

Relationship between DNA Ploidy, Glandular Differentiation, and Tumor Spread in Human Prostate Cancer¹

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ABSTRACT

DNA ploidy was evaluated by flow cytometry for 45 human prostate carcinomas (34 prostatectomy specimens and 11 biopsies). Twenty tumors (44.4%) contained a distinct aneuploid stem line. All 11 tumors confined to the prostate gland (pathological Stage B) were diploid. The frequency of aneuploidy increased with advancing stage, and most tumors with distant metastases were aneuploid. The degree of glandular differentiation was characterized by the Gleason score. One-third of tumors with a Gleason score of 5 to 6 were aneuploid, whereas over 70% of poorly differentiated tumors with a Gleason score of 9 to 10 were aneuploid. Among diploid tumors, 45.5% were localized carcinomas (Stage B), 36.4% were characterized by invasion outside the prostate (Stage C), and 18.2% formed pelvic nodal or distant metastases (Stages D₁ and D₂). In nearly two-thirds of patients with aneuploid tumors, pelvic nodal or distant metastases were found. When tumors were classified according to both DNA ploidy and degree of glandular differentiation, then subgroups of tumors with the highest and lowest degree of malignant potential became apparent. Only 7.1% of diploid tumors with a Gleason score of 5 to 6 formed metastases, but 80% of aneuploid tumors with a higher Gleason score (7 to 10) formed metastases. Diploid tumors with higher Gleason scores and aneuploid tumors with lower Gleason scores had intermediate frequencies of metastases. The presence of an aneuploid stem line in prostate carcinomas indicated that the tumor had spread outside the prostate gland or had metastasized. DNA ploidy may be an important prognostic factor for human prostate cancer. DNA ploidy and the degree of glandular differentiation considered together may improve prognostic evaluation of prostate carcinomas.

INTRODUCTION

An important problem in the management of prostatic cancer is that of predicting the malignant potential of this tumor, especially its ability to spread outside the prostate gland and to metastasize. Histological evaluation of the degree of glandular differentiation using the Gleason or other grading systems is widely used for this purpose (14-16, 21, 24, 28). A grading system which evaluates both the nuclear anaplasia, as well as the mode of glandular growth, has been suggested (13). There is a general consensus that the incorporation of the nuclear or

cytoplasmic characteristics of tumor cells into the Gleason system may improve the prognostic evaluation of prostate tumors (24).

DNA ploidy estimated by FCM⁴ may be used as a cellular characteristic for the evaluation of prostate carcinomas. Tribukait *et al.* (30) have shown that, for prostate tumors, the degree of DNA ploidy correlates with the degree of cellular anaplasia. DNA ploidy is estimated objectively from the measurements of a large number of cells and is readily reproducible. There are numerous examples of a correlation between the degree of malignancy and the degree of aneuploidy for different human solid tumors (4). Most aneuploid bladder tumors are of high grade and display invasive growth (11, 18, 29). There is, however, no detailed investigation on the relationship between DNA ploidy, pathological tumor stage, and degree of differentiation for human prostate cancer. Such investigations may provide useful information for the understanding of the biology of prostate cancer and may have important clinical applications.

In the present investigation, DNA ploidy was determined by FCM in 45 human prostate carcinomas, most of which were prostatectomy specimens with pathological stage determined by histological analysis of the prostate gland and lymph nodes. The relationship between the presence of aneuploid cells and tumor spread, as indicated by tumor penetration through the prostate capsule and metastasis formation, was examined. We also examined the relationship between DNA ploidy and the degree of glandular differentiation (Gleason score) for the evaluation of human prostate cancer. In general, we concluded that DNA ploidy is a useful prognostic factor for prostate carcinoma, especially if it is used in combination with the degree of glandular differentiation.

