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Expression of the c-*myc* Protooncogene in Human Prostatic Carcinoma and Benign Prostatic Hyperplasia[1]

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ABSTRACT

We have examined the level of c-*myc* transcripts in prostate tissue obtained from patients with both benign prostatic hyperplasia and adenocarcinoma of the prostate. A significantly higher level of c-*myc* transcripts is observed in patients with adenocarcinoma ($P < 0.05$). In addition, a subset of patients with adenocarcinoma had levels of c-*myc* transcripts 2-fold higher than the mean level for this group. These preliminary results indicate that the investigation of c-*myc* levels as a prognostic indicator in prostatic carcinoma is warranted.

INTRODUCTION

The expression of the c-*myc* gene has been associated with a variety of human cancers (1). Normal cells induced to undergo proliferation by mitogens also show increased expression of the c-*myc* gene (2). However, the early increase of c-*myc* levels seen in cell culture are not necessarily sufficient to induce proliferation (3). The cellular homologues of several of the transforming retroviral oncogenes show strong phylogenetic conservation from yeast to man (4). This high degree of structural conservation suggests that these genes subserve vital cellular processes and that their association with malignant disease represents an aberration of their normal function. Abnormal c-*onc* gene expression has been attributed to a variety of qualitative changes in the structural gene itself. A point mutation within the coding region of the Ha-*ras* oncogene in the T24 bladder carcinoma results in a single amino acid substitution which confers transforming activity (5). Translocation of the c-*myc* oncogene from chromosome 8 to the immunoglobulin gene heavy chain locus on chromosome 14 in Burkitt's lymphoma (6) and similar translocations in murine plasmocytomas (7) are thought to be of importance in the etiology of these cancers. Quantitative changes in the expression of c-oncogenes have been described in a wide variety of tumors. Amplification of the c-*myc* gene and subsequently elevated levels of c-*myc* expression occurs in myeloid leukemia, Burkitt's lymphoma, carcinoma of the stomach, colon, and breast, and in small cell carcinoma of the lung (8–13). Gene amplification occurs in only a small percentage of these tumors. The more common finding of increased c-*myc* mRNA levels is associated with a normal gene dosage suggesting that defective gene regulation is responsible for the overproduction of these transcripts. The p21[5] protein product of the *ras* oncogene has been quantitated in both malignant and benign colonic disease. Increased levels of the p21 protein are found in carcinoma of the colon and the level of p21 expression correlates with the depth of invasion of these tumors (14). These preliminary studies indicate that the investigation of the level of expression of cellular oncogenes in human cancers may provide both diagnostic tools and prognostic indicators.

Prostatic cancer is the third most common cause of death due to cancer in North American men, with more than 74,000 new cases diagnosed each year (15). The extreme variability of the natural history of this disease coupled with a high frequency of incidental diagnosis of subclinical disease, often following transurethral resection for urinary obstruction, has resulted in considerable controversy in the management of this tumor (16). We have examined the expression of the c-*myc* oncogene in both prostatic carcinoma and benign prostatic hyperplasia in order to determine whether the level of expression of this gene can help in distinguishing between benign and malignant lesions or in predicting the presence of clinically aggresive disease.

MATERIALS AND METHODS

Tissue Samples. Tissue was obtained from patients with biopsy proven adenocarcinoma of the prostate following transurethral prostatectomy. Samples of tissue from patients with BPH were obtained following either suprapubic prostatectomy or transurethral prostatectomy. Two normal prostates were obtained at autopsy. All samples were rapidly frozen and then stored at −70°C. Independent histological evaluation was performed on all samples using the M. D. Anderson (17) and Gleason (18) grading systems.

