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BREAST CANCER TASK FORCE SUGGESTS 12 NEW PROJECTS FOR RFPs, HEARS REPORT ON HORMONES

Breast Cancer Task Force committees, responding to Chairman Pietro Gullino's suggestion that they take a more active role in developing RFPs, have come up with about a dozen possible projects which could result in at least that many and perhaps more new RFPs during the current fiscal year.

The Task Force last week held the second of six two-day meetings a year Gullino has scheduled. The first day was devoted to reports from ongoing research projects involving mammary cells and their dependence on and response to hormones. (See abstracts of those reports following this article.)

The Task Force committees spent the second day working up their

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In Brief

BIOMEDICAL PANEL REPORT WILL SUPPORT CANCER PROGRAM, TARGETED RESEARCH

THE PRESIDENT'S Biomedical Research Panel, when it reports on the state of federally-supported biomedical research, in general will be supportive of the Cancer Program, won't attack targeted research. Some critics of the Cancer Program had hoped the Panel would turn out a report they could use to sell Congress on diverting funds from NCI to other research; they'll be disappointed. The Panel will have some suggestions and criticisms involving technology transfer, but will not recommend that cancer control be taken away from NCI. . . . **NEW PUBLICATIONS:** "Persons at High Risk of Cancer—An Approach to Cancer Etiology and Control," edited by Joseph Fraumeni, chief of NCI's Environmental Epidemiology Branch. Based on an NCI-American Cancer Society conference on risk factors, it is available from Academic Press, $19. Also, a summary of Breast Cancer Task Force Program and related projects is available from the NCI Office of Cancer Communications, in a limited number of copies. It includes summaries of each Task Force research contract plus descriptions of grants related to breast cancer; related contracts supported by the Virus Cancer, Tumor Immunology and Diagnostic Radiology Programs; list of Cooperative Groups conducting breast cancer clinical research; list of drugs used against the disease; and a list of contraceptive research projects supported by all sources. . . . **BENNO SCHMIDT**, chairman of the President's Cancer Panel, defended the Cancer Program in a talk at the Robert A. Welch Foundation Conference on Chemical Research. Schmidt said the program "will require long-term support and great patience on the part of the American people and Congress. We are still far away from being able to put either a date or a price tag on the ultimate conquest of cancer . . . We are making extremely important progress and this effort must be sustained."

GULLINO STRESSES NEED FOR MORE RESEARCH ON BREAST CANCER MARKERS

(Continued from page 1)

suggestions for new research projects. The suggestions are still tentative and did not reach the stage where NCI staff can start writing the RFPs; that will require at least one more meeting. The committees hope to have some of the new RFPs ready by May.

Gullino suggested that the most important or urgent new research might be studies of casein and human chorionic gonadotropin as mammary tumor markers. Both the Diagnosis and Treatment committees will be involved in that project, since the Treatment committee already has some studies under way in biochemical markers.

Gullino said in a memo to Diagnosis committee members:

"The need for 'markers' able to reveal the presence of a small number of neoplastic cells remaining in the host after removal of the primary mammary carcinoma is well recognized. In addition to 'specificity' one also looks at a marker in terms of clinical applicability of the detection procedure. From the literature of the past two years there are two groups of observations to which I want to call your attention.

"Bussolati et al. (Vischows Arch. 365: 15-21, 1975) have described a simple method for detecting casein in mammary tissues by the use of immunofluorescence. The procedure is applicable to frozen as well as paraffin sections and is, therefore, readily applicable to the clinical laboratory. Hendrick and Franchimont (Europ. J. Cancer 10: 725-730, 1974) have developed a radioimmunoassay for detection of casein in serum. Bussolati et al. found that many but not all mammary carcinomas contained cells with casein in the cytoplasm. Hendrick and Franchimont detected casein in the serum of 72% of patients with primary breast cancer and 80% of patients with recurrence. False positives should be expected in patients with mammary dysplasias since Bussolati et al. showed casein in all these lesions. However, it seems promising that one can determine in the serum and from the slides of the primary tumor whether or not casein is produced by the tumor, and before and after mastectomy one can monitor for the presence of casein in serum.

