IMPORTANCE OF CHROMOSOME 8 GAIN AND c-myc GENE AMPLIFICATION IN HIGH GRADE PROSTATE CANCER[1]

Ebru ETEM[1], Halit ELYAS[1], Hüseyin YÜCE[1], Nusret AKPOLAT[2], Mustafa Kemal ATIKELER[3]

[1]Department of Medical Biology and Genetics, Faculty of Medicine, Fırat University, Elazığ - TÜRKİYE
[2]Department of Pathology, Faculty of Medicine, Fırat University, Elazığ - TÜRKİYE
[3]Department of Urology, Faculty of Medicine, Fırat University, Elazığ - TÜRKİYE

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Prostat Kanserinde c-Myc Gen Amplifikasyonu ve Kromozom 8 Kazancının Önemi

Özet

Son zamanlarda yapılan sitogenetik ve moleküler genetik çalışmalarla, 8q24'de lokalize olup özellikle ilerlemiş ve rekürrent prostat kanserlerinde amplifiye olan c-myc geni tanımlanmıştır. Bu çalışmada amacımız, prostat karsinogenezinde kromozom 8 kazancı ve c-myc'nin rolünü tanımlamak ve prostatik intraepitelial neoplazi (PIN) ve prostat kanseri arasındaki genetik ilişkiyi değerlendirmektir. 9 adenokarsinom, 6 PIN ve 15 benign prostatik hiperplazi (BPH)olmak üzere toplam 30 örnek Fluoresans *in situ* Hibridizasyon (FISH)'le değerlendirildi. Adenokarsinomların %88.8'inde tanımlanan kromozom 8 kazancı metastatik prostat kanseriyle beraberlik göstermektedir. PIN ve karsinomada en sık görülen anomali kromozom 8 kazancıdır ve bu anomalinin varlığı yüksek Gleason skoruyla beraberlik göstermektedir. C-myc geninin aşırı ekspresyonunda temel mekanizmanın amplifikasyon olmayabileceğine inanmaktayız. Bulgularımız c-myc gen ekspresyonunda temel mekanizmanın kromozom 8'in basit kazancı veya 8q'nun kazancından kaynaklanabileceğini göstermektedir. Sonuçlarımız PIN'nin karsinomanın prekürsörü olduğunu söyleyen diğer yazarların bulgularıyla tutarlıdır.

Anahtar Kelimeler: Prostat kanseri, gen amplifikasyonu, FISH, c-myc, PIN

Summary

Recently, cytogenetic and molecular biological studies have identified the band 8q24, where the located c-myc gene is commonly amplified in prostate cancer, especially in advanced and recurrent ones. Our objectives in this study were to define the role of c-myc and the gain of chromosome 8 in prostatic carcinogenesis and to evaluate the genetic relationship between prostatic intraepithelial neoplasia (PIN) and carcinomas. We examined a total of 30 specimens, including 9 adenocarcinoma, 6 PIN and 15 benign prostatic hyperplasia (BPH) by Fluorescence *in situ* Hybridization (FISH). The gain of chromosome 8 identified in 88.8% of adenocarcinomas was associated in metastatic prostate cancer. The most frequent anomaly in PIN and carcinoma was a gain of chromosome 8, and the presence of this anomaly strongly correlated with a high Gleason Score. We believe that the basic mechanism in overexpression of c-myc gene may not be amplification. Our results indicate that the basic mechanism of c-myc gene overexpression may be simple gain of chromosome 8 or gain of ''8q''. Our results agree with findings of other authors that PIN is probably a procurser of carcinoma.

Key Words: Prostate cancer, gene amplification, FISH, c-myc, PIN

Introduction

Prostate cancer is the most frequent malignancies and the second leading cause of cancer deaths among males in the Western World. The clinical course of the disease is higly complex and genetic factors underlying tumorigenesis are poorly understood (1). An understanding of the genetic events that accompanied with the progression of the most likely precursor lesion, PIN, to prostatic adenocarcinoma and the subsequent development of metastases may be useful for prevention, early detection and treatment (2- 4).