Northern Blot Analysis. Total RNA was extracted using the guanidinium isothiocyanate/cesium chloride method (19) and enriched for poly(A)$^+$ RNA by oligodeoxythymidylic-cellulose chromatography (20). poly(A)$^+$ RNA was electrophoresed on 1% agarose gels containing 2.2 M formaldehyde (21) and transferred to diazobenzyloxymethyl paper (22). Blots were hybridized at 42°C in 50% formamide with a ^{32}P-labeled human c-*myc* probe (pKW3, a *Pst*I second exon, provided by W. S. Hayward and K. Wiman). Blots were then washed in 0.2 × standard saline citrate (0.15 M sodium chloride:0.015 sodium citrate, pH 7.4) at 65°C and autoradiographs were exposed from 4 to 10 days at −70°C using an enhancing screen (Quanta III).

Quantitation of c-*myc* Levels. Densitometric scanning of Northern gel analysis for c-*myc* specific transcripts was performed as previously described (2). Several RNA samples were run on consecutive gels to serve as internal standards of c-*myc* expression. One sample was

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[5] The abbreviations used are: p21, a protein with a molecular weight of 21,000; BPH, benign prostatic hyperplasia; poly(A)$^+$, polyadenylated.

arbitrarily assigned a value of 100 units and the other samples were expressed relative to this value.

Prostatic Acid Phosphatase Levels. All determinations were made at the time of diagnosis using the enzymatic method described by Roy *et al.* (23). Serum samples were quantitated using a Dupont Automatic Clinical Analyzer.

RESULTS

The presence of the c-*myc* specific transcript in various prostate samples and in the human promyelocytic leukemia cell line HL-60 is shown by Northern blot analysis (Fig. 1). All prostate samples examined demonstrated the characteristic 2.4-kilobase c-*myc* band. The level of c-*myc* expression in poly(A)$^+$ RNA isolated from a total of 19 patients was quantitated by densitometric analysis of the Northern gels. One patient was assigned a level of 100 units and all other samples were expressed relative to this value (Fig. 2). The levels of c-*myc* in 7 patients with adenocarcinoma of the prostate [54 ± 40 (SD)] were significantly higher than that observed in 11 patients with BPH (26 ± 19) ($P < 0.05$). The normal prostates of 2 males aged 22 and 67 yr were obtained at autopsy and demonstrated levels below that observed in patients with either BPH or adenocarcinoma. Subsequent hybridization of these samples with an actin probe indicates that RNA from these samples was not degraded. One patient with adenocarcinoma of the prostate (Fig. 1, *lane 8*) showed a diffuse pattern of hybridization in addition to a faint 2.4-kilobase c-*myc* band. Subsequent hybridization of this sample with the pA1 actin probe (24) indicated considerable degradation of mRNA and this patient was excluded from statistical analysis (data not shown). Probing with actin complementary DNA has been used only to help establish the integrity of the mRNA. The heterogeneous nature of prostate tumors may result in differential expression of actin. Thus, samples were not standardized to actin levels. It was not possible to examine the level of c-*myc* expression in normal aged matched controls due to the high incidence of microscopic evidence of BPH in prostates which are of normal weight (25).

The patients with BPH used in this study, with two exceptions, were treated by suprapubic prostatectomy which involves the removal of all hyperplastic tissue of the lateral and anterior lobes. Levels of c-*myc* expression did not correlate with the mass of prostate tissue removed (range, 60 to 130 g). The levels of c-*myc* in 2 patients over 80 yr of age were less than 35% of that of the mean for the BPH group while the youngest patient in this group (58 yr) had c-*myc* levels more than 3-fold higher than the mean (Table 1).

