ORIGINAL ARTICLE

Heterogenous expression of ERG oncoprotein in Malaysian men with adenocarcinoma of the prostate

Jia Shin Jessica TAN[1], Kien Chai ONG[2], Diana Bee-Lan ONG[1], Azad RAZACK[3], Jasmine LIM[3], Rosna YUNUS[4], Murali SUNDRAM[5] and Anthony RHODES[1,6]

[1]*Departments of Pathology,* [2]*Biomedical Science and Surgery* [3]*Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia,* [4]*Histopathology Unit, Hospital Kuala Lumpur, Kuala Lumpur, Malaysia,* [5]*Department of Urology, Hospital Kuala Lumpur, Malaysia,* [6]*School of Medicine, Faculty of Health and Life Sciences, Taylor's University, Selangor, Malaysia.*

Abstract

Introduction: Prostate cancer is a heterogenous disease and the mechanisms that drive it to behave differently are not well understood. Tumour expression of the ERG oncogene occurs in the majority of patients with prostate cancer in Western studies. This is considered to be oncogenic as ERG acts as a transcription factor to regulate genes involved in tumour proliferation and invasion. In this study we investigated expression of ERG in Malaysian men with prostate cancer. *Methods:* Tissues were collected from 80 patients with clinically detected prostate cancer and treated with radical prostatectomy. Cases were tested for ERG by immunohistochemistry using the mouse monoclonal antibody EP111. All blocks on 48 cases were tested in order to determine the extent of heterogeneity of ERG expression within individual cases. ERG expression was analysed in relation to patient age, ethnicity and tumour stage and grade. *Results:* Forty-six percent of cases were ERG positive. There was no significant association between ERG and tumour grade or stage. Sixty-nine percent of Indian patients had ERG positive tumours; this was significantly higher (p=0.031) than for Chinese (40%) and Malay (44%) patients. Heterogeneity of ERG expression, in which both positive and negative clones were present, was seen in 35% of evaluated cases. Evaluation by tumour foci showed younger patients had more ERG positive tumour foci than older patients (p=0.01). Indian patients were more likely to have the majority of tumour foci with ERG staining positively, compared to either Chinese or Malay patients (P <0.01). *Conclusion:* In this study, tumour expression of ERG was more likely to occur in patients of Indian ethnicity.

Keywords: TMPRSS2-ERG, ERG, ethnic variation

INTRODUCTION

A fundamental goal of cancer research is to identify key mutations in oncogenes which are responsible for driving a tumour into an aggressive cancer that will both invade adjacent tissues locally and metastasize. This is particularly important for prostate cancer as it is well known that a large numbers of screen detected prostate cancers are relatively indolent and unlikely to result in mortality.[1] In addition, prostate cancer is notoriously heterogeneous with respect to exhibiting different clones of cancer within the same prostate gland; some of higher grade and potentially more aggressive than other clones.[2] Consequently, the diagnosis and management of prostate cancer is particularly challenging, as in order to be truly effective, a prognostic test is needed that can reliably differentiate the aggressive tumour foci from the indolent ones. To date, no test is able to achieve this with a high degree of accuracy; consequently there is considerable over-treatment of indolent prostate cancer, by either radical prostatectomy or radiotherapy.[3] Both these treatments can result in unwanted side effects following treatment, such as urinary incontinence and erectile dysfunction, which adversely affect the quality of life of men diagnosed with prostate cancer.

A key oncogenic event occurring in up to sixty-percent of prostate cancers in western

Address for correspondence: Professor Dr Charles Anthony Rhodes, School of Medicine, Faculty of Health and Medical Sciences, Taylor's University, Selangor, Malaysia. Tel: 01133172407 E-mail: carhodes60@gmail.com

cohorts is the fusion of the androgen driven TMPRSS2 gene to the proto-oncogene ERG.[4] ERG protein expression is then greatly increased due to androgen binding to the promoter region of the TMRPSS2-ERG fusion. This increased ERG expression is considered to be oncogenic as it acts as a transcription factor for the downstream regulation of a number of genes, known to be important in tumour proliferation and invasion.[4] It has been shown in numerous studies comparing ERG protein expression to TMPRSS2-ERG gene fusion that up regulation of ERG in tissue samples is caused predominantly by the TMPRSS2-ERG fusion event, and this over expression of ERG can be reliably detected using immunohistochemistry.[5-9]

