The Mary Lasker Conference, on Growth Factors in Hormone-related Tumors

The Mary Lasker Conferences, sponsored by the American Cancer Society, have the goal of sharing information in a critical cancer research field. The Lasker Conference held in December 1989 focused on the subject of Growth Factors in Hormone-related Tumors and was initiated because of rapid progress in the understanding of growth factors, growth factor receptors, hormone action, and oncogenes. The American Cancer Society brought together leaders in this field to see if current knowledge had opened any new routes to the control or therapy of hormone-related tumors in humans. If not, the organizers challenged the workshop participants to identify the research areas that will provide the knowledge necessary for progress toward these goals.

Participants were requested to bring their knowledge of growth factors, hormones, and differentiating agents related to three main areas: how those substances influence the processes of cell replication and differentiation; how they influence the expression of cell cycle-dependent genes and the assembly of G1 interval protein complexes that, as defined in lower cell systems, play an intrinsic role in the G1 to S-phase transition of the cell cycle; and how they influence the mechanisms that lead to cell replication or to terminal differentiation or cell death in these tumors.

One must note that the replication frequency of mammalian cells is largely controlled by molecular events that occur in the G1 interval of the cell cycle. Whether it is the impact of a growth factor, a hormone, or a unique nutrient, the response is usually one of activating a receptor which in turn activates a chain of reactions that culminates in the triggering of S-phase and DNA/chromatin replication. This trigger appears to involve the activation of genes leading to the synthesis and assembly of the DNA replicase system. While our knowledge of this chain of reactions is still fragmentary for mammalian cells, studies of the cell cycle in yeast and oocyte systems have unearthed certain genes and gene products that are essential to this progression.

While knowledge of the molecular events regulating the triggering of cell replication is essential in order to deal with the human problem of hormone-related tumors, it is equally important to understand the processes that lead to terminal differentiation or cell death in these tumors. An oncogenic event leading to a growth advantage and tumorigenesis frequently leaves the cell with a normal complement of processes for terminal differentiation. The challenge to the therapist is to find ways to superinduce these vestigial potentials. Success in this endeavor could spell the ultimate in selective therapy of cancer.

Biological Factors Controlling Cell Proliferation (Gerald Mueller, M.D., PhD., University of Wisconsin, discussion leader)

Steroid hormones are perhaps the most extensively studied and best understood regulators of cell growth. Depending on the cell type and the nature of the steroid, cell growth or cell death can be implemented with low levels of these agents. While estrogens and androgens have been implicated in growth-promoting responses, it is not known how their receptors stimulate specific cell cycle gene expressions that lead to the initiation of replication. This special area, which promises to be critical, is just beginning to be investigated.

A better understood aspect of steroid effect on cell growth is the destruction or induced terminal differentiation of lymphoid cells by glucocorticoids. With selection techniques, clones of lymphoblasts have been isolated that are either highly sensitive to glucocorticoids or highly resistant. Analyses of the sensitivity have revealed that this response is dependent on the presence of functional glucocorticoid receptors, which in the presence of a glucocorticoid turn on the synthesis of RNA and proteins leading to cell death. In contrast, resistant cells have either a deficiency of glucocorticoid receptors or receptors which have been mutated to a form that does not initiate cell destruction. Since cell death depends on the steroid-induced synthesis of RNA and protein, it was suggested that the mutant receptors are somehow defective in addressing the genes involved in the killing response.

Current experiments are directed towards finding which part of the glucocorticoid receptor is required for the cell death phenomenon and identifying the genes that are responsible. Molecular genetic analysis of the role of the receptor has shown that this activity resides somewhere in the DNA-binding region of the receptor and that it is possible by genetic engineering to convert this region to a functionally constitutive state or to place it under the control of another ligand. The present challenge is to design ways in which the cell-killing mechanisms that are activated by this region of the glucocorticoid receptor can be used selectively in the therapy of cancer.