"The second group of observations concerns the detection of human chorionic gonadotropin hCG. Braunstein and his NIH colleagues (Ann. Int. Med. 78: 39-45, 1973) have detected hCG and the β subunit of the protein in the serum of tumor-bearing patients. Moreover, Franchimont et al. (Clin. Endocrin. 1: 315-336, 1972) have detected the α subunit of hCG in 25% of patients with a variety of tumors, including breast tumors. Naughton et al. (Cancer Res. 35: 1887-1889, 1975) have described a method for localizing on frozen sections the β chain of hCG in human tissues. Again, the possibility of testing both serum and mammary tissue at the time of surgery and monitoring after mastectomy for hCG and/or the α and β chains may be a useful approach.

"Neither casein and hCG nor the components of the molecule should be present in the normal, nonpregnant or lactating woman. If serum is monitored before and after surgery for patients with mammary tumors containing casein, hCG, or only the α and/or β chain of hCG, one might have a reasonably good tool to detect metastatic growth.

"I invite the Diagnosis committee to look into the possibility of preparing an RFP to exploit these markers. It is not a question of finding a universal marker for all mammary tumors, but rather of trying to answer the following questions: (1) How many carcinomas can be characterized by the presence of casein and hCG in the cells of biopsied tissue? (2) What is the correlation between the presence of casein and hCG in the neoplastic cells and detectable levels in serum? (3) How effective are the assays for both proteins as monitoring devices for the early detection of metastases?"

The Diagnosis committee came up with these other tentative projects:

- Use of ultrasound for clinical evaluation, with whatever machinery is presently available.
- Cell mediated immunity screening tests and detection of virus structured antigens, in the immunology area.
- Non-invasive means for determination of axillary node status, probably using radiological techniques.
- Studies of early pre-malignant or borderline lesions and their potentiality for breast cancer.

The Treatment committee identified two areas for possible new RFPs:

- Predictive testing for chemotherapy—develop models and new approaches to test whether a specific patient will respond to a specific drug.
- Identification and treatment of high risk breast cancer patients with negative axillary nodes—that is, develop a method to identify those patients with negative nodes but who do have metastatic disease at the time of surgery.

The Treatment committee took no action on the suggestion by Bernard Fisher, chairman of the National Surgical Adjuvant Breast Project, that the time may be ripe for a study using segmental surgery. That approach is still under consideration.

The Epidemiology committee discussed these projects as possibilities:

- Relationship of prolactin to breast cancer.
- Combination of risk factors, a multifactorial study.
- Estrogen receptors—the epidemiology of those rich and those poor in estrogen receptors.
- Exogenous estrogens, and the endocrinology of earlier years.

The Experimental Biology committee could not come up with any definite project suggestions.

ABSTRACTS ON MAMMARY CELL–HORMONE REPORTS FROM BREAST CANCER MEETING

Fluorescence Labeling and Polarization as a Means of Quantifying the Hormone-Receptor Interaction – *W.B. Dandliker, James Brawn and S.A. Levison, Scripps Clinic & Research Foundation*

Fluorescence polarization methodology has been applied to a study of the rates and equilibria of the hormone-receptor interaction. The interaction of estrogens, growth hormone and prolactin with receptors from normal and malignant mammary cells are being examined. The methodology involves the fluorescence tagging of polypeptide and steroid hormones followed by monitoring changes in the polarization of fluorescence which occur when fluorescent labeled hormones are added to receptors. The sensitivity of these techniques is comparable to that of radiolabeling and the measurements can be made on a time scale as short as a few milliseconds. Specificity of the interaction of fluorescent labeled hormones with receptors is evidenced by the very high binding affinities observed together with inhibition of the binding in the presence of the same hormone in an unlabeled form.

Quantitative results for the forward rate constants, k_1, the reverse rate constants, k_{-1}, the equilibrium constants, K, and the receptor site concentrations have been obtained. The data indicate that the primary combination between hormone and receptor behaves like a simple second order process not diffusion controlled. In the case of estradiol a second temperature-dependent, presumably conformational, change has been observed to follow the primary combination.

Direct measurements on the dissociation reaction have been made by dilution jump. Qualitative observations of hormone binding to microsomal receptors and to whole cells have been made by fluorescence microscopy.