There is still considerable debate about whether or not over-expression of ERG in prostate cancer is a marker of aggressive tumours and therefore of poor prognosis.[4, 10-12] However, in the prostate it does appear to be only associated with invasive adenocarcinoma of the prostate and its pre-cursor lesion, prostatic intra-epithelial neoplasia (PIN).[13] Interestingly, there is evidence to suggest that the expression of ERG occurs more frequently in western studies of prostate cancer[13,14] than it does in Asia, where in some cancer cohorts its frequency of occurrence has been reported to be as low as thirty-percent.[15-20]

Epidemiological studies of men with prostate cancer show distinct differences in terms of clinical incidence and mortality rates between ethnic groups.[21] While prostate cancer is the most frequently diagnosed male malignancy in the Western world including Northern Europe, the USA and Canada, it is notably less common in Asian countries[21,22] despite a rapid increase in recent years.[21-23] Specifically, in Malaysia despite having a relatively lower incidence in comparison to Western countries, the incidence of prostate cancer has increased by twenty-percent between the years 2007 to 2011. It is also noted that about 40% of men with prostate cancer have metastatic disease at presentation,[24] unlike western studies where the majority of cases are detected at an earlier stage. Among the major ethnic groups in Malaysia, the Chinese have the highest incidence of prostate cancer (nine-percent), followed by Malaysian Indian (six-percent) and Malaysian Malay (five-percent).[25]

In the current study we seek to investigate the expression of ERG in a retrospective series of prostate cancers from Malaysian men with clinically detected hormone naïve prostate cancer treated by radical prostatectomy at a large city hospital. If future research establishes ERG as a key event promoting aggressive forms of prostate cancer, it is important to determine its relevance to Asian men. Moreover, examining the uniformity of ERG expression between different tumour foci in each radical prostatectomy, will help determine whether ERG expression is a main driving event in these Asian men with prostate cancer.

MATERIALS AND METHODS

Tissue samples
Tissue samples were retrospectively collected from clinically detected prostate cancer patients who were treated with radical prostatectomy at Hospital Kuala Lumpur, Malaysia from 2007 to 2013. A total of 80 cases were selected based on the availability of samples for review. Whole prostatectomy samples were fixed in neutral buffered formalin for 24 hours before processing to paraffin wax and embedding. For the study, the Haematoxylin & Eosin (H&E) stained slides for each tissue block were reviewed and samples showing invasive adenocarcinoma were selected by a pathologist for testing. Patients' data including age and ethnicity were collected for all cases where available from the patient's medical record folder. Histological type and tumour grading according to the Gleason's grading system were determined from the pathologist's report. The American Joint Committee on Cancer (AJCC Cancer) Prostate Cancer Staging Manual 8th edition was used for pathological tumour staging.[26] On 48 of the total 80 cases, all available blocks for each case and containing tumour were tested for ERG expression; on the remaining 32 cases, tissue blocks containing tumour with the primary Gleason grade were chosen for testing. Permission for the analysis of human tissue was obtained from the Medical Research & Ethics Committee, Malaysian Ministry of Health (NMRR-10-1400-7968).