As E. Brad Thompson, M.D. (University of Texas Medical Center), noted, while the actual cell-killing mechanism is not yet understood, there are a few indications that the regulation of the protooncogene, c-myc, is an important component of the response. Correlated with the glucocorticoid-mediated cell killing is a rapid depression of the myc gene expression as measured by the synthesis of both c-myc mRNA and the protein. Somehow the depressed levels of myc seem to lead to the cell death.

When the c-myc is placed in plasmids containing a glucocorticoid response element and the constructs are transfected into sensitive cells, the induced expression of c-myc by added glucocorticoids actually protects against steroid-induced cell death. Accordingly, it appears that a high level of c-myc correlates with continued growth in the transfected cells whereas a depression of c-myc in the target cells by the steroid leads to cell death and lysis. The continued study of the interaction of glucocorticoid receptors and the regulation of c-myc expression promises to yield new insights into cell life or death in response to what is already an important mode of cancer therapy.

Nuclear Oncogenes and Transcription Factors in Growth Responses. Growth factors interacting with cell surface receptors transduce signals to the cell nucleus which activate the expression of a panel of genes coding for transcription regulating proteins. Examples of such genes are genes coding for c-fos, c-myc, erbA, the jun complex, and others. Of particular interest to the problem of cancer is that these genes constitute the protooncogenes which, on mutation and activation in oncogenic viruses, are capable of transforming normal cells to cancer cells.

Received 5/10/91; accepted 5/22/91.
Accordingly, studies of the synthesis, metabolism, and function of these proteins appear essential to an understanding of growth regulation in both normal and malignant cells. Studies of this type have been carried out in the laboratory of Inder Verma, Ph.D. (Salk Institute for Biologic Studies), using the fos and jun proteins as candidates.

Cell studies have shown that the fos gene expression is turned on very quickly in response to a number of growth stimulants and that in normal cells it quickly returns again to a low expression state. Its expression appears required for the activated expression of other growth-related genes. In fos oncogene (v-fos)-transformed cells, a truncated form of this protein continues to be made in a rather constitutive manner, facilitating a growth response. This distinction in the performance of the c-fos and the v-fos genes has been shown to be due to properties of the proteins themselves. c-fos protein, upon phosphorylation of the carboxyl end of the protein, returns to the fos gene as a suppressive element. v-fos protein, missing the phosphorylation site due to truncation, is unable to downregulate the expression of the fos gene. The downregulation in the normal cell has now been shown to be due to the ability of c-fos and c-jun proteins to form complexes with each other, which in dimeric form combine with the fos gene and prohibit its transcription.

The protein/protein interaction between c-fos and c-jun appears to be mediated via the leucine zipper sequences in the hydrophobic regions of each protein. Interruption of the leucine zipper sequences by molecular genetic approaches shows that the sequences are important in both proteins, because the formation of the protein heterodimers exposes basic sequences on the jun protein for interaction with DNA. In the case of the c-fos gene, the interaction of the heterodimer is inhibitory to transcription, whereas similar interactions with other genes containing the same response element lead to stimulated expression.

The findings from these experiments now provide criteria for the search for other genes in a growth response cascade, ones which play a distinctive role in normal and malignant cell responses to growth factors in their environment. Knowledge of these genes, their interactive proteins, and the functions of the gene products therefore promises to provide new strategies in the development of cancer therapy.

Transforming Growth Factors. In the late 1970s and early 1980s, a set of peptides was isolated from tumor cells that influenced the growth of cells in culture. They were initially referred to as “transforming growth factors” and “tumor growth factors” because they made fibroblasts look transformed in vitro cultures. With time it has become clear that the initial results were due to two classes of these peptides, TGF-α1 and TGF-β. The TGF-α class is produced by many cells, binds to the epidermal growth factor receptor, and stimulates the growth of many cells in an autoregulated manner. The TGF-β family of peptides turns out to be almost exclusively growth inhibitory when tested in defined culture systems.