Hormone Dependency in Human Breast Cancer – *E.V. Jensen, S. Smith and E.R. DeSombre, Univ. of Chicago*

On the basis of the minimum estrophilin levels required for response in 123 patients undergoing endocrine therapy for advanced breast cancer, about 70% of the 1,100 women whose primary and/or metastatic tumors have been analyzed may be classified as estrophilin-poor and 30% as estrophilin-rich. Since the receptor-poor group has little chance of benefit from endocrine therapy, whereas nearly two-thirds of the receptor-rich group show objective response, determination of the estrophilin content of excised tumor specimens can suggest the proper type of therapy for 85 to 90% of women with advanced breast cancer. Current goals of our research project are: 1). Elucidation of the reason or reasons why not all patients with receptor-rich tumors are responsive; 2). More precise definition of the critical estrophilin level is required for response; 3). Development of a simple but dependable assay procedure; and 4). Evaluation of the ability of an estrophilin assay on the mastectomy specimen to predict response to endocrine therapy if metastases appear at a later time.

Failure of some receptor-positive patients to respond may result from various causes. Tumor heterogeneity or the presence of both receptor-rich and receptor-poor metastases in the same patient probably is responsible in some instances, emphasizing the need for multiple specimens whenever possible. Studies of induced rat mammary tumors demonstrate that an occasional autonomous tumor will contain a substantial amount of estrophilin, even though the RNA polymerase system of its nuclei is insensitive to stimulation by estrogen-receptor complex. This finding suggests that a simple test to evaluate the susceptibility of tumor nuclei to stimulation in vitro might provide a more direct indication of hormone dependency than does the receptor content. On the basis of McGuire's suggestion that responding cancers might require the presence of both estrogen and progestin receptors, we have determined both in 55 breast cancers. The highest binding of R-5020 was seen in 7 of 12 estrophilin-rich tumors, although 4 of 43 estrophilin-poor tumors also showed a significant content of progestin receptor. Correlations with clinical response to endocrine therapy are not available as yet.

Experiments are continuing in an attempt to develop a simple radioimmunoassay for estrophilin, using antibodies to the pure receptor. Rabbits have been immunized with an apparently homogenous preparation of receptor from calf uterus, and efforts are being made to accumulate enough purified receptor to immunize a goat. Purification of estrophilin from human uterus by similar techniques is just beginning.

More than 650 primary breast cancers have been analyzed from patients without evident metastases, who are being followed for recurrence. So far two patients with estrophilin-rich cancers both responded to endocrine treatment 14 and 24 months later, respectively, whereas 10 of 11 patients with estrophilin-poor primary tumors failed to respond to treatment 4 months to 5 years later. One patient showed response to diethylstilbestrol 67 months after mastectomy.

Hormone Dependent Breast Cancer – *William McGuire, Univ. of Texas (San Antonio)*

Breast cancers are often hormone responsive; 60% of estrogen receptor-positive tumors regress following endocrine therapy. However, since binding to receptors is only the first step in hormone action, the failure of hormone control in 40% of estrogen receptor-positive tumors may be the result of lesions in later steps. We have proposed that the ideal marker of

hormone dependence would be a measurable product of hormone action rather than the initial binding step and that progesterone receptor, a product of estrogen action, may be such a marker. To test this hypothesis we sought to determine whether and when breast tumors contain progesterone receptor, how progesterone receptor was related to estrogen receptor, and what correlation, if any, progesterone receptor had with behavior of the tumor following endocrine therapy.

Before demonstrating progesterone receptor in mammary tumors we required a reliable assay for the receptor in a known target tissue.

With the use of ^{3}H-R5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione-6, 7-^{3}H), a potent synthetic progestin, we have demonstrated progesterone receptor in rat uterine cytosols. The receptor sediments at 7-8S in sucrose density gradients and binds hormone with high affinity (dissociation constant $\sim$ 1.2 x 10^{-9}M). Its concentration can be increased 10 fold by estrogen injection in hypophysectomized, ovariectomized and adrenalectomized rats.

Using R5020, a specific progesterone receptor, distinct from corticosteroid binding globulin, glucocorticoid and estrogen receptor is present in human breast tumors. In the same tumors demonstration of progesterone receptor using ^{3}H-progesterone is difficult. The affinity of R5020 binding (dissociation constant $\sim$ 0.83 $\pm$ 0.38$_{(SD)}$x 10^{-9}M) was 5 times higher than progesterone binding. 236 human breast tumors were assayed. Only 8% of estrogen receptor-negative tumors contained progesterone receptor whereas 62% of estrogen receptor-positive tumors contained the receptor. This distribution is similar to the response rate to endocrine therapy for estrogen receptor-positive and estrogen receptor-negative tumors and supports the hypothesis that progesterone receptor may be a marker of hormone dependent tumors. Preliminary clinical correlations of the presence of progesterone receptor and tumor response to endocrine therapy are encouraging.