Immunohistochemical testing for ERG Oncoprotein Expression
Tissue sections were cut at 4 micrometers using a rotary microtome and the sections mounted onto Superfrost Plus Slides (Thermo Scientific, USA) for maximum adhesion. Immunohistochemical detection of ERG oncoprotein expression was achieved using the previously validated[4] ERG monoclonal antibody Clone EP111 (Dako, Denmark). Briefly, the paraffin wax embedded sections were dewaxed in xylene and rehydrated in a series of graded alcohols before antigen

retrieval in Tris EDTA (pH 9) for 30 minutes at 100°C. The sections were then treated with 0.3% H_2O_2 to block endogenous peroxidase for 10 minutes. Incubation with the ERG mAb (Clone EP111, Dako, Denmark) diluted at 1:100 was carried out for 1 hour at room temperature. Primary antibody was detected using DAKO REAL EnVision Detection System with a horse radish peroxidase label (Dako, Denmark) for 1 hour at room temperature and visualised using 3,3-diaminobenzidine tetrahydrochloride (DAB) chromogen (Dako, Denmark). Nuclei were counterstained with Harris's haematoxylin (Leica, Germany) for 1 minute. The expression of ERG oncoprotein was evaluated microscopically and recorded as positive when the tumour nuclei stained positively, regardless of the proportion of tumour cells stained or the staining intensity. Experimental runs contained negative controls in which the primary antibody was omitted whilst the staining of vascular endothelial cells of small vessels functioned as the internal positive control. In addition, a tissue section from a prostatic adenocarcinoma known to stain strongly for ERG was included in each staining run. A case, comprising a number of different blocks, was considered to be positive if at least one tumour block showed positivity for ERG expression.

Statistical analysis
Statistical analyses were performed using SPSS 16.0 software for Windows (IBM, Inc., New York, NY, USA). Clinical and pathological features of the cases were compared across groups of patients using frequencies and percentages. The difference of distribution of clinical and pathological characteristics across different ethnicity groups were evaluated using the Chi square test. Results were considered to be significant if the P-value was less than 0.05.

RESULTS

The median age of the patients was 67.0 years (range, 53-78 years). The majority of the patients comprised Chinese (50.0%), followed by Malay (33.8%) and Indian ethnicities (16.3%) (Table 1).

ERG expression
Overall, 37/80 (46.3%) of the cases were positive for ERG expression (Fig. 1). With respect to ethnicity; 9 out of 13 Indian patients (69.2%) had ERG positive tumours; this proportion is significantly higher (p=0.031) than in the other two ethnicities, with 16/40 (40.0%) of Chinese patients and 12/27 (44.4%) of Malay patients having ERG positive tumours, respectively. ERG was not found to be associated with tumour grade or stage (Table 2).

Heterogeneity for ERG oncoprotein expression, in which both ERG positive and negative clones were present, was seen in 35.4% (17/48) of cases, in which all tissue blocks were tested. Evaluation by tumour foci showed that patients younger than the median age of 67 years had more ERG positive tumour foci than older patients (p=0.01). Similarly, Indian patients were not only more likely to have ERG positive tumours than the other two ethnicities, but when positive for ERG were also more likely to have the majority of tumour foci staining (P<0.01) (Table 3, and Fig. 2).

TABLE 1: The patient age, and the stage and grade of the prostate cancers included in the study (n=80)

Variable	Chinese		Malay		Indian		*P* Value
N	40		27		13		
Age at Surgery							0.262
Median (Range)	67	(54-77)	64	(54-78)	66	(53-73)	
Gleason Score - N (%)							0.812
6	7	(8.75)	3	(3.75)	2	(2.50)	
7	27	(33.75)	17	(21.25)	8	(10)	
8 or above	6	(7.50)	7	(8.75)	3	(3.75)	
Pathological T Stage - N (%)							0.817
T2	27	(33.75)	16	(20)	8	(10)	
T3a*, b	13	(16.25)	11	(13.75)	5	(6.25)	

*PT3a tumours comprised 5 cases only.

TABLE 2: Evaluation of the association of ERG oncoprotein expression status with clinical and pathological parameters

Parameter	ERG Negative		ERG Positive		P Value
	No.	%	No.	%	
Total N	43	54	37	46	
Age					
<67	18	49	19	51	0.5
≥67	25	58	18	42	
Ethnicity					
Chinese	24	60	16	40	0.031*
Malay	15	56	12	44	
Indian	4	31	9	69	
Path T Stage					
2	30	59	21	41	0.25
3a*, b	13	45	16	55	
Gleason Score					
6	7	58	5	42	0.76
7	29	56	23	44	
≥8	7	44	9	56	

*PT3a tumours comprised 5 cases only.

