CONF-8911174--1

UCRL- 102862
PREPRINT

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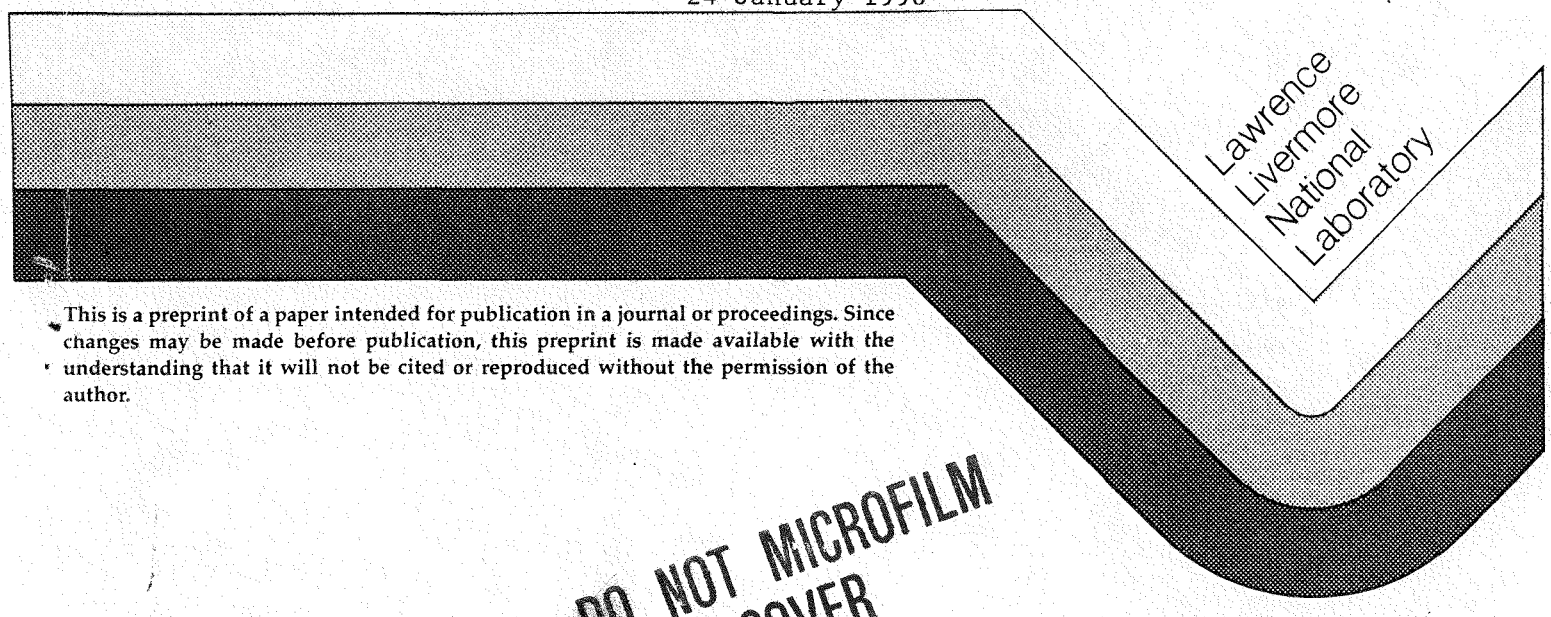
Advances in Analytical Cellular Pathology
Excerpta Medical International Congress Series
Proceedings of European Society for Analytical
Cellular Pathology
Schloss Elmau, West Germany
12-17 November 1989

24 January 1990



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This work was performed under the auspices of the University of California Program for Analytical Cytology, the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract Number W-7405-ENG-48, the Laboratory for Cell Analysis, University of California San Francisco, and The Geraldine Brush Cancer Research Institute, 3330 Clay St., San Francisco, 94115.

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INTRODUCTION

We are investigating the potential utility of cellular markers, including cellular proliferation and DNA cytometry, as independent diagnostic and prognostic markers in human breast cancer. However, as the clinical laboratory is responsible for providing physicians with data relevant to the patient, it is essential first to establish the validity of such markers before their use is recommended. Prospective validation is time-consuming and costly for tests of human malignancies, such as breast cancer, which may follow a lengthy and indolent course requiring patients to be followed for a decade or more before their clinical outcome is known. Therefore, retrospective studies on archival material are used whenever possible.