Finally, we have found that the human mammary carcinoma cell line (MCF-7) has receptors for four classes of steroid hormones that influence mammary gland growth and function. In addition to estrogen receptor (dissociation constant $\sim$ 0.6 x 10^{-10}M) and progesterone receptor, there were receptors for androgens (dissociation constant (dihydrotestosterone) $\sim$ 2.8 x 10^{-10}M) and glucocorticoids (dissociation constant (Dexamethasone) $\sim$ 8 x 10^{-9}M). We had predicted that progesterone receptor would be present in these cells derived from an estrogen receptor-positive hormone dependent tumor. The demonstration of four classes of steroid receptors is unique and means this line will be useful for studying not only the mechanisms of tumor endocrine response but also the complex interrelationships between binding and biological actions of these four steroids.

Medical Adrenalectomy with Aminoglutethimide: Development of a Practical Regimen to Eliminate Drug Interaction – ***Richard Santen, Dept. of Medicine, Div. of Endocrinology, Hershey Medical Center***

Aminoglutethimide (AG) and dexamethasone (DM) can be used in patients with carcinoma of the breast to abolish adrenal steroidogenesis. Initial studies with this regimen, however, revealed an unsuspected drug interaction in which aminoglutethimide markedly accelerated the metabolism of dexamethasone. This problem necessitated careful titration of DM and AG dosage and close monitoring of steroid levels in individual patients. We wished then, to develop a more practical and easily administered drug regimen which would eliminate the problem of drug interaction and facilitate wide applicability of medical adrenalectomy.

We suspected that hydrocortisone might be metabolized differently than potent synthetic glucocorticoids such as dexamethasone. To evaluate this possibility, initial studies were designed to compare the effect of aminoglutethimide on hydrocortisone and DM metabolism in treated patients. Whereas the DM half-life was reduced two-fold during aminoglutethimide administration, the clearance of hydrocortisone was not altered (T½ before AG - 77 minutes, during AG administration - 73 minutes). To confirm the results of these kinetic studies, we then compared the biopotency of dexamethasone and hydrocortisone. Glucocorticoid activity was quantitated by monitoring adrenal steroid output and ACTH levels in patients receiving varying doses of DM or hydrocortisone in addition to aminoglutethimide. These studies confirmed the conclusion that aminoglutethimide does not significantly alter the biologic activity of hydrocortisone. It was appropriate, then, to develop a new "medical adrenalectomy" regimen consisting of aminoglutethimide and standard glucocorticoid replacement amounts of hydrocortisone. Thirteen patients were treated with 30-40 mg of hydrocortisone and 1,000 mg of aminoglutethimide daily for one to 19 months. Complete adrenal blockade was achieved in each.

Conclusions: (1) Aminoglutethimide does not alter hydrocortisone metabolism in contrast to its effects on dexamethasone metabolism. (2) The use of aminoglutethimide in combination with standard replacement amounts of hydrocortisone is an effective regimen for "medical adrenalectomy". (3) This newly developed method should allow the wide applicability of medical adrenalectomy with aminoglutethimide since it is simple and effective.

Hormone Induction of Postsynthetic Modifications of Chromosomal Proteins in Mammary Neoplasia – ***Kenneth McCarty, Duke Univ. Medical Center***

It is becoming increasingly apparent that an important aspect of hormone induction of gene regulation in eukaryotic cells occurs at the level of RNA

transcription and appears to be limited to a small fraction of the total DNA specific for those cells that have hormone receptors. Thus the best evidence that steroid action is at the level of the chromosome is from those in vitro systems in which a hormone-receptor-chromatin complex demonstrates the capacity to induce a tissue response in transcription of specific mRNAs.

Evidence is also beginning to accumulate to suggest that a primary mechaism of hormone induced gene activation in mammary tissue represents the consequence of postsynthetic modifications of chromosomal proteins. Thus it is reasonable to propose that this level of control may reflect gene derepression as a result of the alterations in "specific charge interactions" of chromosomal proteins and DNA. These alterations probably represent the first step in a cascade of events closely coupled to and dependent on the phase of the cell cycle, to include cytoplasmic steroid receptors, peptide hormone plasma membrane receptors, cyclic nucleotides, kinases, acetylases, etc. in addition to specific activator or repressor acidic proteins of the type already demonstrated to perform an essential role of prokaryotic.