DISCUSSION

Malaysia comprised the 3 most populous ethnicities in Asia; Malay, Chinese and Indians. Therefore, with respect to its people and its cultures it represents a microcosm of Asia. In comparing the expression of the ERG oncogene in men with prostate cancer from different Asian ethnicities, our study has the advantage that the men all attended the same medical center. Therefore, the processing and testing of the tissue samples was standardised and uniform throughout. This is not necessarily the case when comparing the results from different studies, conducted at different centres and in different countries.

Prostate cancer is notoriously multifocal. Morphologic and molecular analysis carried out in the past has demonstrated that up to eighty percent of prostates can harbour multiple separate cancers by the time of diagnosis.[2,27-30]

TABLE 3: Evaluation of heterogeneity of ERG expression in all tissue blocks of 48 cases of prostate cancer and association with patient age and ethnicity

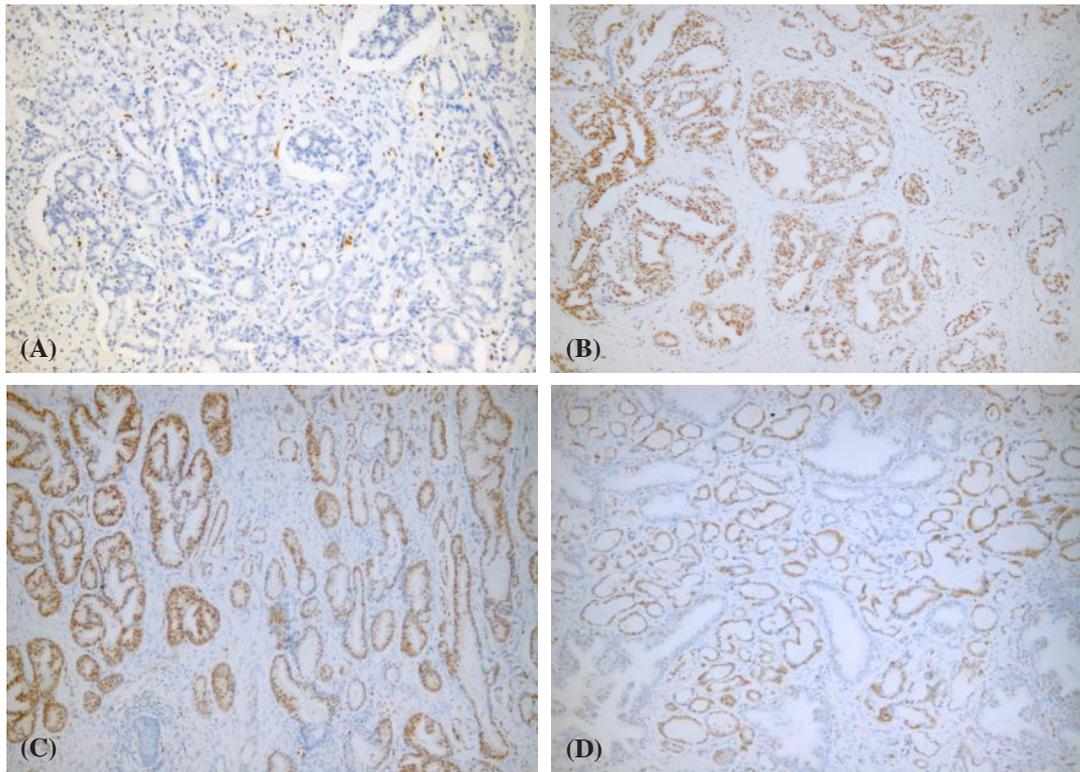| Parameter	ERG Negative		ERG Positive		P Value
	No.	%	No.	%	
Total N	149	67	73	33	
Age					0.01*
<67	58	58	42	42	
≥67	91	74.6	31	25.4	
Ethnicity					<0.001*
Chinese	69	87.3	10	12.7	
Malay	63	65.6	33	34.4	
Indian	17	36.2	30	63.8	