The TGF-β peptide family is a large family of related peptides, with about 10 subclasses, and is broadly represented in different tissues, species, and interactive growth responses. This family includes such diverse factors as the müllerian inhibitory factor, activins, inhibins, and decapentaplegic factor. All members of this family carry a high level of gene and amino acid sequence homology. In mammals, three forms of TGF-β are produced, but their production varies dramatically from tissue to tissue and in juxtaposed cells in different biological responses.

Produced as a 390-amino acid pre-pro protein, TGF-β is processed to a small active peptide that is initially released as a noncovalent dimer. The latter is inactive until dissociated by an as yet unknown, but apparently regulated, process. The active peptide elicits the TGF-β responses in turn by interacting with a cell surface receptor which may, in fact, be a set of receptors.

There are two hallmarks of TGF-β action: (a) chemotaxis of macrophages, fibroblasts, and epithelial cells at very low concentrations; and (b) the reversible inhibition of growth and differentiation of a wide variety of cells at high levels. The latter response is associated with a remarkable stimulation in the production of extracellular matrix components such as collagens, proteoglycans, fibronectin, and integrin proteins. The accumulation of these entities is also correlated with an increased production of protease inhibitors that may contribute to the response. How the growth inhibition and interruption of cell replication are accomplished is not actually known, but it is interesting that they are not cytotoxic and are reversible on removal of the TGF-β.

As Harold Moses, M.D. (Vanderbilt University), noted, the two parameters of TGF-β actions are revealed in the phenomenon of wound healing. In both the epithelization of wound areas in pigskin and the angiogenesis response of injured chick chorioallantoic membranes, the low levels of TGF-β in the periphery of the lesions appear to stimulate the immigration of epithelial, fibroblastic, and endothelial cells, which on migration respond to the growth-stimulatory action of other, as yet, unknown growth factors. As they replicate and fill up the cell deficiency, the deposition of extracellular matrix in areas of high TGF-β appears to stop growth as the lesion is repaired.

The manner in which high levels of TGF-β block cell replication is not completely known; however, it appears to function in part by the suppression of c-myc expression, thereby preventing cells from passing from the G1 phase of the cell cycle into the DNA replication of the S phase. The way in which c-myc expression is repressed is not known, but presumably it involves an effect of TGF-β on the availability of a properly activated protein for assembly into an inhibitory complex about the c-myc gene. The available information on the interaction of TGF-β with its cell surface receptor and the known structure of response element in the c-myc gene has set the stage for a fundamental explanation of this picture and, it is hoped, for the use of this knowledge for growth restriction in malignant cells.

FGF-related Growth Responses. FGFs, the heparin-binding growth factors, constitute a family of cationic peptides, ranging in molecular weight from 18,000 to 24,000, that are rather ubiquitous in tissues and are potent mitogens for mesenchymal tissue cells. In normal tissues, there are two subclasses of the fibroblastic growth factors, the basic and acidic FGFs. They are highly homologous and are easily isolated by the affinity of their basis segments to heparin affinity columns. There are also FGF-like peptides that derive from oncogenes such as the Int; these products differ from the normal FGFs in having a signal peptide. As a result, the latter can be secreted but is found largely localized to the cell membrane at which site it appears to interact with specific FGF receptors. The oncogene product is highly transforming and cells transfected with an expressible construct of the oncogene form become highly malignant, even in syngeneic animals.
In addition to being potent mitogens for mesenchymal cells, the FGF peptides stimulate neurite outgrowth for neuronal cells and exhibit high chemotactic activity for mesenchymal cells. While these actions are not fully understood, the responses correlate with the induced synthesis and secretion of a M, 92,000 collagenase. The latter digests extracellular matrix components and appears to be responsible for the low affinity of the cells transformed with the basic FGF-like oncogenes to attach to growth surfaces. Inhibition of the protease with protease inhibitors such as TIMP and suramin changes the growth morphology of transformed cells from a loose aggregate form to well-defined monolayers. Such cells soon exhibit growth inhibition whether studied in surface or in suspension cultures.