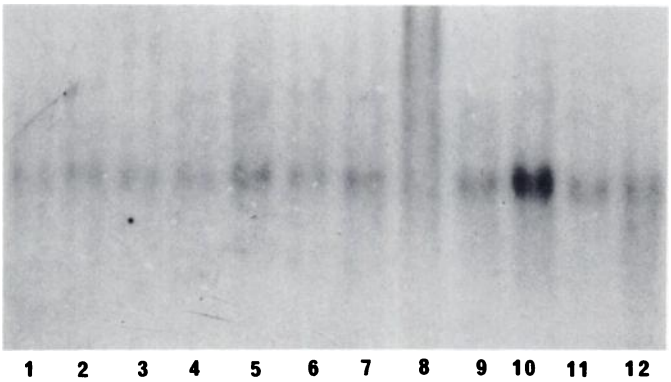

Fig. 1. Expression of the c-*myc* gene in human prostatic tissue. *Lane 1*, normal prostate; *Lanes 2–6*, benign prostatic hyperplasia; *Lanes 7–11*, prostate carcinoma; *Lane 12*, HL-60 leukemia cells. Each lane contains 6 μg of poly(A)$^+$ RNA except *Lane 12* which contains 6 μg of total RNA. Northern blots were probed with the human c-*myc* probe pKW3.

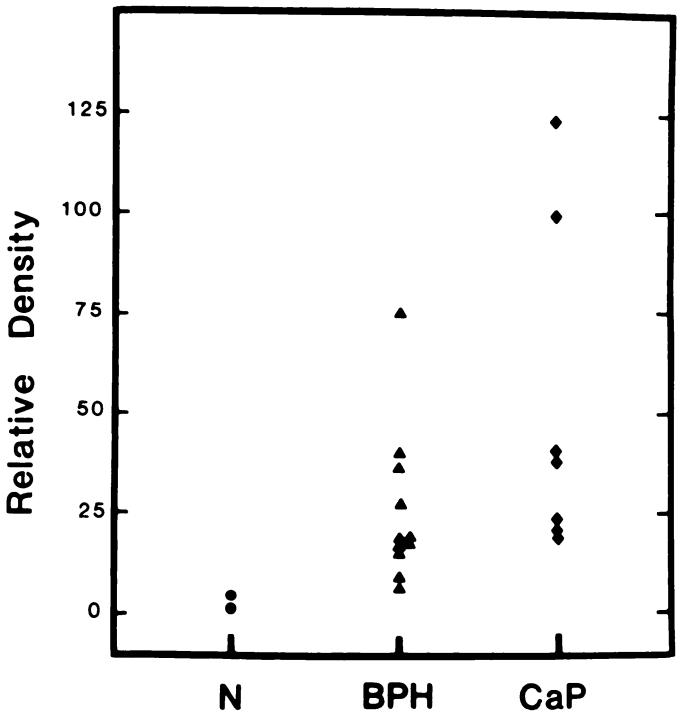

Fig. 2. Levels of c-*myc* mRNA in human prostate tissue. Densitometric scanning of Northern gel analysis of prostate tissue from 20 patients expressed in relative units. The mean levels of c-*myc* in 11 cases of BPH is 26 ± 19 and in 7 cases of carcinoma of the prostate (*CaP*) is 54 ± 40, $P < 0.05$. *N*, normal prostate.

Table 1

c-myc expression in BPH

Three BPH patients were not included because the mass of tissue removed was not available.

Age of patient (yr)	Prostate wt (g)	c-*myc* expression
85	85	9.1
81	90	7.1
79	80	36.2
78	85	18.3
78	105	39.0
74	85	27.0
73	73	16.0
58	60	75.0

The levels of prostatic acid phosphatase at the time of diagnosis of prostate carcinoma did not correlate with c-*myc* levels (Table 2). Two patients (1 and 2) had high c-*myc* levels, normal prostatic acid phosphatase levels at diagnosis, and no evidence of metastatic disease. Six mo following diagnosis patient 2 developed systemic disease with bone metastasis. Two other patients (3 and 5) presented with Stage D clinical disease, the former having a moderately elevated level of c-*myc* whereas the latter showed a level consistent with BPH.