FIG. 1: Immunohistochemical staining for the expression of truncated oncoprotein erythroblast transformation-specific-related gene (ERG). Representative images demonstrating: (A) The endothelial cells of small vessels showed positive endogenous ERG expression in the context of surrounding ERG-negative tumour. (B, C, D) Nuclear staining of the tumour cells is apparent in ERG positive tumours; original magnification x200.
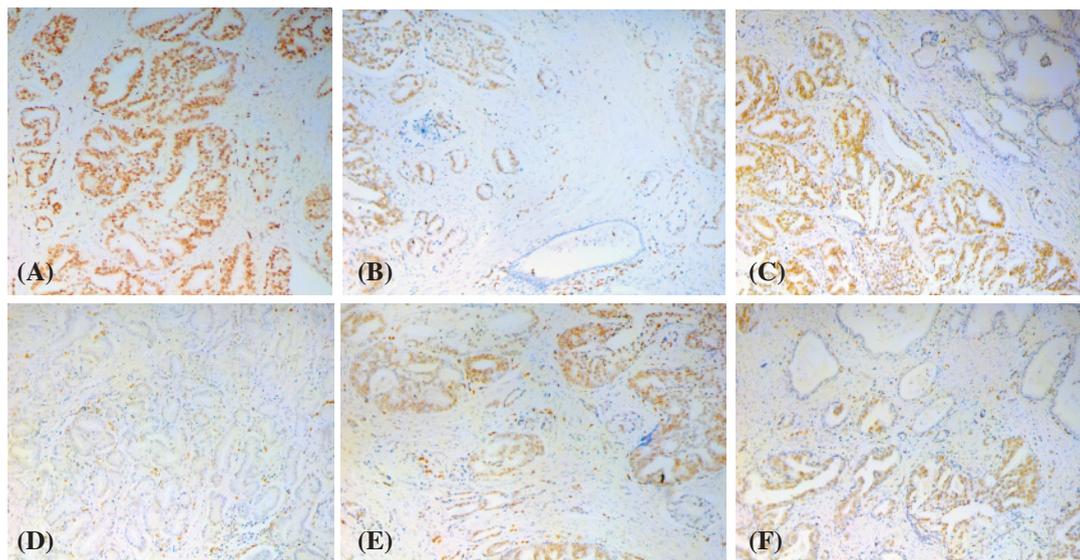

FIG. 2: Inter-focal heterogeneity of ERG oncoprotein expression in different tissue blocks from one patient. Blocks A, B, C, E and F exhibited ERG oncoprotein expression with intense nuclear staining; (D) Absence of ERG staining in the tumour but positive endogenous ERG expression on the endothelial cells can be seen; original magnification x200.

Previous studies have also reported inter-focal and intra-focal variability in ERG protein levels in prostate cancer.[31-33] Therefore, we considered it important to our investigations to systematically evaluate the question of heterogeneity of ERG expression in the samples. Overall, we observed a high rate (35%) of inter-focal heterogeneity for ERG oncoprotein expression in our cases.

Similar to other studies investigating the frequency of ERG expression in radical prostatectomy patients,[7,34,35] the overall frequency of ERG over-expression in our study was 46.3%. Studies from the west, especially from the United States and Europe have shown slightly higher TMPRSS2-ERG fusion and ERG expression rates (>50%)[13,14] in comparison to Asian countries with some studies showing less than 30% of cases exhibiting TMPRSS2-ERG fusion or ERG expression.[15-20]