The observation that the FGF-oncogene-derived peptides localize to a large extent in cell membranes in combination with specific receptors has suggested that the FGF may function in some sort of an internal autocrine circuit; thus, it contrasts with most other secretable oncogene-derived growth factors. Michael Klagsbrun, Ph.D. (Harvard Medical School), suggested that the repeated interaction of the oncogen-derived FGF with the cell surface (albeit inside surface) is required for perpetuation of the mitogenic response. In the presence of an agent such as suramin, FGF binding to the receptors is blocked; this appears to account for the growth inhibition of this new class of cancer therapeutics. In fact, sensitivity to suramin seems to distinguish two oncogenic states, the RAS/NEU-dependent transformations versus the FGF-like oncogene transformations.

The intriguing observation in these studies is that FGF-like oncogenic states, operating through an internal autocrine loop-regulated transformation, can be reverted to normal growth character by levels of suramin that interrupt the FGF/receptors interaction.

It is of considerable fundamental interest that FGF and TGF-β have opposing actions in vivo and thereby provide a possible mechanism for homeostasis in tissue growth responses. The available data from a spectrum of cancer states suggest that derangements in either metabolic area can contribute to the malignant properties of oncogenically transformed cells.

**Biological Factors Controlling Cell Proliferation (Harold Moses, Vanderbilt University, discussion leader)**

To date, three oncogenes derived from growth factor receptors have been shown to play a role in human neoplasia. They include the EGF receptor (also known as c-erbB), its close relative, the erbB2 gene, and the trk protooncogene. Several other genes encoding cell surface receptors are also known to acquire transforming properties when either mutated, overexpressed, or submitted to ectopic expression. Most of these genes, including 

\[ \text{fms (colony-stimulating factor-1 receptor), } k\text{ir}, \text{ros, met, ret, and trkB}, \]

are members of the tyrosine protein kinase family. Others, such as the mas oncogene and the 5HT1C serotonin receptor, are members of the G protein-coupled receptor family prototyped by the β-adrenergic receptor which is characterized by the possession of seven transmembrane domains. Accumulating evidence suggests that some of these receptors (e.g., erbB2 in mammary carcinomas) may induce neoplasia by improper stimulation of normal signal transduction pathways. In contrast, other oncogenes such as trk are likely to induce transformation by interacting with nonphysiological substrates. Strategies to attempt to interfere with the neoplastic properties of any of these oncogenes will have to take into consideration these properties. For instance, in the case of erbB2, approaches leading to the downmodulation of the receptor may prove useful. However, in the case of trk, it might be possible to design specific inhibitors that will block the oncogene without affecting protooncogene function. Unfortunately, either approach will require detailed knowledge of the signal transduction pathways in their normal and mutated stages.

Mariano Barbacid, Ph.D. (National Cancer Institute), described the molecular structure and mechanism of activation of the trk and trkB tyrosine kinase cell surface receptors. Thus far, all trk oncogenes found in human tumors have had their extracellular domain replaced by unrelated sequences (non-muscle tropomyosin sequences in at least two independent isolates). Similarly, a series of in vitro-generated trk and trkB oncogenes also present gross alterations in their respective extracellular domains. However, Dr. Barbacid’s laboratory observed that trk can become activated by more subtle mutations, including short deletions and single point mutations. Expressions of the trk and trkB protooncogenes appear to be limited to neural cells. For instance, trk is exclusively expressed in trigeminal and dorsal root ganglia. In contrast, trkB transcripts have been found in many structures within the peripheral and central nervous systems. In addition, trkB appears to code for at least two types of molecules, a classical tyrosine protein kinase transmembrane receptor and a shorter molecule containing the extracellular and transmembrane domains, but not the kinase catalytic region.

**The Rb Tumor Suppressor Gene.** The Rb gene was first identified through its involvement in retinoblastoma. Inactivation of one gene copy in the conceptus predisposes to retinoblastoma. In such genetically afflicted children, tumors arise when the surviving gene copy is lost somatically in one or another retinal cell. In contrast to this “familial” (also termed “germinal”) disease, a sporadic form of retinoblastoma occurs through somatic loss of both Rb gene copies. All this suggests that the normal version of the Rb protein acts to retard or constrain cell proliferation. The gene encodes a M, 105,000 nuclear phosphoprotein that binds to DNA and may act as a regulator of transcription or replication. The gene is inactivated in other types of tumors, including small cell lung carcinomas, sarcomas, and bladder carcinomas.

