11.1)	

* *Fisher Exact's* test

cancer and in patients with recurrent endometrial cancer in contrast to those without recurrence (Ma et al. 2010). Deletion at 3p mapping to this gene was also frequent in other cancer including lung cancer (Lerman & Minna 2000).

CHROMOSOMAL GAINS

A gain at 17q was the most frequent chromosomal gain in our study, with a minimal region occurring at 17q12, the region in which the oncogene *HER2* is located. We have detected *HER2* amplification in 31 out of 36 (86.1%) cases, however this was not statistically significant when correlated with the clinicopathological parameters. The role of *HER2* in prostate cancer remains controversial (Bubendorf et al. 1999; Ross et al. 1997), although amplification of *HER2* is frequently found in breast cancers. The frequency of 17q12 amplified region found in our study was slightly higher than that in other studies.

A gain of 12p13.31 was also detected in our cases. This region showed significant correlation with bone metastases. One of the genes contain in this region is *CD9*. *CD9* gene encodes the protein that belongs to the tetraspanins family, which involved in many cellular processes including differentiation, adhesion and signal transduction. *CD9* has been implicated in various tumor types such as colon (Hashida et al. 2003), pancreatic (Sho et al. 1998) and bladder cancer (Mhawech et al. 2003) and loss of *CD9* expression was suggested to be one of the steps that promote cancer progression in most cases analyzed. However, in prostate cancer, CD9 did not appear to affect tumorigenesis *in vivo* (Zvieriev et al. 2005). In our study, we detected the gain of *CD9* (12p13.31) in 16 cases and 11 (68.8%) of these showed bone metastases. This suggests the role of this gene in the behaviour of tumor progression to metastasis.

Gain of 16q was detected in our cases with minimal region of gains at 16q24.3, observed in 19 (52.8%) out of 36 cases. This region showed significant correlation with tumor size (>4.5 cm). *CDK10* gene is one of the genes contain in this region. *CDK10* (cyclin-dependent kinase 10) is a member of Cdc2-related kinases family that plays a role of the G2/M phase transition in the cell cycle (Zvieriev et al. 2005). This gene was reported to be implicated in breast cancer however the gain of this gene in prostate cancer has not been reported in previous studies. Our study also showed that *CDK10* was more frequent in cases with bone metastasis (7/19; 36.8%), suggesting that this gene may have a possible involvement in prostate tumor progression, although no significant correlation was found.

Array CGH also detected amplification at Xq12 in our cases (7/36; 19%), the region in which the *androgen receptor* (*AR*) gene is located. However, our findings were at lower frequencies than those found in previous studies. We found that Xq12 amplified region correlated significantly with high tumor grade (>Gleason 7). Previous study had demonstrated that *AR* gene seems to be rarely amplified in primary tumors (Bubendorf et al. 1999) while other study found that AR gene was amplified in 20–50% of hormone-refractory prostate cancers (Brown et al. 2002). Despite the amplification at the Xq12 region, a frequent Xq28 amplification was also noted in our cases but at fairly low frequencies, in line with the finding of previous study which identified the second cancer susceptibility locus on Xq27-q28, suggests that there might be also other genes on chromosome X may involve in the prostate tumorigenesis.

In summary, array CGH analysis of a series of 36 prostate adenocarcinomas identified deleted and amplified chromosomal aberrant regions, some of which correlated with clinicopathological parameters. We also found no significant chromosomal aberration according to racial distribution as the number of each races analysed wasn't equally proportioned. Future study on larger series of prostate cancer patients would be of valuable data to correlate the chromosomal aberrations according to race. Several genes within the aberrant chromosomal sites such as *myopodin* (4q26-q27), *ROBO1* (3p13-p11.2), *ERCC5* (13q33.1-q33.3) and *CD9* (12p13.31) have not been reported in previous array CGH studies. Our array CGH profile may provide important clues in identifying putative genes within these altered regions. Our chromosomal aberration results deserve further evaluation of these genes on their role in prostate tumorigenesis of Malaysian population.

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