MATERIALS AND METHODS

Tumors. Tumor tissue was obtained by radical retropubic prostatectomy from 34 patients and by biopsy from 11 patients. A piece of the tumor for FCM analysis from the prostatectomy specimens was selected by the surgeon. Presence of tumor cells in material used for DNA ploidy determination was confirmed by histological analysis of frozen sections. Staging of patients with prostatectomies was done by detailed histopathological analysis of the prostate gland and lymph nodes in addition to preoperative metastatic screening by bone scans and serum acid phosphatase measurements. Eleven patients with tumors confined to the prostate without evidence of capsular invasion or lymph node involvement were classified into Stage B. Tumor penetrating the prostatic capsule was observed in 12 patients, and invasion of the capsule without its penetration, in one patient. These 13 patients were classified into Stage C₁. In 2 patients, invasion of seminal vesicles was detected (Stage C₂) with no lymph node involvement. Pelvic lymph node metastases were

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⁴ The abbreviation used is FCM, flow cytometry.

found in 8 patients (Stage D₁). In addition, needle biopsies were obtained from prostate tumors of 8 patients with distant metastases and from metastatic tumors of 3 patients (Stage D₂). Glandular differentiation in the tumor specimens from 34 prostatectomies and in 8 biopsies of prostate tumors was evaluated according to the Gleason system (14, 15) during routine histopathological examination.

FCM. Methods for FCM analyses of human solid tumors were described in detail previously (10, 12). Briefly, cell suspensions from larger tumors were obtained by mechanical disaggregation. For small tumors and biopsies, tissue was cut into small pieces and incubated in 0.2% EDTA in 137 mM NaCl-3 mM KCl-6.5 mM Na₂HPO₄-1.5 mM KH₂PO₄ for 2 to 16 hr at 4° before disaggregation. Cell suspensions were stained with a DNA fluorochrome, 4',6-diamidino-2-phenylidole, in the presence of 0.2% Triton X-100. DNA distributions were obtained on an ICP-22 flow cytometer (Ortho Instruments, Westwood, MA). Human blood leukocytes were used as a diploid standard. The DNA index was calculated as the ratio of the peak channel of aneuploid cells to that of diploid cells. The DNA index of diploid cells was equal to 1.0 (1).

Representative DNA histograms of human prostate tumors from this study are shown in Chart 1. Diploid tumors are characterized by the presence of one G₀-G₁ peak. Tumors were considered to be aneuploid if a separate G₀-G₁ peak of aneuploid cells was present. Tumors were classified as tetraploid if an apparent subpopulation of G₀-G₁ cells with a DNA index of 2.0 was present, and if S and G₂-M cells of this population were visible in the DNA histograms. This is in contrast to the criteria applied by Tribukait *et al.* (30), who considered a tumor as tetraploid if the percentage of G₂-M cells in an apparently diploid population was above 7.0%. This difference in the definition of aneuploidy may explain the higher proportion of tetraploid stem lines among aneuploid tumors (64%), as reported by Tribukait *et al.* (30), in comparison with the present study (24%).

Data Analysis. Linear logistic regression (6), Kaplan-Meier life table analysis (17), and the log rank test (25) were conducted with the software package, FRENDS (8), an adjunct to the Statistical Package for Social Sciences (23), on a Univac 90/80 mainframe computer.

RESULTS

DNA ploidy, degree of glandular differentiation (Gleason score), and pathological stage for 45 patients with prostate carcinoma are shown in Table 1. The frequency of aneuploidy for all specimens was 44.4% (25 diploid and 20 aneuploid tumors). DNA indices for aneuploid tumors varied from 0.91 to 3.42. Only one tumor was hypodiploid, and 2 tumors had 2 aneuploid stem lines. The median DNA index for all aneuploid stem lines was 1.87.

The frequency of aneuploidy increased with advancing stage

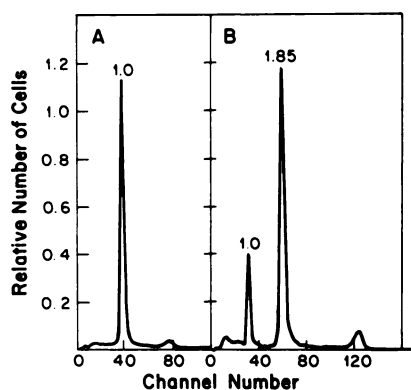


Chart 1. DNA distribution of the human prostate carcinomas. A, diploid tumor; B, aneuploid tumor. DNA index is indicated above G₀-G₁ peaks. Diploid cells have a DNA index of 1.0.

