

ANALYSIS OF DNA PLOIDY HETEROGENEITY IN PROSTATE CANCER

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ABSTRACT

The objectives of this study were to assess DNA ploidy heterogeneity and thus the specificity of digital cytometry for prostate adenocarcinoma, as intratumoral variation of DNA content is believed to exist among these cells, as occurs with histological differentiation.

Fifteen prostate adenocarcinoma patients were selected. They underwent radical prostatectomy and the tissue obtained had 3 distinct areas analyzed to determine cellular ploidy and tumor grade (Gleason score). Statistical analysis has described the values in the Gleason score in the different areas, both in the diploid and aneuploid groups, in relation to average, standard deviation, median, and maximal and minimum values.

The prostatic tissue studied showed only diploid tumors in some patients and only aneuploid tumors or an association between diploid and aneuploid tumors in others. There were 11 tumors where the areas were diploid (73.3%), 3 aneuploid tumors (20%) and a tumor with 2 diploid areas and an aneuploid one (6.7%). There was thus homogeneity among the areas studied in 93.3% of the prostatic tumors. The values in the Gleason score were compared with the ploidy of each area in the several tumors. The average of values in the Gleason score in the tumor areas of the diploid group was significantly smaller ($p < 0.05$) than the observed average in the areas of aneuploid group of tumors. This relationship occurred globally in the areas of tumors.

In conclusion, ploidy analysis of the cellular DNA in prostate adenocarcinoma through digital cytometry shows high specificity (93,3%) and cellular differentiation rate was significantly lower in diploid tumors.

Key words: prostate, prostatic neoplasms, DNA ploidy, heterogeneity

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INTRODUCTION

Neoplasias are widely recognized by inter and intratumoral heterogeneity characterized by several aspects: morphological, phenotypic and genetic. This diversity does not, however, imply necessarily in chaotic behavior. If there is any structure in a system, and if anybody understands the rules and interactions of this system, they may understand the ways of its evolution and its potential behavior (1). The natural history of the prostatic adenocarcinoma is still poor known. The factors that trigger prostate cancer, that make it develop, penetrate the prostatic capsule, send metastases or become refractory to hormone therapy are still hardly known. Some of these tumors evolve rapidly, while others

stay quiescent (2). It is early to assert that all prostate cancers begin with the same rate of cellular multiplication and become more aggressive with the passage of time or whether they begin already at different growth rates (3). The forecast of the potential malignity of the tumors is thus one of the greatest problems in handling prostate cancer. The clinical and pathological stage, the tumor grade, volume and PSA levels are the principal methods used. However, they cannot be considered to be consistently objective and trustworthy. This is the context in which DNA ploidy analysis has emerged as a promising technique for the classification of the malignity potential of the tumor. Tumor cells with a nucleic acid content similar to normal cells are believed to have a less aggressive behavior.

The objectives of this study were to assess DNA ploidy heterogeneity and thus, the specificity of digital cytometry for prostate adenocarcinoma, besides verifying the existence of a corresponding of this with the variation of cellular differentiation.

MATERIAL AND METHODS

The group studied consisted of 15 patients with prostate adenocarcinoma, verified by transrectal biopsies, who underwent radical prostatectomy. The tissue obtained in these surgeries was submitted to histopathological routine preparation and kept in paraffin.

In order to evaluate the heterogeneity of prostate tumors, the cellular ploidy and the tumor grade (Gleason score) were determined in 3 distinct areas of each tumor, area A being the most expressive and area C the least frequent.

The quantitative DNA analysis was made in 5- μ m sections of prostatic material by Feulgen stoichiometric method. The new slides were observed in an optic videomicroscopy system joined to an IBM-PC compatible microcomputer. The tumor images generated by the camera were frozen and fed into software specifically designed for nucleic tracing, named W.ING.

The total optical density of the nucleus represents its DNA content. In this manner, when measuring the amount of DNA of a certain cellular population, a chart is obtained, called a DNA histogram, representing the ploidy pattern of this cellular population. The determining of the control histograms was made by the analysis of DNA content of the cells of either normal prostatic tissue or tissue with benign hiperplasia in the sample of each patient. Comparing the histograms of the DNA of neoplastic cells to those of normal cells we obtained its DNA index, meaning the proportion between the DNA of cells at rest (G0/G1) in neoplasia and in the normal population. So, a DNA index equal to one marks a diploid pattern, while a DNA index markedly different from one determines an aneuploid pattern.