Interestingly, we observed significant differences in ERG expression in the prostate cancer of Malaysian men. As with a recent study on TRUS biopsies,[36] we found expression of ERG to be more common in the radical prostatectomy samples of Indian Malaysian men (69%), than Malay (44%) or Chinese Malaysian men (40%) with prostate cancer. Moreover, when occurring in the tissue samples of Indian men, ERG expression tended to occur in the majority of the tumour foci i.e. there was less heterogeneity of expression; suggesting that ERG is associated with the main driver of prostate cancer in these cases. This is in contrast to ERG expression in the prostatectomy samples of Chinese and Malay Malaysian men, where it occurred less frequently and when it did occur tended to be in a minority of the tumour foci (tissue blocks). Whilst the numbers are small, these are important observations as it suggests that an oncogenic event other than ERG expression, is the main driver of prostate cancer in Chinese and Malay men. This is particularly relevant in Malaysia where the data shows prostate cancer to occur more commonly in Chinese men, than the other two main Malaysian ethnicities.[25]

The other main observation was that ERG expression occurred more frequently in samples of patients less than the median age of 67 years of age, and was significantly associated with greater uniformity of expression i.e. when ERG positive, the majority of foci in the radical prostatectomy were positive. These findings are in agreement with studies that have similarly demonstrated an association of TMPRSS2 ERG fusion status with younger age at diagnosis.[37,38]

Future studies are required to establish the prognostic relevance of ERG in prostate cancer. However, a number of studies to date have shown an association of ERG expression with worse prognosis indicated by higher tumour stage and metastasis or tumour-specific mortality.[39-47] In addition, there is a need to determine the events responsible for the occurrence of the fusion gene, such as the activity of the androgen receptor, and how this in turn may reflect ethnic differences in ERG expression in prostate cancer.[48-51] These events are likely to involve both genetic and environmental factors.

REFERENCES

1. Cooperberg MR, Broering JM, Carroll PR. Time Trends and Local Variation in Primary Treatment of Localized Prostate Cancer. J Clin Oncol. 2010; 28: 1117-23.
2. Arora R, Koch MO, Eble JN, Ulbright TM, Li L, Cheng L. Heterogeneity of Gleason grade in multifocal adenocarcinoma of the prostate. Cancer. 2004; 100: 2362-6.
3. Schröder FH, Hugosson J, Roobol MJ, et al. Screening and Prostate-Cancer Mortality in a Randomized European Study. N Engl J Med. 2009; 360: 1320-28.
4. Hagen RM, Adamo P, Karamat S, *et al*. Quantitative analysis of ERG expression and its splice isoforms in formalin-fixed, paraffin-embedded prostate cancer samples: association with seminal vesicle invasion and biochemical recurrence. Am J Clin Pathol. 2014; 142: 533-40.
5. Miettinen M, Wang ZF, Paetau A, *et al*. ERG transcription factor as an immunohistochemical marker for vascular endothelial tumors and prostatic carcinoma. Am J Surg Pathol. 2011; 35: 432-41.
6. van Leenders GJ, Boormans JL, Vissers CJ, *et al*. Antibody EPR3864 is specific for ERG genomic fusions in prostate cancer: implications for pathological practice. Mod Pathol. 2011; 24: 1128-38.
7. Furusato B, Tan SH, Young D, Dobi A, Sun C, Mohamed AA. ERG oncoprotein expression in prostate cancer: clonal progression of ERG-positive tumor cells and potential for ERG-based stratification. Prostate Cancer Prostatic Dis. 2010; 13.
8. Park K, Tomlins SA, Mudaliar KM, Chiu YL, Esgueva R, Mehra R. Antibody-based detection

of ERG rearrangement-positive prostate cancer. Neoplasia. 2010; 12.

9. Chaux A, Albadine R, Toubaji A, et al. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. Am J Surg Pathol. 2011; 35: 1014-20.

10. Spencer ES, Johnston RB, Gordon RR, *et al*. Prognostic value of ERG oncoprotein in prostate cancer recurrence and cause-specific mortality. Prostate. 2013; 73: 905-12.

11. Leinonen KA, Saramäki OR, Furusato B, *et al*. Loss of PTEN Is Associated with Aggressive Behavior in ERG-Positive Prostate Cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2013; 22: 2333-44.

12. Hoogland AM, Jenster G, van Weerden WM, *et al*. ERG immunohistochemistry is not predictive for PSA recurrence, local recurrence or overall survival after radical prostatectomy for prostate cancer. Mod Pathol. 2012; 25: 471-79.