Recent cytogenetic and molecular biological studies have established that band 8q24 is commonly

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amplified in prostate cancer, especially in advanced and recurrent prostate cancer. c-myc activation usually occurs at later stages of the carcinoma in human and is usually a poor prognostic marker (4). The c-myc gene is mapped to this region, and the gene family appears to play an important role in the regulation of cellular proliferation and differantiation. Aberrrant expression of these genes contributes to the pathogenesis of numerous human neoplasms and has been implicated in the apoptotic process (5, 6). A significant association was found between elevated htert (human telomerase reverse transcriptase) expression and c-myc overexpression. It is likely that the ability of c-myc protein to stimulate expression of htert and thereby enhance telomerase activity represents an important step in prostate tumorigenesis (7, 8)

FISH analysis of interphase cells with centromere specific and region-specific probes is useful for the detection of numerical chromosomal abnormalities and genetic alterations in solid tumors such as prostatic carcinomas that are often difficult to analyze by conventional cytogenetic analysis (2, 9). The most common alterations in prostate cancer are loss of 8p and gain of 8q, which have been detected in more than 80% and 90% of the cases, respectively. The gain of 8q is often associated with the loss of 8p especially in hormone-refractory carcinomas and in distant metastases (10-12).

Our aims in this study were to define the role of c-myc and the gain of chromosome 8 in prostatic carcinogenesis and evaluate the genetic relationship between PIN and adenocarcinomas.

Materials and Methods

Patient Selection

Prostate cancer, PIN and BPH cases were collected for our study at Firat Medical Center between March 2001 and August 2002. Prostate adenocarcinomas and PIN were histopathologically diagnosed as high-grade.

Sample Preparation and Histopathological Evaluation

We analyzed a total of 30 cases, including 9 prostate adenocarcinoma, 15 benign prostatic hyperplasia and 6 prostatic intraepithelial neoplasia. Two patients with prostate cancer had metastases (One case had bone metastases and the other one had pelvic lymph node metastases), two patients had recurrent and this patients had received hormone therapy. All remaining cases included 26 newly diagnosed untreated patients. To determine the criteria for FISH anomalies, 15 samples of benign prostatic hyperplasia tissue obtained by retropubic prostectomy were also analyzed. The tumors selected had high Gleason Scores (6-10). Tumor grade was classified according to the Gleason Score and Cell Cytology. May Grunwold-Giemsa (MGG) and Papanicolaou Gynaekologie (PAP) was made for cell cytology. Cell cytology and Gleason Score were evaluated by expert pathologist.

Fluorescence *in situ* Hybridization (FISH)

Slides were prepared so as to use touch preparation protocol. Specimens were touched lighty on precleaned slides, which can be performed in a short time. After air-drying at room temperature, these slides were fixed with fixing solution (3:1 methanol:acetic acid) and stored at -20°C until use. Target slides were denatured in 2XSSC /70% formamide, pH 7, at 67 °C for 6 min and dehydrated in graded ethanol. Dual-labeling hybridization was performed using 10µl of the hybridization mixture containing fluorescein direct labeled chromosome 8 alpha-satellite probe and rhodamine direct-labelled c-myc probe. Probes were denatured at 76 °C for 10 min and applied to the target slides. Hybridization was performed overnight at 37°C in moist chamber. Posthybridization washes were performed with %50 formamide/ 2XSSC three times for 10 min, 2XSSC for 5 min, and 2XSSC/Nonidet P-40 for 5 min at 42°C. Counterstaining was specifically prepared by mixing 2µl of PI to 8µl of DAPI and then used. The number of FISH signals was counted with a Nicon microscope equipped with a three color filter. At least 100 nuclei was evaluated. FISH signals were counted according to the criteria described previously (13). Gain of Chromosome 8: ≥ 20% nuclei with three or more signals for centromeric 8. C-myc gene amplification: more c-myc signals than sentromeric 8 signals or c-myc/centromeric 8 ratio ≥ 1.10.

Statistical Analysis

Statistical analysis was carried out using the SPSS programme (Statistical Packages of Social Sciences, SPSS for Windows, Version 9.0, Inc, Chicago, IC, USA). Fischer's exact test was used to determine the association between copy number aberrations and tumor recurrence as well as metastases and Gleason Score. The p value of less than 0.05 was considered significant.

Results

Total of 30 cases were successfully analyzed. We found an 88.8% gain of chromosome 8 centromere and 100% c-myc extra copy number of adenocarcinomas exhibiting 3 or more positive signals for chromosome 8 centromere or c-myc in

20% or more of the cells. The gain of chromosome 8 and c-myc gene extra copy number are summarized in Table 1. Figure 1 shows typical FISH results for chromosome 8 and c-myc gene.

Table1. Classification of patients with prostate adenocarcinoma on the basis of FISH findings.