Tyler Jacks, of Robert Weinberg’s laboratory (Whitehead Institute), noted that the Rb-encoded protein appears to sit at a central point in the growth regulatory pathways of the cell. The oncoproteins encoded by three different DNA tumor viruses are found complexed with p105-Rb. Their transforming powers would seem to be due, at least in part, to the ability of the oncoproteins to complex the Rb protein in a manner that inactivates it, thereby achieving a phenocopy of the state seen in cells that have lost Rb function due to gene inactivation.

**New Therapeutic Strategies in the Control of Malignant Growth.** Receptors on cell surface membranes for polypeptide growth factors are often overexpressed on solid tumors and may be involved in autocrine/paracrine stimulation of tumor growth. The laboratory of John Mendelsohn, M.D. (Memorial Sloan-Kettering), has produced mAbs 225 IgG1 and 528 IgG2a against the human receptor for EGF, which can inhibit binding of EGF and TGF-α to the receptor and prevent activation of receptor tyrosine protein kinase activity. These mAbs can inhibit EGF and TGF-α-dependent proliferation of normal and malignant human cell lines. Furthermore, the growth of a number of cell lines, particularly those expressing elevated EGF receptors, is directly inhibited by antireceptor mAb, both in...
culture and in xenografts. Apparently, the mechanism of this inhibition is related to blocking of an autocrine loop. Antibodies labeled with $^{111}$In can selectively concentrate in and image xenografts bearing high numbers of EGF receptors.

A Phase I clinical trial with $^{111}$In-225 IgG1 was carried out in patients with advanced squamous lung cancer, which invariably expresses high levels of EGF receptors. $^{111}$In-225 (4 mg; 5 mCi) was administered by itself or coinfused with 16, 36, 116, or 296 mg of unlabeled mAb in groups of three patients each. No toxicity occurred at any dose level. Tumor imaging was observed in all but 1 of 10 patients receiving more than 20 mg mAb and was optimal 3 days after injection. Significant liver imaging was also observed. Serum clearance of mAb was dose related. At a dose of 120 mg, mean serum levels were maintained at greater than 10% ID/liter for greater than 24 h, and the distribution of $^{111}$In-225 to the tumor site reached 2% ID at 72 h, compared with 28% ID to the liver, and 77% ID in the whole body. All patients produced human anti-mouse antibodies, foreshadowing a pressing need for nonantigenic or human type antibodies for effective repeat therapy of such malignancies. The preliminary studies show, however, that 225 IgG1 mAb against the EGF receptor can be administered safely in the dose and schedule studies and that it can localize squamous lung cancer, a tumor known to express elevated levels of receptor.

Control of Invasion and Metastasis: A Parameter of Tumor Growth. Metastasis is the process by which tumor cells escape from the original lesion, spread throughout the body, and colonize and proliferate in distant sites. The spread of cancer is one of the major causes of morbidity in cancer patients, since it increases the tumor burden, destroys the function of important organs, and is associated with the appearance of an even more aggressive and drug-resistant population of tumor cells. Tumors, when they arise, are composed of benign cells which proliferate uncontrollably but do not have the capacity to metastasize. Subsequently, a population of malignant cells appears in the tumor, probably due to the activation of additional oncogenes or other chromosomal changes. Metastasis of tumor cells has been found to be a complex multistep process which consists of the escape of tumor cells from the original lesion, dissemination of these cells via the vasculature and lymphatics, arrest and invasion in target tissues, and proliferation to form new lesions.

Current concepts suggest that the steps by which tumor cells metastasize are not random and that different types of cancer cells utilize similar mechanisms. It is the purpose of research on tumor cell metastasis to identify the specific cellular and molecular activities used by the malignant tumor cells, to model these activities in vitro and in vivo, and to use this information to screen for drugs that are antimetastatic.