Table 1
Stage of disease, DNA ploidy, and Gleason score in human prostate carcinomas

Patient	Pathological stage	Type of specimen	DNA index ^a	Gleason score
1	B	PX ^b	1.00	4
2	B	PX	1.00	5
3	B	PX	1.00	5
4	B	PX	1.00	5
5	B	PX	1.00	5
6	B	PX	1.00	5
7	B	PX	1.00	6
8	B	PX	1.00	6
9	B	PX	1.00	6
10	B	PX	1.00	6
11	B	PX	1.00	6
12	C ₁	PX	1.00	6
13	C ₁	PX	1.00	6
14	C ₁	PX	1.00	6
15	C ₁	PX	1.00	7
16	C ₁	PX	1.00	7
17	C ₁	PX	1.00	7
18	C ₁	PX	1.00	7
19	C ₁	PX	1.00	9
20	C ₁	PX	1.93	6
21	C ₁	PX	2.04	7
22	C ₁	PX	2.26	6
23	C ₁	PX	1.81 + 3.42 ^c	6
24	C ₁	PX	2.00	6
25	C ₂	PX	2.00	6
26	C ₂	PX	1.87	7
27	D ₁	PX	1.00	6
28	D ₁	PX	1.00	7
29	D ₁	PX	1.00	7
30	D ₁	PX	1.42	6
31	D ₁	PX	1.80	7
32	D ₁	PX	1.93	7
33	D ₁	PX	2.03	6
34	D ₁	PX	2.00	6
35	D ₂	BX	1.00	3 ^d
36	D ₂	BX	1.00	—
37	D ₂	BX	1.00	10
38	D ₂	BX	0.91	10
39	D ₂	BX	1.12	—
40	D ₂	BX	1.51	8
41	D ₂	BX	1.68	—
42	D ₂	BX	1.75	10
43	D ₂	BX	1.85	10
44	D ₂	BX	1.94	10
45	D ₂	BX	1.76 + 2.00 ^c	10

^a DNA index of diploid tumors is 1.00.

^b PX, radical prostatectomy; BX, biopsy.

^c Two aneuploid stem lines.

^d —, Gleason score not determined from biopsies of metastatic tumors.

Table 2
DNA ploidy in 45 prostate carcinomas related to tumor stage

Pathological stage	No. of tumors		Frequency of aneuploidy (%)
	Diploid	Aneuploid	
B	11	0	0
C	8	7	46.7
D ₁	3	5	62.5
D ₂	3	8	72.7

(Table 2). All tumors confined to the prostate gland (Stage B) were diploid. Among tumors which spread outside the prostate (Stage C) and among tumors with pelvic lymph node metastases (Stage D₁), both diploid and aneuploid tumors were encountered. Aneuploid tumors predominated in patients with distant metastases (Stage D₂).

Tumors were arbitrarily classified into 3 groups according to glandular differentiation, with Gleason scores of 5 to 6, 7 to 8, and 9 to 10, respectively (Table 3). All tumors confined to the

DNA PLOIDY AND TUMOR SPREAD IN HUMAN PROSTATE CANCER

Table 3

Glandular differentiation (Gleason score) in 40 prostate carcinomas related to tumor stage

Pathological stage	No. of tumors with Gleason score of			Frequency of tumors with Gleason score >6 ^a (%)
	5-6	7-8	9-10	
B	10	0	0	0
C	8	6	1	46.7
D ₁	4	4	0	50.0
D ₂	0	1	6	100

^a Gleason score was determined during routine histopathological examination from prostatectomy specimens for tumors at Stages B, C, and D₁ and from biopsy specimens of prostate tumors at Stage D₂.

Table 4

Frequency of aneuploidy among prostate carcinomas with different degrees of glandular differentiation

Gleason score	No. of tumors		Frequency of aneuploidy (%)
	Diploid	Aneuploid	
5-6	14	8	36.4
7-8	6	5	45.5
9-10	2	5	71.4

prostate gland were moderately differentiated (Gleason score of 5 to 6), whereas almost all tumors from patients with distant metastases were poorly differentiated and had a Gleason score of 7 or more. Among patients at Stages C and D₁, a significant proportion of tumors had a Gleason score of 5 to 6. Tumors with the lowest Gleason score (3 to 4) were seen in only 2 cases. Groups of moderately and poorly differentiated tumors were selected for the analysis of the relationship between glandular differentiation, DNA ploidy, and tumor spread.

The relationship between degree of glandular differentiation and frequency of aneuploidy is presented in Table 4. Diploid and aneuploid tumors were encountered in all 3 tumor groups, characterized by different values of Gleason score. Among the least differentiated tumors (Gleason score of 9 to 10), most of which were from patients with distant metastases, aneuploid tumors were predominant. Approximately one-third of moderately differentiated tumors (Gleason score of 5 to 6) were aneuploid.