Patients No	Gleason Score	AverageSignals for c-myc	Average Signals for Cent 8	Myc/ Cent 8 Signal Ratio	FISH C-Myc	Classification Cent 8
1	5+4	373	363	1.03	Gain	Gain
2	5+4	272	308	1.13	Gain	Gain
3	5+4	378	385	1.01	Gain	Gain
4	5+5	384	400	1.04	Gain	Gain
5	5+5	596	618	1.03	Gain	Gain
6	5+5	318	389	1.22	Gain	Gain
7	5+5	387	391	1.01	Gain	Gain
8	3+3	395	225	1.75	Gain	Normal
9	4+3	250	271	1.08	Gain	Gain
10	PIN3	214	218	1.01	Gain	Gain
11	PIN2	180	174	1.03	Normal	Normal
12	PIN2	178	191	1.07	Normal	Normal
13	PIN1	186	197	1.05	Normal	Normal
14	PIN2	181	209	1.15	Gain	Normal
15	PIN1	180	183	1.01	Normal	Normal

Cent: Centromere, FISH: Fluoresans in situ Hybridization, PIN: Prostatic Intraepithelial Neoplasia

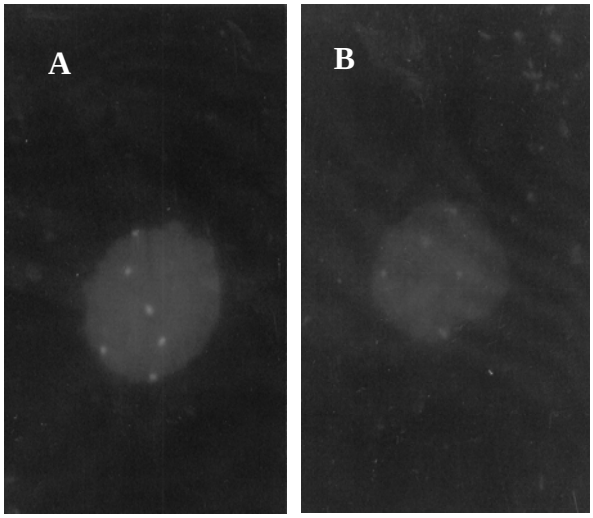

Figure 1: Dual-color FISH with chromosome 8 centromere (green signal) and c-myc gene specific probe (red signal). A: Nucleus of prostate cancer cell with 6 signals for green, indicating gain of chromosome 8. B: Nucleus of prostate cancer cell with 5 red signals, indicating amplification of c-myc.

According to the extra copies of c-myc, specimens could be divided into three groups; i. those with simple gain of a whole chromosome 8 (no increase in c-myc copy number relative to the chromosome 8 centromere), which was identified in 88% and 26.6%, in adenocarcinoma and PIN, respectively. ii. those with an intermediate increase in c-myc copy number relative to the chromosome 8 centromere, which was found in adenocarcinomas and PIN in 22.2% and 16.6%, respectively. iii. those with substantial amplification of c-myc (large increases in c-myc copy number relative to the chromosome 8 centromere), which was found in 11.1% of prostate adenocarcinoma, but not in PIN and BPH. Case 6, 8, and 14 are not included in the amplification evaluation to the study because of the existence of extra copy number of c-myc in centromeric 8 signals.

There was no correlation between gain of chromosome 8 or c-myc extra copy number and recurrence or metastases (p=0.12, p= 0.95 and p=0.14, p=0.95), respectively. However, the correlation was found between gain of basic chromosome 8 centromere and metastases with linear correlation test (p=0.038). Average signals numbers seen in most cells for chromosome 8 centromere and c-myc in cases with adenocarcinoma are illustrated in

Table 2. A statistically significant difference was found between PIN and carcinomas for signals obtained from c-myc and chromosome 8 in most of cells (p<0.05).

Patients No	The signal count seen in more than 20% cells	
	c-myc	Cent
1	4	4
2	3	3
3	4	3
4	4	4
5	6	6
6	4	3
7	4	4
8	3	2
9	3	3

Cent: Centromere

Discussion

Recent studies have indicated that several chromosomes (7, 8, 10 and Y) play important roles in tumorigenesis and tumor progression of prostate cancer (1, 14). Numerical chromosomal anomalies were found in 67%, 68%, and 96% of foci of PIN, carcinoma, and metastases, respectively. Chromosome 8 alterations, including loss of 8p21-22 and gain of 8q24, are commonly observed in prostate carcinoma. Sato et al. reported that alterations of c-myc were associated with both systemic progression and patient deaths (15). We found extra copies of c-myc in chromosome 8 centromere in 16% of PIN (case14), 22% of cancer (case 6 and 8).