Our initial observations demonstrated a hormone induced phosphorylation of histone H2a. We have evidence now that this phosphorylation is exclusively on serine residue 19 as a result of mitogenic activity of insulin. In addition we have now been able to resolve this histone into two subfractions using Triton gel electrophoresis. Both of these fractions are phosphorylated.

Morphological and Biochemical Responsiveness of a Mammary Cell Culture – *S. Sizemore, G. Lawson and David Cole*

The NMuMG cell line derived from normal mouse mammary epithelial cells is being tested for responsiveness to hormones. The hormones studied included insulin, glucocorticoids (cortisole or dexamethasone) and prolactin. The cells were found to possess 8,000 membrane bound insulin receptors and 20,000 cytoplasmic glucocorticoid receptors per cell, but the steroid receptors could only be demonstrated with the use of serum which had been treated with charcoal. The extraction of endogenous hormone was done at 37 degrees because high temperatures destroy the ability of the serum to support cell growth. Cells grown with charcoal-treated (37 deg.) serum grow at essentially normal rates but a combination of insulin, dexamethasone, and prolactin enhances growth in the earliest stages after plating the cells, perhaps by increasing plating efficiency. Moreover, about seven days after confluence is reached the combination of hormones produces many large or flat cells that are stable for at least two weeks. The steroid alone produces fewer such cells and more slowly while insulin treatment shows no such effect at all.

Assays of these mammary cells for lactose synthetase seem to show a wave of induction of both A and B subunits of the enzyme when the cells are treated with insulin, glucocorticoid, and prolactin, but a partial effect is obtained with insulin alone. The magnitude of the enzyme induction is about three to four fold.

Insulin induces a doubling of the activity of glyceraldehyde phosphate dehydrogenase.

Experiments carried out before we learned how to remove endogenous hormones from serum with charcoal without inactivating the serum indicated modest hormonal effects on cytochrome C reductase, DNA synthesis, and histone synthesis.

DMBA Induction of Mammary Carcinomas in Neonatally Masculinized Female Rats – *Susumu Ohno and James Kan, City of Hope*

Our study on *Tfm/+* heterozygous mice that are natural mosaics indicated that the androgen suppression of mammary gland growth is mainly through the hypothalamus-pituitary axis, certainly involving pituitary prolactin. Thus, we became interested in the observations by others that DMBA induction of mammary carcinomas in female rats is the prlactin dependent process.

We have reasoned that female rats with the neonatally masculinized central nervous system should then prove to be resistant to this DMBA effect, for their mammary glands are not exposed to periodical bursts of pituitary prolactin. Our interest was further enhanced by the report of Stern, Mickey and Gorski (1969) that DMBA, either implanted directly to the hypothalamic region or administered through a stomach tube, restores cyclic ovarian function to masculinized female rats. Indeed, when 10 mg of DMBA was given to masculinized female mice in persistent estrus, cyclic formation of ovarian corpora lutea accompanied by the abolishment of masculine aggression was noted. The subsequent study using ^{3}H-DMBA, however, showed that DMBA is incapable of crossing the bload-brain barrier, while it preferentially concentrates in the mouse ovary. Thus, the apparent feminization of masculinized female mice was due to the direct DMBA effect on mouse ovaries, not involving the central nervous system.

Next, we have divided the neonatally masculinized S-D strain female rats in confirmed persistent estrus into three groups and given 25, 35 and 50 mg of DMBA via stomach tubes at the 50 and 60th days. While vaginal smears indicated irregular ovarian cyclic function, such changes were not accompanied by the corpora lutea formation, and blood samples indicated no cyclic bursts of pituitary LH as well as prolactin. Thus in rats, we failed to observe even the direct effect of DMBA on the ovarian function. However, all of them were free of mammary carcinomas up to the 70th day after DMBA administration. Thus, when this experiment is completed, we might confirm our

original postulate that female rats, with a masculinized central nervous system, are resistant to DMBA induction of mammary carcinomas.