Statistical analysis has described the values in the Gleason score in the different areas, both in the diploid and aneuploid groups, in relation to average,

standard deviation, median, and maximal and minimum values. The non-parametric test of Mann-Whitney was used for determining the significance, with the adoption of "p" values smaller than 0.05 as statistically significant.

RESULTS

The prostatic tissue studied showed only diploid tumors in some patients and only aneuploid tumors or an association between diploid and aneuploid tumors in others. There were 11 tumors where the areas were diploid (73.3%), 3 aneuploid tumors (20%) and a tumor with 2 diploid areas and an aneuploid one (6.7%). There was thus homogeneity among the areas studied in 93.3% of the prostatic tumors.

The values in the Gleason score were compared with the ploidy of each area in the several tumors. The average of values in the Gleason score in the tumor areas of the diploid group was significantly smaller ($p < 0.05$) than the observed average in the areas of aneuploid group of tumors. This relationship occurred globally in the areas of tumors (Table-1).

Table 1 – Average of values in the Gleason score in each area of the groups of diploid and aneuploid prostate tumors

	Aneuploid Average	Diploid Average	(Significance)
Area A	7.67	6.08	p = 0.0176
Area B	8.33	6.00	p = 0.0176
Area C	8.00	6.36	p = 0.0059

DISCUSSION

The real value of ploidy in estimating the potential malignity of prostate tumors and the survival of patients remains controversial. Some studies show a strong forecast value while others failed in attempt of proving the significance of this relationship.

As a group, diploid tumors present a disease-free period after treatment greater than shown by the

group of patients with aneuploid tumors. Determination of the ploidy of a given tumor cannot, however, be useful to determine the survival chance of a particular patient (4).

Some 30% of the tumors restricted to the gland are "pure" non-diploid (4). In this study, there were 73.3%, which were homogeneously diploid tumors. This predominance is probably due to our having studied the tumors localized in the prostate, for which surgery was indicated.

In a recent consensus meeting on ploidy of prostate tumors, the conclusion was reached that there is a significant relationship between DNA content and the evolution, survival and response to hormone therapy. It was also established at the meeting that there were tumors heterogeneous as to ploidy, with diploid and aneuploid areas, and that this variation could compromise the results of ploidy determination in prostatic tumors (5). Studies of ploidy heterogeneity of prostate tumors through digital cytometry have shown that patients with a localized disease, restricted to the prostate, aneuploid tumors are rare (13%) and that ploidy heterogeneity occurs in less than 10% of these tumors (2,6).

We demonstrated that, as far as the correspondence of ploidy with the Gleason score is concerned, the values of Gleason score of diploid tumors are in average significantly smaller than the values for aneuploid tumors. As the degree of cellular differentiation is a well-established parameter in determining the aggressiveness of tumor cells, despite its subjective component, the association of ploidy gives probably greater objectivity to the forecast of the potential malignity of the tumor.

CONCLUSIONS

We conclude that ploidy analysis of cellular DNA in prostate adenocarcinoma through digital cytometry shows high specificity (93.3%). This permits cellular ploidy to be determined by simple transrectal biopsy, while yet in the pre-operative phase. Biopsy cannot determine the degree of histological differentiation as reliably (6). We have also concluded that the cellular differentiation rate

was significantly higher in diploid tumors, which probably represent diseases that are really less aggressive than aneuploid tumors.

REFERENCES

1. Shackney SE, Shankey TV: Genetic and phenotypic heterogeneity of human malignancies: finding order in chaos. *Cytometry*, 21: 2-5, 1995.
2. Shankey TV, Jia-Kuan J, Dougherty S, Flanigan RC, Graham S, Pyle JM: DNA ploidy and proliferation heterogeneity in human prostate cancers. *Cytometry*, 21: 30-39, 1995.
3. Alers JC, Krijtenburg PJ, Vissers CJ, Bosman FT, Van der Kwast TH, Van Dekken H: Cytometry heterogeneity and histological tumor growth patterns in prostatic cancer. *Cytometry*, 21: 84-94, 1995.
4. Shankey TV, Kallioniemi OP, Koslowski JM: Consensus review of the clinical utility of DNA content cytometry in prostate cancer. *Cytometry*, 14: 497-500, 1993.
5. Epstein JI, Pizov G, Steinberg GD: Correlation of prostate cancer nuclear deoxyribonucleic acid, size, shape and Gleason grade with pathological stage at radical prostatectomy. *J Urol*, 148: 87-91, 1992.
6. Ross JS, Figge H, Bui HX: Prediction of pathological stage and post-prostatectomy disease recurrence by DNA ploidy analysis of initial needle biopsy specimens of prostate cancer. *Cancer*, 74: 2811-2818, 1994.

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