George Martin, Ph.D. (National Institute of Aging Gerontology Research Center), and other researchers are particularly interested in the mechanisms by which circulating metastatic tumor cells identify their target tissue, arrest at the site, and invade through tissue barriers. His studies suggest that this is a concerted process, almost a cascade, initiated when the tumor cells encounter the capillary beds in their target tissue. It is important to understand that tumor cells do not have free access to tissues. Rather blood vessels, muscles, peripheral nerves, gland, and other epithelial tissues are completely encircled by solid extracellular sheets known as basement membranes. Basement membranes provide physical support to the cells and tissues that they surround and maintain the separation of different types of cells. The membranes include collagen IV, laminin, and heparin sulfate proteoglycan. Collagen IV is actually the major structural framework of basement membranes, interwoven with laminin and heparan sulfate proteoglycan.

Malignant cells, but not benign, have the capacity to degrade and to invade through these basement membrane barriers and then grow into surrounding tissue. Still another basement membrane barrier must be crossed when the cancer cells reach the blood vessels and lymphatics to become blood borne.

Dr. Martin's laboratory has devised in vitro systems which have allowed him to model the interaction of tumor cells with such basement membranes. These studies have shown a good correlation between the behavior of the tumor cells in vitro and their metastatic activities in vivo. In such studies, malignant tumor cells bind avidly to basement membranes via laminin which in turn induces the production of an enzyme, collagenase IV, that is mainly responsible for the degradation of the membrane.

The initial interaction of the tumor cells with the basement membrane involves a "metastasis-associated receptor" for laminin on the tumor cell surface. Some studies suggest that this receptor recognizes and binds to a sequence of five amino acids on one of the laminin chains, since cyclic peptides bearing the sequence tyrosine-isoleucine-glycine-serine-arginine inhibit the interaction of the cells with laminin and reduce the number of metastatic lesions in mice given i.v. injections of tumor cells; subsequent binding of the cells to a second site in laminin induces collagenase IV production. This activity of laminin has also been duplicated in small synthetic peptides, and such biologically active peptides could lead to therapeutically useful agents.

Role of Growth Factors in Breast Cancer (Robert B. Dickson, Ph.D., Lombardi Cancer Center, Georgetown University, discussion leader)

The session on the role of growth factors in breast cancer pointed to the need for a coherent view of the pathophysiology of the disease.

Kenneth S. McCarty, Jr., M.D., Ph.D. (Duke), provided the overview, emphasizing pathology and diagnosis of breast cancer. To understand the changes in the malignant state, an appreciation of the normal adult breast is required. It is also important that the breast undergoes cyclic changes in morphology during the follicular and luteal phases of the estrous cycle.

With epithelial hyperplasias, however, there is an associated risk of developing breast cancer, whether of lobular or of ductal origin. A first order family history combined with such hyperplasias increases the cancer risk to 4-fold. The presence of nuclear or cellular atypia markedly increases the risk to 11-fold if it is also associated with family history.

Cystic changes, which are not associated with increased risk for cancer, occur when secretion is greater than resorption. Sclerosing adenosis, like the proliferation of myoepithelial cells, is also not associated with risk or malignancy.

Breast cancer is further classified as in situ or invasive. In situ lobular carcinoma is often multicentric and multifocal; it retains an intact basal lamina. Invasive breast cancer may retain differentiated morphology and tissue features in both the primary and metastatic sites.

Survival is 70–75% at 10 years for node-negative patients but less than 60% for node-positive patients. The mean age of initial observation of the atypical hyperplasia usually precedes invasive breast cancer by 10 years.
Amplification and Expression of Protooncogenes as Prognosticators of Mammary Cell Growth. Dennis Slamon, M.D., Ph.D. (UCLA), introduced one of the most promising new markers of breast malignancy, an increased level of the oncogene HER-2, or erbB2. The protooncogene codes for EGF receptor. An elevated level of HER-2 expression is associated with poor prognosis regardless of tumor estrogen receptor status. Over-expression of HER-2 is due, in many cases, to gene amplification. One study shows that coexpression of EGF receptor and HER-2 in breast tumors is associated with a poorer prognosis than for tumors expressing either entity. The search for the HER-2 ligand(s) still remains an elusive area of investigation.