13. Tomlins SA, Rhodes DR, Perner S, *et al*. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005; 310: 644-8.

14. Petrovics G, Liu A, Shaheduzzaman S, *et al*. Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome. Oncogene. 2005; 24: 3847-52.

15. Lee K, Chae JY, Kwak C, Ku JH, Moon KC. TMPRSS2-ERG gene fusion and clinicopathologic characteristics of Korean prostate cancer patients. Urology. 2010; 76: 1268.e7-13.

16. Mao X, Yu Y, Boyd LK, Ren G, Lin D, Chaplin T. Distinct genomic alterations in prostate cancers in Chinese and Western populations suggest alternative pathways of prostate carcinogenesis. Cancer Res. 2010; 70.

17. Miyagi Y, Sasaki T, Fujinami K, *et al*. ETS family-associated gene fusions in Japanese prostate cancer: analysis of 194 radical prostatectomy samples. Mod Pathol. 2010; 23: 1492-8.

18. Suh JH, Park J-W, Lee C, Moon KC. ERG Immunohistochemistry and Clinicopathologic Characteristics in Korean Prostate Adenocarcinoma Patients. Korean J Pathol. 2012; 46: 423-28.

19. Qi M, Yang X, Zhang F, *et al*. ERG Rearrangement Is Associated with Prostate Cancer-Related Death in Chinese Prostate Cancer Patients. PLoS ONE. 2014; 9: e84959.

20. Rawal S, Young D, Williams M, *et al*. Low Frequency of the ERG Oncogene Alterations in Prostate Cancer Patients from India. J Cancer. 2013; 4: 468-72.

21. Gronberg H. Prostate cancer epidemiology. Lancet. 2003, 361: 859-64.

22. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010; 60: 277-300.

23. Sim HG, Cheng CW. Changing demography of prostate cancer in Asia. Eur J Cancer. 2005; 41: 834-45.

24. Lim J, Bhoo-pathy N, Sothilingam S, *et al*. 1023 Prostate cancer in multi-ethnic Asian men. Eur J Cancer. 2015; 51: S152-S53.

25. Azizah A, Nor Saleha I, Noor Hashimah A, Asmah Z, Mastulu W. Malaysian National Cancer Registry Report 2007-2011. Malaysia Cancer Statistics: Data and Figure. Edited by Institute NC. June 2015 ed. Ministry of Health, No 4, Jalan P7, Presint 7, 62250 Putrajaya, MALAYSIA: National Cancer Institute, 2015. p. 74.

26. Buyyounouski MK, Choyke PL, McKenney JK, *et al*. Prostate cancer - major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. 2017; 67: 245-53.

27. Greene DR, Wheeler TM, Egawa S, Dunn JK, Scardino PT. A comparison of the morphological features of cancer arising in the transition zone and in the peripheral zone of the prostate. J Urol. 1991; 146: 1069-76.

28. Cheng L, Song SY, Pretlow TG, *et al*. Evidence of independent origin of multiple tumors from patients with prostate cancer. J Natl Cancer Inst. 1998; 90: 233-7.

29. Qian J, Bostwick DG, Takahashi S, *et al*. Chromosomal anomalies in prostatic intraepithelial neoplasia and carcinoma detected by fluorescence in situ hybridization. Cancer Res. 1995; 55: 5408-14.

30. Sakr WA, Macoska JA, Benson P, *et al*. Allelic loss in locally metastatic, multisampled prostate cancer. Cancer Res. 1994; 54: 3273-7.

31. Ayala G, Frolov A, Chatterjee D, He D, Hilsenbeck S, Ittmann M. Expression of ERG protein in prostate cancer: variability and biological correlates. Endocr Relat Cancer. 2015; 22: 277-87.

32. Mehra R, Han B, Tomlins SA, *et al*. Heterogeneity of TMPRSS2 gene rearrangements in multifocal prostate adenocarcinoma: Molecular evidence for an independent group of diseases. Cancer Res. 2007; 67: 7991-5.