Characterization of Putuitary Mammotrophs from Humans and Rats Bearing Mammary Tumors – ***W.C. Hymer, W. Wilfinger and H. Asawaroenchai, Pennsylvania State Univ.***

We have used the R13762-b mammary ascites tumor (MAT) line that is carried in the Fisher 344 rat to study the influence of the tumor on the structure and function of the host's pituitary prolactin cells (mammotrophs). In this animal metastases of the tumor cells to lymph nodes, lungs, abdominal organs, and occasionally the brain are common; animals die 10-12 days after tumor implantation. The tumor shows hormone responsiveness in that administration of estradiol benzoate (E_2), but not testosterone propionate (TP), significantly prolongs life of the tumor-bearing animals.

Our results show that the mammotrophs from tumor-bearing rats, when compared to their counterparts from normal animals a) contain significantly less hormone, b) are smaller and c) release significantly less prlactin over a two-week culture period. On the other hand, mammotrophs from tumor-bearing animals injected with E_2 a) contain significantly more hormone b) are larger and c) release significantly more prolactin in culture compared to mammotrophs from normal animals. Mammotrophs from TP injected tumor bearing animals are generally similar to those from the E_2treated animals.

We have also begun to characterize the tumor cells separated by velocity sedimentation. Our preliminary results show a) that tumor cells can be separated from leukocytes and b) that tumor cells from E_2 treated animals tend to be smaller while a fraction of cells from TP treated animals are hypertrophied.

These results will

Role of Prolactin and Estrogen in Breast Cancer – ***O.H. Pearson, A. Manni and J. Trujillo, Case Western Reserve Univ.***

Clinical trials of Tamoxifen (T) (ICI United States Inc.), which is a potent antiestrogen and binds competitively to estrogen receptors (ER), and Lergotrile Mesylate (L) (Lilly), which is a potent inhibitor of prolactin secretion, were carried out in selected patients with progressive disease. T was given in doses of 20 mg PO q 12 h to 31 patients for periods of 1 to 1 to 15 months. Tumor regression occurred in 14 (45%), no progression in 9 (29%) and 8 (26% were failures. Remissions have lasted 8.3+ months with only 2 relapses; no progression cases average 7.6+ months with no relapses. Tumor regression or arrest was seen in skin (6/9), bone (13/17), lung (7/8), breast (2/4), lymph node (2/6), pleura (3/3) and liver (0/1). ER was measured in 12 patients of which 6 of 9+ ER responded and 3 of 3- ER failed. T had no effect on serum prolactin levels. Two premenopausal patients improved even though menses were not completely suppressed. One of 4 post-hypophysectomy patients obtained objective remission. One who failed on anti-estrogens had regression after hypophysectomy. Side effects of T were minimal.

L was given in doses of 2 mg PO q 8 h to 12 patients with progressive disease for periods of 2 to 9 months. Serum prolactin was suppressed throughout the day and night to levels of 1 ng/ml or less, but L had no effect on growth hormone secretion or other pituitary hormones. None of the 12 patients had objective regression but 4 had no progression for a period averaging 6 months. Six of 8 who failed and 3 of 4 who had no progression on L, obtained objective remissions from other modalities of endocrine treatment. Prolactin receptors have been detected in low titer in about 30% of human breast cancers, but this finding has not yet been correlated with responses to endocrine therapy. L produced minimal side effects.

The results indicate that T is an effective antitumor agent in stage IV breast cancer and that L, by itself, had minimal antitumor effects. Since human growth hormone has lactogenic properties, a drug to suppress the secretion of this hormone would be of interest. ER appears to have predictive value in the response to treatment with T. Progesterone and prolactin receptors are also being studied for their potential predictive value. Further studies are needed to determine whether combination of T and L might yield synergistic effects, and whether anti-hormone therapy can produce results comparable to surgical hypophysectomy.

The Androgen Response of the Embryonic Mammary Rudiment: Development of Responsiveness and Tissue Interaction in the Response – ***Klaus Kratochwil***

The explanted mammary rudiment of the mouse responds directly to testosterone. This response is characterized by high sensitivity (5×10^{-10}M) and very good steroid-specificity. The rudiment becomes responsive to androgens at 13½ days (two days after its formation) and loses its responsiveness again only two days later (still before outgrowth of the primary sprout). Both acquisition and loss of responsiveness occur in explants at the same time as in utero, thereby reflecting an intrinsic developmental process in the gland itself.

Both tissues of the rudiment are involved in the response: The mesenchyme condenses around the epithelial bud, the gland epithelium separates from the epidermis and undergoes extensive necrosis. Through experimental tissue combinations, taking advantage of the androgen-insensitive mutant (Tfm) we could establish that testosterone acts directly only on the mesenchyme. The effect on the gland epithelium must be mediated by testosterone-activated mesenchymal cells.

Estriol Dynamics and Breast Cancer – *C. Longcope and Howard Pratt, Worcester Foundation and Boston Univ.*

It has been suggested that in women estriol may play a protective role against the development of breast cancer. Among the evidence cited for this has been the finding of high ratios of the metabolites of estriol to estrone and estradiol in the urines of women who do not have breast cancer compared to the low ratios of the estrogen metabolites in the urines of women who have breast cancer. Estrogen metabolites which are measured in the urine do not necessarily reflect the dynamics of the free steroid circulating in the blood. We have therefore been studying the dynamics of free estriol in the blood in women with high and low ratios of estrogen metabolites in the urine and in women with breast cancer.

We have found no significant difference between the metabolic clearance rates, plasma concentrations and blood production rates of estriol in normal reproductive-aged women who have high as compared to low ratios. For both groups of women the follicular phase concentrations and blood production rates were lower than those found in the luteal phase of the corresponding group.

The sources of estriol did not differ significantly between the groups although somewhat more estriol arose from conversion from androstenedione in the high as compared to low ratio groups in the follicular phase.

The production rates of androstenedione, estrone and estradiol did not differ between the high and low ratio groups of normal women when measured in the respective follicular or luteal phases.

In a small group of women who had undergone a mastectomy for breast cancer and had no clinical evidence of recurrence, mean estriol metabolic clearance rates,concentrations and production rates did not differ significantly from a control population, and androstenedione, estrone and estradiol production rates were similar in these two groups of women.

Biochemical Nature of Hormone Dependence in Mammary Cancer – *Eugene DeSombre, Univ. of Chicago*

It has been known for some time that certain rat mammary tumors, like their human counterparts, regress following endocrine ablation (ovariectomy). In the DMBA induced mammary tumors of the rat the majority of the tumors regress after host ovariectomy and these tumors contain significant amounts of estrogen receptor. In normal estrogen-responsive tissues there is considerable evidence implicating estrogen receptors in the tissue response to this hormone. Until recently it was generally found that the hormone-independent rat tumors which continue growing in the castrate host were devoid of estrogen receptors. The recent evidence from our laboratory and elsewhere indicates that there are rat tumors which do not regress on host castration but have, nonetheless, appreciable amounts of estrogen receptor. Studies of the uptake of cytosol estrogen-receptor complex by nuclei of hormone-dependent and autonomous tumors have shown that nuclei of both types of tumors, whether receptor positive or negative, take up estrogen receptor when incubated with it. Therefore neither the presence of cytosol estrogen receptor nor nuclear uptake of receptor complex correlate exclusively with hormone-dependence, although all dependent tumors show both. It is therefore possible that the presence of estrogen receptor may be a necessary but not sufficient condition for tumor response. In this case an assay of some biochemical effect of estrogen receptor may be a more accurate predictor of response. Studies of estrogen receptor mediated stimulation of nuclear RNA synthesis suggest that this biochemical endpoint may be of value in understanding the tumor responses. Although autonomous tumor nuclei take up estrogen receptor they do not show the characteristic stimulation of RNA synthesis.

Another biochemical effect of hormone being studied is the induction of peroxidase activity. This enzyme activity appears following estrogen stimulation of normal tissues showing a growth response to the hormone. We have shown that this enzyme activity, demonstrated histochemically, is also characteristic for growing hormone dependent rat mammary tumors.

RFPs AVAILABLE

Requests for proposal described here pertain to contracts planned for award by the National Cancer Institute, unless otherwise noted. Write to the Contracting Officer or Contract Specialist for copies of the RFP. Some listings will show the phone number of the Contract Specialist, who will respond to questions about the RFP. Contract Sections for the Cause & Prevention and Biology & Diagnosis Divisions are located at: NCI, Landow Bldg. NIH, Bethesda, Md. 20014; for the Treatment and Control Divisions at NCI, Blair Bldg., 8300 Colesville Rd., Silver Spring, Md. 20910. All requests for copies of RFPs should cite the RFP number. The deadline date shown for each listing is the final day for receipt of the completed proposal unless otherwise indicated.

RFP NCI-CM-67064

Title: *Relationship of antineoplastic drug kinetics to in vivo effects*

Deadline: *Approximately March 8*

The contractor shall carry out a series of studies designed to obtain detailed information on the pharmacology of adriamycin derivatives to be selected by the contractor. These drugs will be studied both as single agents and in combination with other agents. These studies will be carried out in tumor-bearing mice at various stages of tumor growth (spontaneous

mammary carcinoma and Lewis lung carcinoma with proper strains of non-tumor bearing mice as controls).

It will require the ability to develop sensitive techniques for drug and metabolite detection and quantitation. Effort will be made to correlate the concentrations of these agents and their active metabolites in blood, tumor and normal tissues (particularly the small intestine and bone marrow) with their toxic activity. Measurement of toxic activity should include: depression of bone marrow proliferative capacity, damage to intestinal epithelium, degree of tumor cell kill and immunosuppression. Both the time course of initiation of these effects and the time course of recovery from such effects are to be determined. The comparison of the effects of significant metabolites found in vivo will also be made with the parent drug in vitro employing a cell kill assay technique.

The principal investigator should be competent in pharmacology and show evidence by previous publications of interest in kinetic analysis of in vivo biological phenomenon. The principal investigator would coordinate a staff in several specialties such as the following: 1) A cancer biologist capable of handling both in vivo and in vitro tumor systems and determining effects of antitumor agents on these systems. 2) An immunologist with expertise in quantitation of measurements of immune response from in vivo experiments. 3) A consulting chemist for purposes of help in determining analytical techniques for the drug derivatives and synthesizing these derivatives when called for. 4) A consulting pharmacokineticist with a working knowledge of statistics as well as compartmental kinetic analysis. This individual should be a dynamic person with capabilities of dealing directly with the above individuals. He should be motivated in developing new concepts which relate drug kinetics to drug effect.

Contract Specialist: J.M. Cooper
Cancer Treatment
301-427-7463

RFP NCI-CM-67080-26

Title: *Determinative and diagnostic microbiological studies*

Deadline: *Feb. 12*

The Div. of Cancer Treatment will make available to interested organizations an RFP to supply high quality microbiological studies on patient and environmental samples by means of a clinical support contract for the care of cancer patients who receive chemotherapy.

These patients undergo severe depression of host defense mechanisms which protect them against infection. Data obtained by means of this contract will provide specific identification of the infecting microorganism to enable the use of optimal therapy, disclose the patients' endogenous flora (present at admission and hospital-acquired), allow monitoring of sources of hospital acquired infection and provide the basis for evaluation of protective environments for immunosuppressed patients.

Specifically, the contractor will utilize current culture methods for bacteria (aerobic and anaerobic) fungi, and mycoplasma, when indicated, to monitor patient and environmental samples. Complete identification to species will be required as well as subspecification in certain cases. Antibiotic susceptibility tests will also be performed. Approximately 60 patient diagnostic and surveillance samples daily and 15 to 20 on weekends as well as approximately 70 environmental samples per week will be provided for culturing.

The contractor must supply all of the trained personnel, facilities, equipment, labor and materials necessary to carry out the work required by this proposed contract. Due to the nature of this project, the contractor must be located within an area sufficiently close to both the BCRC, Baltimore, Md. and the Pediatric Oncology Branch, Bethesda, Md. to provide timely daily pick-up and delivery of samples as specified in the RFP.

Contract Officer: George Summers
Cancer Treatment
301-427-7463

RFP NO1-CP-65760-62

Title: *Data and information resources*

Deadline: *March 23*

The proposer is to develop a resource that would allow data and information to be made available to scientists and scientist administrators in a timely manner and in a useful format. The involvement can range from supplying bibliographies with and without a screen for relevance (formats to be specified) to supplying critical analysis of defined areas of the scientific literature with varying degrees of contractor involvement.

Contract Specialist: D.J. Longen
Cause & Prevention
301-496-6361

CONTRACT AWARDS

Title: Breast cancer detection demonstration project

Contractor: New Jersey College of Medicine & Dentistry, $265,161.

Title: Isolation and chemical characterization of soluble human tumor specific antigens

Contractor: Scripps Clinic & Research Foundation, $71,909.

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