The frequency of aneuploidy and degree of glandular differentiation had a similar relationship with pathological stage (Tables 2 and 3). All localized tumors without invasion of the prostate capsule (Stage B) were diploid and belong to the group of moderately differentiated tumors. Among tumors at Stage C or Stage D₁, approximately half were aneuploid and had a Gleason score of 7 or above. Aneuploid tumors and poorly differentiated tumors predominated among tumors with distant metastases.

The relationship among DNA ploidy, degree of glandular differentiation, and tumor spread is summarized in Table 5. To analyze this relationship, tumors were divided into groups according to DNA ploidy and Gleason score. Tumors were separated into 2 groups according to the degree of glandular differentiation, those with a Gleason score of 5 to 6 and those with a score of 7 to 10. Selection of these values for separation of tumors into 2 groups is justified, since localized tumors were encountered only among tumors with a Gleason score of 6 or below (Table 3). For characterization of tumor spread, prostate carcinomas were divided into 3 groups: localized tumors (Stage B); tumors that had spread outside the prostate gland (Stage C); and tumors that had metastasized (Stages D₁ and D₂). Tumors

Table 5

Relationship between DNA ploidy, glandular differentiation, and tumor spread in human prostate cancer

Tumor characteristics			No. of tumors		
DNA ploidy	Gleason score	Total	Localized to the prostate (Stage B)	Invasion outside the prostate (Stage C)	Metastasis (Stages D ₁ and D ₂)
Diploid	5-10	22	10 (45.5) ^a	8 (36.4)	4 (18.2)
Aneuploid	5-10	18	0	7 (38.9)	11 (61.1)
Diploid + aneuploid	5-6	22	10 (45.5)	8 (36.4)	4 (18.2)
Diploid + aneuploid	7-10	18	0	7 (38.9)	11 (61.1)
Diploid	5-6	14	10 (71.4)	3 (21.4)	1 (7.1)
Diploid	7-10	8	0	5 (62.5)	3 (37.5)
Aneuploid	5-6	8	0	4 (50.0)	4 (50.0)
Aneuploid	7-10	10	0	2 (20.0)	8 (80.0)

^a Numbers in parentheses, percentage of total.

with pelvic nodal and distant metastases were combined for the analysis due to the relatively small number of cases in each group.

Among diploid tumors, localized tumors were most frequent, and only 18.2% of the tumors had metastasized. Two-thirds of aneuploid tumors had formed metastases, and none was localized. Thus, the presence of aneuploid cells indicated that tumor cells had spread outside the prostate. Metastatic tumors were less frequent among tumors with a Gleason score of 5 to 6 than among those with a score of 7 and above when diploid and aneuploid tumors were considered together (Table 5).

When tumors were divided into 4 groups according to DNA ploidy and glandular differentiation, then subgroups of tumors with the lowest and highest degree of cancer became apparent (Table 5). Only 7.1% of diploid tumors with lower Gleason scores, but 80% of aneuploid tumors with higher Gleason scores formed metastases. Diploid tumors with high Gleason scores and aneuploid tumors with lower Gleason scores had intermediate percentages of metastases.

Degrees of glandular differentiation for tumors at Stages B, C, and D₁ as shown in Tables 1, 3, 4, and 5 were determined from prostatectomy specimens with evaluation based on relatively large amounts of tissue. In 29 of the prostatectomy patients, Gleason score determination was possible in both the needle biopsy and radical prostatectomy specimens. The Gleason score from the biopsy was lower than that from the corresponding prostatectomy specimens in 18 cases and was similar in 11 cases. For 8 tumors which had a Gleason score of 7 or above from prostatectomy specimens, the score determined from the biopsy was 5 to 6. All 8 tumors had invaded tissues outside the prostate or had metastasized. As a result, the Gleason score estimated from biopsies was less informative for the biological characterization of tumors than was Gleason score estimated from prostatectomy specimens.

The relationship between Gleason score, DNA ploidy, and pathological stage was examined more formally with linear logistic regression. Gleason score was treated as a continuous predictive variable ranging from 5 to 10, ploidy was treated as a binary predictive variable with values of diploid or aneuploid, and pathological stage was treated as a binary outcome variable with values of no metastases (Stages B and C) or metastases (Stages

D₁ and D₂). There were significant relationships between Gleason score and stage, and between ploidy and stage ($\alpha = 0.05$). Moreover, using the 2 predictive variables simultaneously, in the predictive equation was significantly better than using either Gleason score or ploidy alone ($\alpha = 0.05$).

