Report

The functional relationship between in vivo bromodeoxyuridine labeling index and Ki-67 proliferation index in human breast cancer

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Summary

Proliferation indices are used, along with other parameters, to estimate the risk of recurrence of breast cancer for individual patients. Because it is unlikely one index will be practical for all patients, it is important to understand the relationship between various indices of proliferation. For this reason, we compared a proliferation index based on in vivo labeling of S-phase tumor cells with the thymidine analog bromodeoxyuridine (BrdUrd), to a proliferation index based on an estimate of the growth fraction with the MIB-1 antibody to the Ki-67 antigen. With informed consent, we gave 145 patients 200 mg/m² BrdUrd intravenously just prior to surgical removal of breast cancer. On histology sections, we visually counted S-phase cells which had incorporated BrdUrd using the Br 3 antibody which is specific to DNA incorporated BrdUrd, and we counted cells in the growth fraction using the MIB-1 antibody to the Ki-67 antigen. We found that both indices were positively correlated with tumor size, number of positive nodes, and tumor grade, and both were negatively correlated with age and estrogen-progesterone receptor positivity. Using a linear functional relationship model, we found that the best (i.e. the maximal) fit between the two indices (correlation coefficient 0.79; p < 0.0001) occurred when each index was square root transformed, as is appropriate when counts follow a Poisson distribution. When we used the median as a cutpoint for each index, the classification of 19 percent of data pairs changed depending upon which index was used. We also estimated that the Ki-67 intercept (1.02 ± 0.25) was significantly greater than zero. We conclude that the BrdUrd index of DNA synthesis in S-phase correlates highly with the MIB-1 index of the growth fraction, and both indices correlate well with other parameters of tumor aggressiveness. Because this correlation is driven by concordance of the extremes of high and low counts, clinical comparison will be necessary to determine which is the better prognostic marker for human breast cancer.

Introduction

In 1957, Bloom and Richardson observed that clinical staging '... although of considerable value does not take into full account the nature of the tumor itself' [1]. Stated another way, classification of

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breast cancers by the size of the primary tumor and the presence and extent of lymph node metastases does not adequately explain differences in the clinical outcome of individual patients.

Bloom and Richardson proposed a histologic grading system to supplement the prognostic information available to women with different pathologic stages of cancer. Innumerable other authors have proposed supplementing pathologic stage with information derived from markers of hormone responsiveness, genetic instability, tumor secreted proteases, tumor vascularity, programmed cell death, growth fraction, etc.

Measures of cell proliferation have been widely used to estimate the 'nature of the tumor itself'. The standard to which all indices of cell proliferation are compared is labeling of S-phase cells with tritiated thymidine [2]. Because 3H-thymidine is not practical for routine in vivo use [3], in vitro labeling is usually used for human subjects. Availability of the thymidine analog bromodeoxyuridine (BrdUrd), which can safely be used in vivo in quantities sufficient to be detected with monoclonal antibodies specific to DNA incorporated BrdUrd, allows in vivo labeling of S-phase tumor cells in human subjects [4, 5]. We, and others, have studied patients with breast [5–7], brain [8], colon [9], ovarian [10], head and neck [11], lung [12], and bladder [13] cancers using in vivo BrdUrd.

MIB-1 is a monoclonal antibody which has been used to detect cells expressing the nuclear antigen Ki-67 [14]. MIB-1, and the Ki-67 antibody before it, have been proposed as measures of cell proliferation for estimation of prognosis in human breast cancer and other human cancers. Ki-67 was first isolated from a Hodgkin's disease cell line. It is located in the nucleus, possibly associated with the nucleolus and/or fibrillar components; but its function is poorly understood. In continuously cycling cells, it is present in all cells in the growth fraction. However, it may be absent in some S and G2/M cells of some tumors even without treatment [15].

In this paper we explore the relationship between a labeling index for human breast cancers obtained with in vivo bromodeoxyuridine (BrdUrd) and the percent of tumor cells in the growth fraction as indicated using the MIB-1 antibody, which recognizes an epitope of the Ki-67 antigen. We compare each index to clinical and pathologic characteristics, define a functional relationship between the indices, compare the data with different transformations, and consider how use of one index or the other might give a different impression of the prognosis of a specific tumor. We conclude that these indices are highly related, but that clinical comparison will be necessary to determine which is the more reliable index of prognosis.

Methods

This study was approved by the Committee on Human Research at the University of California San Francisco. All subjects gave written informed consent. In the thirty minutes prior to surgery for removal of their cancer, 142 women and 3 men received 200 mg/m² bromodeoxyuridine (BrdUrd, National Cancer Institute, Bethesda, MD, and more recently Neopharm, Lake Forest, IL) in 250 ml of normal saline. Anesthesia, surgery, and all pre- and postoperative care were as was customary. Histology was graded using the Scarff-Bloom-Richardson scale by one of us (B.M.L.), who was unaware of the indices. Hormone receptors were determined in the clinical laboratory of U.C.S.F. and more recently by immunohistochemistry. Treatment recommendations were not based on BrdUrd labeling index.

Tissue was prepared, stained, and counted as described previously [4–6]. During routine cutting of fresh tissue, portions of the tumor were excised, fixed in 70% alcohol or 10% neutral buffered formalin, and embedded in paraffin. Tissue sections were treated one hour with 2N HCl to denature the DNA. Alternatively, cross-linked proteins in formalin fixed tissue sections were cleaved for one minute with 0.1% Protease XXIV (Sigma Chemical Company, St. Louis, MO) before denaturing with HCl. The Elite ABC kit (Vector Laboratories, Burlingame, CA) was used for indirect immunoperoxidase staining using the IU4 antibody (45 minutes; 1:250 dilution with 5% horse serum; courtesy of Dr. Joe Gray, University of California, San Francisco, CA) for DNA incorporated BrdUrd. Light