33. Tsourlakis M-C, Stender A, Quaas A, *et al*. Heterogeneity of ERG expression in prostate cancer: a large section mapping study of entire prostatectomy specimens from 125 patients. BMC Cancer. 2016; 16: 641.

34. Barry M, Perner S, Demichelis F, Rubin MA. TMPRSS2-ERG Fusion Prostate Cancer Heterogeneity: Clinical and Biologic Implications. Urology. 2007; 70: 630-33.

35. Rosen P, Pfister D, Young D, Petrovics G, Chen Y, Cullen J. Differences in frequency of ERG oncoprotein expression between index tumors of Caucasian and African American patients with prostate cancer. Urology. 2012; 80.

36. Kelly GM, Kong YH, Dobi A, *et al*. ERG oncoprotein expression in prostate carcinoma patients of different ethnicities. Molecular and Clinical Oncology. 2015; 3:23-30.

37. Schaefer G, Mosquera JM, Ramoner R, *et al*. Distinct ERG rearrangement prevalence in prostate cancer: higher frequency in young age and in low PSA prostate cancer. Prostate Cancer Prostatic Dis. 2013; 16: 132-8.

38. Kimura T, Furusato B, Miki J, *et al*. Expression of ERG oncoprotein is associated with a less

aggressive tumor phenotype in Japanese prostate cancer patients. Pathol Int. 2012; 62: 742-8.

39. Deplus R, Delliaux C, Marchand N, *et al*. TMPRSS2-ERG fusion promotes prostate cancer metastases in bone. Oncotarget. 2017; 8: 11827-40.

40. Pettersson A, Graff RE, Bauer SR, *et al*. The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2012; 21: 1497-509.

41. Font-Tello A, Juanpere N, de Muga S, *et al*. Association of ERG and TMPRSS2-ERG with grade, stage, and prognosis of prostate cancer is dependent on their expression levels. Prostate. 2015; 75: 1216-26.

42. Mehra R, Tomlins SA, Yu J, *et al*. Characterization of TMPRSS2-ETS Gene Aberrations in Androgen Independent Metastatic Prostate Cancer. Cancer Res. 2008; 68: 3584-90.

43. St. John J, Powell K, Conley-LaComb MK, Chinni SR. TMPRSS2-ERG Fusion Gene Expression in Prostate Tumor Cells and Its Clinical and Biological Significance in Prostate Cancer Progression. Journal of cancer science & therapy. 2012; 4: 94-101.

44. Demichelis F, Fall K, Perner S, *et al*. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene. 2007; 26: 4596-99.

45. Perner S, Mosquera JM, Demichelis F, *et al*. TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. Am J Surg Pathol. 2007; 31: 882-8.

46. Perner S, Demichelis F, Beroukhim R, *et al*. TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. Cancer Res. 2006; 66.

47. Nam RK, Sugar L, Yang W, *et al*. Expression of the TMPRSS2:ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer. 2007; 97: 1690-5.

48. Lange EM, Sarma AV, Ray A, *et al*. The androgen receptor CAG and GGN repeat polymorphisms and prostate cancer susceptibility in African-American men: results from the Flint Men's Health Study. J Hum Genet. 2008; 53: 220-6.

49. Li J, Mercer E, Gou X, Lu Y-J: Ethnical disparities of prostate cancer predisposition: genetic polymorphisms in androgen-related genes. Am J Cancer Res. 2013; 3: 127-51.

50. Bratt O, Borg A, Kristoffersson U, Lundgren R, Zhang QX, Olsson H. CAG repeat length in the androgen receptor gene is related to age at diagnosis of prostate cancer and response to endocrine therapy, but not to prostate cancer risk. Br J Cancer. 1999; 81: 672-6.

51. Chen C, Lamharzi N, Weiss NS, *et al*. Androgen receptor polymorphisms and the incidence of prostate cancer. Cancer Epidemiol Biomarkers Prev. 2002; 11: 1033-40.