The effect of DNA ploidy on the survival time of patients was examined with a Kaplan-Meier life table analysis and log rank test. Although it was clear that patients with diploid tumors survived significantly longer than did patients with aneuploid tumors ($p = 0.02$), the explanation for this finding was trivial: only patients at pathological Stage D₂ had died; and there was a higher proportion of patients with aneuploid tumors in Stage D₂ than in earlier stages. Since our median follow-up time for survivors was only 5 months, and our sample size was too small to allow stratification by stage, we decided to defer extensive analyses of survival to a later time.

DISCUSSION

DNA Ploidy as a Prognostic Factor. DNA ploidy as measured by FCM is emerging as an important prognostic factor for malignant human tumors (3, 4, 11, 12, 18, 29). To develop and justify the application of a new prognostic tool, it is necessary to establish its relationship with accepted prognostic indicators, such as tumor grade and stage of disease.

For human prostate cancer, the degree of glandular differentiation, which often is measured by the Gleason score, and pathological stage, which characterizes tumor spread, are useful predictors of disease course (13–15, 21–24). The prediction of prognosis is improved with 2 parameters are used in combination (15, 16). It has been reported that, in human prostate cancer, DNA ploidy correlates with the degree of differentiation (5) and with cytological grade (30) and has predictive value for response to hormonal therapy (34).

In this study, the frequency of aneuploidy was compared among tumor groups characterized by pathological stage and degree of glandular differentiation. All tumors confined to the prostate gland were diploid. The frequency of aneuploidy increased with the advancement of stage, and most tumors with distant metastases were aneuploid. Among tumors at pathological Stage C, which are characterized by heterogeneity in terms of prognosis (16), one-half were aneuploid. It will be of interest to compare the long-term survival of patients with diploid Stage C tumors with those with aneuploid Stage C tumors. One might expect a worse prognosis for aneuploid Stage C tumors since, in general, aneuploidy is associated with dissemination of prostate tumors.

Our patient follow-up was too short to allow firm conclusions about the effect of ploidy on patient survival after prostatectomy. Therefore, we attempted using the present material to answer the question of whether DNA ploidy, Gleason score, or a combination of both may be used to predict tumor spread, as characterized by pathological stage; the pathological stage in turn affects patient survival.

The negative prognostic characteristics, *i.e.*, the presence of aneuploid cells or high Gleason score (≥ 7), were associated with tumors which had spread beyond the prostate gland locally in 100% of cases and with tumors which had metastasized in approximately two-thirds of cases. Thus, for identifying tumors with poor prognosis, both DNA ploidy and Gleason score had similar predictive value. However, it should be noted that DNA

ploidy is an objective parameter derived from automated measurements, whereas tumor grading is a subjective process with variable results among investigators.

The prediction of localized disease is more challenging than the prediction of advanced tumor stage. The prognostic characteristics which should indicate a favorable prognosis, *i.e.*, a relatively low Gleason score (5 to 6) and the absence of detectable aneuploid cells, were not necessarily associated with localized tumors, as 54.5% of relatively well-differentiated tumors and 54.5% of diploid tumors proved to have penetrated the prostatic capsule or metastasized. This is in accordance with data of Wilson *et al.* (32), showing retention of metastatic potential in some well-differentiated prostate tumors. In contrast, among tumors which had a Gleason score of 5 to 6 and in which also no aneuploid cells were detected, only 21.4% had microscopic capsular invasion, and only 7.1% had metastasized.

This indicates that, if a tumor is both moderately differentiated (Gleason score of 5 to 6) and diploid, it has a lower chance of having metastasized at the time of surgery, than a tumor which is only either diploid or well differentiated. A tumor which is both poorly differentiated and aneuploid almost certainly has extraprostatic involvement at the time of evaluation. The majority of these tumors have already metastasized to pelvic lymph nodes or distant sites.

Thus, DNA ploidy as measured by FCM and characterized by the absence or presence of distinct aneuploid stem lines appears to be an important prognostic factor for human prostatic cancer. DNA ploidy and the degree of glandular differentiation considered together may improve prognostic evaluation of prostate tumors. This information may be used to modify patient treatment according to the predicted risks.

