HORMONE RECEPTOR STATUS AND CHEMOSENSITIVITY IN BREAST CANCER

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In order to investigate the response of breast cancer cells to anticancer drugs as a function of hormone receptors, 69 specimens obtained from the primary tumor of invasive breast cancer patients were cultivated using the human tumor clonogenic assay. Successful growth in vitro (≥30 colonies per plate) was obtained in 50 (72.5%) of the 69 viable samples, with a median cloning efficiency of 0.17%. Five drugs (adriamycin, vincristine, 5-fluorouracil, carmustine, methotrexate) were tested for each specimen resulting in 250 drug assays. The overall drug sensitivity was 47.2%. Of these, 5-fluorouracil had the greatest effect in vitro with a sensitivity of 68.0%. There was no significant correlation between hormone receptor status and response to anticancer drugs either overall or for agents analyzed individually. It is concluded that hormone receptors of breast cancer have no predictive value for response to chemotherapy.

The treatment of metastatic breast cancer has been better defined in recent years. Chemotherapeutic and hormonal modalities produce an objective disease remission rate of 40% to 60% and 25% to 35% of unselected cases, respectively. It is well established that estrogen receptor (ER) status is the most important indicator for predicting the response of breast cancer to hormonal therapy. The addition of progesterone receptor (PR) assay improves the predictive ability of ER. Furthermore, the presence of both the ER and PR in a tumor will result in a 50% to 70% response rate to hormonal therapy. Chemotherapy on the other hand, has been an empirical science. Drugs are selected on the basis of past experience. Recently, several reports have suggested that ER levels may be a predictor of chemosensitivity in breast cancer too. We evaluated the response of breast cancer cells to cytotoxic drugs as a function of hormone receptors, by the application of the in vitro human tumor clonogenic assay (HTCA). This study had two major objectives: 1) to determine whether the hormone receptors of breast cancer could influence the ability of tumor cells to grow colonies; 2) to determine whether the hormone receptors of breast cancer could predict the tumor response to chemotherapy.

MATERIALS AND METHODS

Clonogenic Assay

Specimens were obtained from the primary tumor of female breast cancer patients undergoing mastectomy. None of the patients had prior anticancer therapy. Tumor specimens were processed under aseptic conditions in a laminar
flow hood within one hour of excision. A single cell suspension was made by mincing the tissue finely and passing it through a 0.1mm wire mesh gauze. The cell pellet was washed, centrifuged, and resuspended in Hank's balanced salt solution. Cell counts and determination of cell viability using trypan blue dye exclusion and the hemocytometer were done. A total of 69 specimens with a cell viability $\geq 30\%$ were used in this study.

The culture system utilized in this study was a modification of the double-layer soft agar system from Hamburger and Salmon. The top layer consisted of RPMI 1640 and fetal calf serum in 0.3% agar. This layer was plated on a basal layer of 0.5% agar in enriched Eagle's medium supplemented with fetal calf serum on a 35mm Petri dish. The cell concentration was adjusted to 1 to $2 \times 10^5$ mononuclear cells per ml and inoculated on the top layer. All plates were screened under an inverted microscope to ensure the absence of cell clumps. The cultures were then incubated at 37°C in a humidified incubator with 5% carbon dioxide. Serial examination was performed using an inverted microscope after plating. Maximum colony size was achieved by 10 to 14 days of incubation. The colonies were defined as aggregates of 40 or more cells in this study to indicate any single cells which were capable of dividing 5 or 6 times in culture. The colonies had to show a collection of cells recognizable as human breast cancer cells. To verify the histologic structure of the clones, the entire top agar layer was prepared by the slide technique after Buick and Salmon, and stained with haematoxylin, eosin, or Giemsa.

Chemosensitivity Testing

Stock solutions of standard anticancer drugs commonly used clinically, including adriamycin (ADR), vincristine (VCR), 5-fluorouracil (5FU), carmustine (BCNU), and methotrexate (MTX), were prepared and stored at -70°C until the time of experimentation. A continuous exposure of drug incubation in vitro with cells was used in this study. Each drug was tested at two dose levels to evaluate the dose-response relationship. A low concentration of approximately 10% of peak plasma concentration in human pharmacokinetic studies was used. The following drug concentrations (in $\mu$g/ml) were used for testing: ADR 0.1 and 10; VCR 0.01 and 0.1; 5FU 5 and 10; BCNU 0.1 and 3; MTX 0.3 and 4. Cells were plated in triplicate for each drug concentration. Three plates without drug were used as controls. The cloning efficiency (CE) was calculated as the number of colonies formed divided by the number of living cells plated without drugs.

$$\text{CE (\%)} = \frac{\text{number of colonies}}{\text{number of cells seeded}} \times 100$$

After 14 days in culture, an average of the number of colonies on the triplicate control plates and of the triplicate drug treated plates were counted under an inverted microscope. At least 30 colonies per control plate were required for evaluation of chemosensitivity. The mean colony count for the control was taken as 100% survival in each case. Tumors were considered to be sensitive to a given drug in vitro if there was greater than 50% inhibition of colony formation. Less than 50% inhibition of tumor colonies was defined as in vitro resistance. The percent inhibition of colony formation was determined by the following formula: