Capture-RT-PCR Assay of mRNA in Breast Tumors: *int*-2 and HERV-K *env* mRNA

David Gillespie, PhD[1], Stephanie Poirer[1], Justin Poley[1], Kevin Cuddy[1], Isadore Brodsky, MD[1], and Jeffrey Brodsky[2]

[1] Hahnemann University, Department of Neoplastic Diseases, Philadelphia, PA 19102-1192
[2] Hahnemann University, Department of Surgery, Philadelphia, PA 19102-1192

Abstract Gene expression at the mRNA level is likely to be a rapidly responding intermediate biomarker for use as a surrogate endpoint in Phase II clinical trials of breast cancer. Using a technology called "capture-RT-PCR," we have been able to show ectopic *int*-2 mRNA expression in 7/9 breast tumors, including two fibroadenomas. The level of expression varied, high expression being observed in three tumors. Frequent expression of *int*-2 in breast tumors is contrary to published reports, possibly reflecting differences in technology. *int*-2 expression in fibroadenomas suggests that the marker may precede malignancy. Conversely, if fibroadenomas are not premalignant, *int*-2 expression may be gratuitous. These alternatives need to be tested in a clinical trial. Human endogenous retrovirus K (HERV-K) *env* gene expression was detected in only 1/7 breast tumors. Expression of this retroviral gene is more restricted than *int*-2 expression. c-*myc* was expressed in all 10 tumors studied, albeit at widely varying levels. Expression of *prad*1 and *erb*B-2 are under study. Gene expression will be compared with gene amplification. It is anticipated that a capture-RT-PCR assay simultaneously signifying levels of expression of several oncogenes/anti-oncogenes will be a most informative surrogate endpoint biomarker.

In particular, chaotropic salt methods that simplify sample preparation and mRNA isolation and reduce these combined steps to a 10 minute procedure will be presented.

Dehydroepiandrosterone: A Chemoprotective Steroid

Gary B. Gordon, MD, PhD, Kathy J. Helzlsouer, MD, MHS, Trudy L. Bush, PhD, and George W. Comstock, MD, DrPH

Johns Hopkins University, Departments of Oncology, Pharmacology, and Epidemiology, Baltimore, MD 21205

Abstract Dehydroepiandrosterone (DHEA) and its sulfate conjugate, DHEAS, are steroids found in high concentration in human plasma. Levels peak in young adulthood and decrease steadily with age as the incidence of cancer increases. Several studies have found that low serum levels of DHEA or DHEAS or their urinary metabolites were associated with the presence or development of breast cancer. Dietary administration of DHEA significantly reduces the incidence of spontaneously and chemically induced mammary tumors in animals. DHEA also has potent chemoprotective actions in several other target organs against a variety of carcinogens. DHEA's mechanism of action is not known but may be due to its uncompetitive inhibition of glucose-6-phosphate dehydrogenase. Animal studies and tissue culture

models support this hypothesis. DHEA and synthetic analogues of DHEA have been proposed as possible chemoprotective agents in selected groups.

A nested case-control study was conducted using serum samples from a population-based serum bank to examine the association between serum DHEA and DHEAS levels and the risk of developing breast cancer. Blood samples were collected from 20,305 Washington County, MD residents. Incident breast cancer cases were identified through the Washington County Register. Fifteen premenopausal and 30 postmenopausal breast cancer cases were identified and matched to two controls by age and time since last menstrual period. Controls were alive and free of other cancers except for nonmelanoma skin cancer at the time the case was diagnosed. Serum steroid levels were determined by radioimmunoassays (RIAs). The associations between DHEA and DHEAS levels and the risk of developing prostate, bladder, and gastric cancers have also been examined.

Prediagnostic serum levels of DHEA were lower in women who developed premenopausal cancer compared to the controls, while postmenopausal cases had significantly higher prediagnostic levels of DHEA than the controls. DHEAS levels were not significantly different between cases and controls in either group. These associations between DHEA and the risk of developing breast cancer did not vary by time to diagnosis. This discrepancy between the results for premenopausal and postmenopausal women may result from the contribution of these steroids to the synthesis of estrogens in postmenopausal women. In similar nested case-control studies, we have shown low levels of DHEA to be a risk factor for subsequent bladder and gastric cancer, but not for prostate or ovarian cancer.

These associations between serum levels of DHEA and DHEAS and the risk of developing specific cancers should be considered in the decision to employ DHEA or a synthetic analogue in selected high-risk populations.

Chemoprevention of Rat Mammary Carcinogenesis: Exceptional Activity of Dietary Dehydroepiandrosterone (DHEA)

David L. McCormick, PhD[1], William D. Johnson, PhD[1], Kandala V.N. Rao, DSc[1], Teresa Bowman-Gram, BS[1], Richard C. Moon, PhD[1], Vernon E. Steele, PhD[2], and Gary J. Kelloff, MD[2]

[1] IIT Research Institute, Life Sciences Department, Chicago, IL 60616
[2] National Cancer Institute, National Institutes of Health, Bethesda, MD 20852

Abstract A major determinant of progress in human breast cancer prevention is the identification of agents with significant anticarcinogenic activity and acceptable levels of toxicity in experimental animals. Over the past 20 years, more than 50 experimental regimens have been shown to have significant chemopreventive activity in the rat mammary gland. The most effective approaches to mammary cancer chemoprevention in rats involve surgical endocrine ablations such as bilateral ovariectomy. However, prophylactic surgical ablations are unlikely to be acceptable to the majority of the general public. All chemicals evaluated to date are less effective, and none has been shown to reduce mammary cancer incidence to zero. As a result, efforts continue to identify chemical agents whose protective activity is comparable to that of endocrine ablation. DHEA is an adrenal steroid with chemopreventive activity in several animal models for human cancer. In the present studies, the chemopreventive efficacy of DHEA was evaluated in rats exposed to the mammary gland carcinogen, *N*-methyl-*N*-nitrosourea (MNU). Groups of 20 female Sprague-Dawley rats were fed an AIN-76A diet supplemented with 0, 400, or 800 mg DHEA per kg diet; one week later, all rats received a single i.p. injection of 35 mg MNU per kg body weight. Animals were palpated weekly to monitor mammary tumor development, and all mammary tumors were histologically confirmed. When administered at 800 mg/kg diet, DHEA reduced