Histological classification of adenocarcinoma of the prostate indicated that the 2 patients with high levels of c-*myc* expression were M. D. Anderson grades 1 and 3 (Table 2). Patients (6 and 7) with grade 2 carcinoma and no evidence of systemic disease

Table 2
c-myc mRNA levels in prostatic carcinoma

Patient	Histological diagnosis: M. D. Anderson	Histological diagnosis: Gleason	Histological diagnosis: % tissue involved	Prostatic acid phosphatase (μ/ml)	c-*myc* expression
1	1	2/2	45	0.36	124.0
2	3	3/4	90	0.40	100.0
3[a]	2	2/3	100	0.60	41.0
4	3	5/3	100	0.60	38.0
5[a]	3	3/4	60	2.80	27.1
6	2	3/2	50	0.52	25.3
7	2	2/3	70	0.26	24.9

[a] Patients who presented with Stage D clinical disease.

had c-*myc* levels comparable to that observed in patients with BPH.

DISCUSSION

Expression of the c-*myc* oncogene in normal cells following mitogen stimulation and in a variety of cancers has given rise to the idea that the c-*myc* gene product is fundamental to cell proliferation. Recent studies indicate that the c-*myc* gene is expressed throughout the cell cycle and that both the mRNA and protein levels are constant (26, 27). This suggests that a minimum threshold level of c-*myc* may be required for cells to pass through the cell cycle. The c-*myc* gene has also been shown to confer an immortalized phenotype on normal fibroblasts in presence of the Ha-*ras* oncogene (28). A large study of fresh hematological cancers indicates that 20% of these tumors have increased levels of c-*myc* gene transcripts (29).

Our data demonstrate the expression of the c-*myc* oncogene in both benign and malignant prostatic tumors. Interpretation of the significance of the level of expression in BPH is complicated by the observation that a large proportion of patients with this tumor have concomitant prostatitis. It has been reported that the level of c-*myc* transcripts in resting lymphocytes is 20-fold higher than that observed in fibroblasts (2). An infiltrate of lymphocytes such as occurs in both acute and chronic prostatitis may significantly increase the c-*myc* level. Histological evaluation of the BPH samples used in this study indicated a only mild diffuse prostatitis in all samples.

A recent review of the development of benign prostatic hyperplasia indicates that the amount of hyperplastic tissue removed at surgery increases with advancing age (25). Calculation of the doubling time of hyperplastic tissue in these patients shows a progressive increase with increasing age, ranging from a doubling time of 4.5 yr in subjects between 31 and 50 yr of age to a doubling time of 100 yr in men over 70 yr of age. In our study both subjects over 80 yr had the lowest levels of c-*myc* expression. The youngest patient in our study (58 yr) had a level of c-*myc* expression of more than 3 times the mean value of the BPH group. These results are consistent with the hypothesis that c-*myc* levels are proportional to the growth fraction of a particular tumor.

Two patients in our study with carcinoma of the prostate had levels of c-*myc* which were 2-fold higher than the mean for the carcinoma group and 5-fold higher than the average subject with BPH. While neither of these patients demonstrated systemic disease at diagnosis, one patient (patient 2) has since developed bone metastasis following a 6-mo interval. This is consistent with the outcome predicted by histological evaluation indicating a high Gleason score (Table 2).

Our data demonstrate that levels of expression of the c-*myc* oncogene is significantly higher in adenocarcinoma of the prostate than in BPH. In addition a subset of patients with adenocarcinoma of the prostate and no evidence of systemic disease have high levels of c-*myc* expression. One major difficulty in evaluating the significance of elevated levels of c-*myc* transcripts is the variable infiltration of the prostate with tumor cells (Table 2). Further studies using DNA probes for *in situ* hybridization and immunocytochemical methods are being conducted to directly evaluate the level of c-*myc* expression. These techniques will eliminate the effect of variable involvement of the gland by carcinoma and permit evaluation of the c-*myc* levels in individual tumor cells. The prognostic significance of elevated levels of c-*myc* expression in prostatic carcinoma expression warrants further investigation.

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