Importance of nuclear characteristics, other than DNA ploidy, for the prognostic evaluation of human prostate tumors was described in 2 studies. The nuclear roundness, which reflects the degree by which the nucleus in cross-section deviates from a perfect circle, was significantly higher for patients with Stage B₁ disease who had metastases, than in patients with Stage B₁ disease who had no evidence of metastases 14 to 15 years after their prostates had been surgically removed (7). The addition of nuclear morphology to glandular differentiation for the assessment of tumor grade improved the prognostic evaluation of prostate carcinomas as characterized by the mortality rate (13). Probably, the deviation of the nucleus from a round shape and the presence of nuclear anaplasia in prostate tumors reflect nuclear characteristics determined by the abnormalities in DNA content.

DNA Ploidy and Tumor Progression. Progression of tumors, as defined by Foulds (9), is a neoplastic development which occurs by permanent, irreversible, qualitative change in one or more tumor characteristics. Progression usually results in the display of a more malignant (metastatic) behavior of tumors. Tumor stage, which reflects the advancement of the disease toward the involvement of surrounding tissues and the development of distant metastases, may be considered an important indicator of tumor progression. The relationship between karyotypic changes and progression of disease to a more malignant state was demonstrated in human leukemia (26).

It may be assumed that, in prostate tumors detected at advanced stage, progression is characterized by rapid and increasing development of new characteristics associated with

malignant behavior, whereas in prostate tumors detected at earlier stages, these characteristic develop at much slower rates. In this study, the relationship between pathological stage of prostate carcinomas and DNA ploidy was described. Based on these data, one of 2 possible roles for DNA aneuploidy in the progression of prostate tumors is likely: (a) DNA aneuploidy (numerical chromosomal abnormalities) appears during initial tumor formation and remains stable during the natural history of the disease; or (b) DNA aneuploidy develops during tumor progression as one of the tumor's characteristics.

The hypothesis that assumes the appearance of DNA aneuploidy at the time of tumor origin implies that DNA aneuploidy is one of the factors determining the rate and direction of progression. The fact that all aneuploid prostate carcinomas in our study were at the most advanced stage of disease would be explained by a very fast rate of progression towards metastatic spread. A role for DNA aneuploidy as a factor influencing tumor progression may be explained by gene dosage effect, proposed by Klein (19). Possibly, the increased expression of oncogenes in aneuploid cells facilitates progression to a more malignant stage. The role of gene dosage effect in tumor development is supported by data showing that, at least in some types of carcinogenesis, tumor formation results from numerical chromosomal abnormalities without apparent chromosomal aberrations (31).

Stability of ploidy characteristics during growth, progression, and development of metastases in solid tumors was shown in a number of cytogenetic and FCM studies (2, 3, 12, 33). The stability of DNA ploidy during tumor progression is also supported by the limited number of DNA stem lines in human solid tumors. In the present data set, only 2 of 45 patients had a tumor with more than one aneuploid stem line. We reported previously that the proportion of tumors with 2 aneuploid stem lines in other solid tumors did not exceed 10 to 20% (12). Three DNA stem lines were observed in less than 1% of tumors. Also, the presence of more than one stem line may reflect multifocal carcinogenesis, rather than evolution of new stem lines during progression. Such stability is a surprising finding, since the number of chromosomes in tumor metaphases varies significantly (20), and this variability may provide material for the selection of new stem lines. Evidently, most karyotypic variants are eliminated during mitosis, as indicated by a much narrower distribution of DNA content in anaphases than in metaphases (1) and by DNA constancy in the face of chromosome variability (20). Possibly, compatibility of numerical chromosome changes with cell survival is a very rare event which may occur only at early stages of carcinogenesis.

The second model describing the relationship between DNA ploidy and the progression of prostate carcinomas suggests that all tumors are diploid at the origin and that the development of aneuploidy during progression is linked to the appearance of a more malignant phenotype and the development of metastases. In this context, aneuploidy may be considered as a secondary karyotypic change (27). The secondary appearance of aneuploidy during tumor progression is, however, difficult to reconcile with the observed stability of DNA ploidy in solid tumors. Evidently, detailed serial analysis of DNA content and modal chromosome number during the early stages of tumor development is necessary to resolve the relationship between ploidy and tumor progression. Whichever the sequence of events, it is obvious that

DNA aneuploidy is linked to the progression of prostate carcinomas to a more malignant state.

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DNA PLOIDY AND TUMOR SPREAD IN HUMAN PROSTATE CANCER

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