Cell proliferation is recognized as an important diagnostic and prognostic marker for human breast cancer (1), and a tritiated thymidine DNA labeling index greater than 5% is associated with a markedly less favorable outcome (2). Incorporation of bromodeoxyuridine (BrdUrd) into the DNA of S phase cells gives a similar labeling index (3). Unfortunately, paraffin-embedded archival material is rarely pre-labeled, and so DNA cytometry of either whole nuclei disaggregated from thick sections (4) or partial nuclei in thin sections (5) must be used as an indirect approach to estimate cellular proliferative activity. We are particularly interested in validating the DNA cytometry of thin sections and in relating the DNA histogram to *in vivo* BrdUrd labeling index, which is our standard for cellular proliferation.

MATERIALS AND METHODS

Patients with breast cancer are given BrdUrd (200mg/mm^2) at the time of surgery. Labeled cells are identified immunocytochemically, using the monoclonal antibody IU-4. Cellular proliferation is expressed as the BrdUrd labeling index, based on counts of 2000 cells. Tumors also are analyzed by DNA image cytometry of 4μm thick sections, stained for DNA by the azure A Feulgen reaction. 200 tumor cells are measured for each section, and the measurements are displayed as a histogram.

RESULTS

Histograms from 12 tumors were evaluated subjectively by two independent observers. They ranked the histograms based on evidence of DNA aneuploidy, presence of cells with very high DNA content ("megaploid cells"), and continuity of DNA distribution. These criteria are similar to those developed by Auer (6), adjusted for effects of sectioning through nuclei. Comparison of the rankings (Spearman rank correlation) shows close agreement ($p<0.05$) between the two observers, and suggests that the histograms have underlying and recognizable patterns. However, there is virtually no correlation ($p>0.4$) between histogram rank and cellular proliferation as measured by *in vivo* BrdUrd labeling index. Tumors whose histograms rank adjacently often have great differences in labeling indices (See Figure 1).

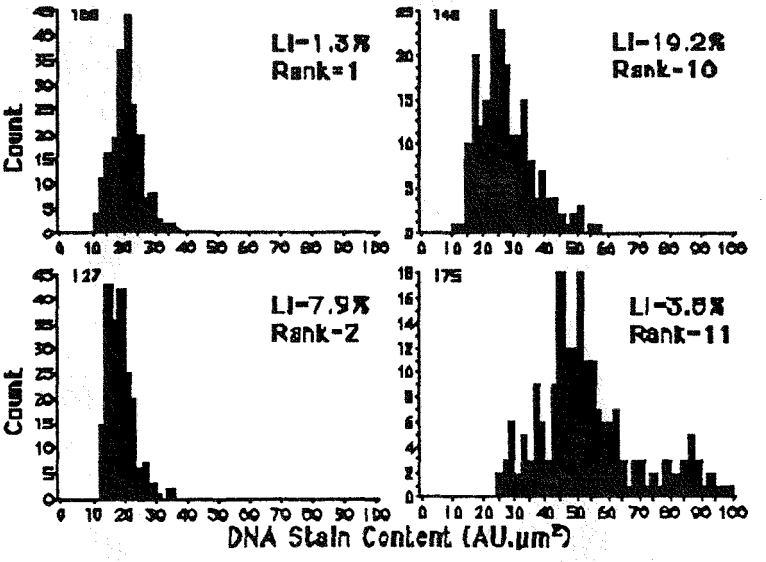

Figure 1. DNA histograms having adjacent ranks. Normal breast epithelial cells have a modal DNA value of 18-20 units. The similar histograms on the left both appeared to be DNA diploid and ranked lowest; they had LI=1.3% & 7.5% respectively. Those on the right appeared markedly abnormal and ranked highest; they had LI=19.2% & 3.0% respectively.

DISCUSSION

There is no obvious relationship between histogram rank and BrdUrd labeling index. This finding is both unexpected and disturbing. DNA histograms, even allowing for the uncertainty of measuring incomplete nuclei in sections, still reflect the underlying distribution of DNA content; hyperdiploid values come only from cells having a hyperdiploid DNA content. However, we find tumors with many DNA hyperdiploid cells but low BrdUrd labeling index. This suggests that these hyperdiploid cells are not S phase cells, but rather are hyperdiploid and megaploid cells with random cytogenetic paneuploidies. If this is so, then the estimation of S phase fraction from DNA histograms, whether by image or flow cytometry, may be a very uncertain and in some cases highly misleading measure. Our results also raise important questions about the biological and clinical significance of these DNA hyperdiploid cells. We are evaluating them prospectively as an independent prognostic marker for human breast cancer.

ACKNOWLEDGEMENT

This study was supported by USPHS grant CA-44768 and by the UC PAC.

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