A variety of factors may contribute to gain of c-myc. Simple gain of the whole chromosome 8 can account for many cases with extra copies of c-myc. Chromatid separation in proliferative cells will result in an apparent increase in the number of region-specific probe signals. Some studies have also reported loss of 8p concurrent with gain of the long arm of chromosome 8 (8q) sequences in advanced prostatic cancer. This combination of events occurring on the same chromosome–loss of 8p seguences and gain of 8q sequences-suggests formation of i(8q) chromosomes in advanced prostate tumors (16). Alers et al. (1997) reported that overrepresentation of 8q sequences, most likely by isochromosome 8q formation, is involved in metastatic spread to the bone (17).

Brown et al reported that anomalies of chromosomes 8 and/or 7, present in 14 of the 16 cases (88%) aneusomic by FISH and high-grade tumors, were more likely to be aneuploid on FISH (18). The present study showed similar findings with an 88.8% gain of chromosome 8 in adenocarcinomas

Oncogene amplification is one mechanism that leads to stepwise progression in solid tumors. Moreover, oncogene amplification may be a useful indicator of progressional prognosis in various human cancers (19). c-myc amplification is not common in prostate cancer specimens, although FISH has been demonstrated to be a sensitive technique for detecting changes in gene copy number. Miyoshi et al. reported that c-myc gene amplification was detected in 8%, 19%, and 46% of PIN, carcinoma and metastases of prostate (20). Bubendorf et al. reported no cases of high level myc amplification in primary tumors (21). Alers et al. previously found c-myc amplification in 8% of primary prostate tumors and c-myc gene amplification which correlates with high levels of myc protein expression (22). Mark et al. reported that an increased copy number in c-myc oncogene copy number was not a prominent finding in their cohort of prostate cancer patients (13). In our study, c-myc gene amplification was found in 11.1% of prostate cancer cases. Interestingly, c-myc gene amplification has been shown to occur in a case with primary prostate cancers without metastases and untreated prostate cancers in the present study. Our results support the findings of others that c-myc gene amplification or copy number increase is not common in prostate cancer specimens.

Jenkins et al. found extra copies of c-myc in 50% of PIN foci, 44% of cancer, and 92% of lymph node metastases and these were usually observed simultaneously with gain of chromosome 8 centromere (4). Our study demonstrated that extra copies of c-myc were found in 100% of adenocarcinoma and 33.3% PIN.

Bastacky et al. have been show 36% in group B (high-grade PIN, HGPIN/no PC) and 69% in group A (HGPIN/PC) for chromosome 8/c-myc. Using a cutoff of 4, between Group A and Group B were found statistically different (23). The present study found statistically significant difference between PIN and carcinomas for signals obtained from c-myc and chromosome 8 in most cells ($p<0.05$). This finding is important because it helps to differentiate between adenocarcinoma and PIN.

This report suggests that amplification and overexpression of c-myc alone with another gene(s) mapped to 8q, may play a key role in the progression and evaluation of prostatic carcinoma. Overall frequencies of extra c-myc copy anomalies and numeric chromosomal anomalies in PIN and carcinoma were similar, suggesting that they share a similar underlying pathogenesis. Thus, these findings suggest that PIN is a precursor of carcinoma. We conclude that an increase in c-myc oncogene copy number was not a prominent finding in our cohort of prostate cancer patients. We believe that the basic mechanism in overexpression of c-myc gene may not be amplification. Our results indicate that the basic mechanism of c-myc gene overexpression may be gain of simple chromosome 8 or gain of ‘’8q’’. Our results concerning c-myc amplification and gain of chromosome 8 in prostate cancer in Turkish patients are consistent with the results observed in prostate cancer in Western countries and suggest that these genetic and chromosomal changes may be associated with the development and progression of prostate cancers.

Conclusions

Hyperploidy of chromosomes 8 are a common finding in high grade prostate cancer and predictive of follow-up prostate cancer. This gain is associated with distant tissue metastases. FISH is a powerful method to definition in chromosomal gain or loss in cancer tissue. But, cellular proliferation studies are necessary when using interphase cytogenetics to ascertain gene amplification. We advice that FISH studies with probes specific for 8p, 8q, and chromosome 8 centromere are used.

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