Growth Factor Control of Normal and Malignant Mammary Cell Growth. Dr. Dickson addressed the role of growth factors and their receptors in the growth and differentiation of normal and transformed human mammary epithelial cells. He reported that TGF-α, a functional homologue of EGF, and its receptor are frequently synthesized in high amounts by mammary epithelial cells. The system tends to act in an autocrine manner, stimulating its own growth. The TGF-α-EGF receptor system also appears to be critical in the proliferation of normal human mammary epithelial cells. To date, however, no evidence exists to implicate the closely related HER-2 products in the proliferative processes that work in the normal human mammary epithelial cell. Its biological functions remain to be determined.

In addition, another locally acting but inhibitory growth factor, TGF-β, is also synthesized by human mammary epithelial cells. This growth factor inhibits epithelial proliferation, promotes basement membrane synthesis, and appears to promote differentiated function. A balance of TGF-α and TGF-β and their receptors may be involved in control of epithelial proliferation in all situations, normal, premalignant, or malignant mammary epithelial cells. Using a series of carcinogen- and oncogene-transformed human mammary epithelial cells, it can be demonstrated that full malignant transformation attenuates the function of both TGF-α and TGF-β in the mammary epithelial cells.

Design and Use of Transgenic Systems for Studying Breast Cancer. Bernd Groner, Ph.D. (Friedrich Miescher Institute; Basel, Switzerland), continued the theme of growth factor/oncogene interactions in malignant transformation. Experiments were carried out with continuous but untransformed cultures of mouse mammary epithelial cells. EGF was required for proliferation and supported the subsequent induction of the milk casein gene when treated with glucocorticoids and prolactin. Nuclear factors associated with tissue specificity and hormonal derepression of the latter genes have been identified. Malignant transformation of the cells with v-raf oncogene or the growth factor TGF-α was associated with loss of differentiated function (casein expression) while transformation of the cells with HER-2 oncogene did not compromise the differentiated status.

Antiestrogens in Control of Breast Cancer. V. Craig Jordan, Ph.D., D.Sc. (University of Wisconsin, Madison), discussed the roles of antiestrogens and hormonal resistance in breast cancer therapeutic responses. Estrogens and antiestrogens alter growth factor synthesis in hormone-dependent human breast cancer cells, but the mechanism(s) of antiestrogen resistance appears not to be associated with loss of growth factor regulation. An interesting in vivo model of antiestrogen resistance was presented which demonstrated that MCF-7 human breast cancer cells implanted in the nude mouse could begin to recognize the mild or weak estrogenic character of the antiestrogen tamoxifen with prolonged treatment. The tamoxifen-maintained tumor growth could be abrogated by treatment with a fully estrogenic compound that appears to neutralize receptor function.

Role of Growth Factors in Endometrial Cancer (Saul B. Gusberg, M.D., Mt. Sinai School of Medicine, discussion leader)

In introducing the subject, Dr. Gusberg, reviewed the history of human endometrial cancer, which has become the most common cancer of the female genital tract yet has the lowest mortality. He noted that many years ago, scientists learned that the onset of such cancers was gradual rather than cataclysmic, that steroid hormones have a powerful effect on target tissues, and that an endometrial change, adenomatous hyperplasia, bore a constant relationship to later invasive endometrial cancer. Adenomatous hyperplasia can appear at any time in a woman's reproductive life, most commonly in the perimenopausal era and in other circumstances when ovulation fails and the endometrium is stimulated by estrogen without progesterational modification. Physicians were led to this conclusion by the simple epidemiological facts that high risk for endometrial cancer included infertility due to the failure of ovulation, obesity, dysfunctional bleeding of menopause, prolonged estrogen administration, estradiol-producing granulosa-theca cell tumors of the ovary, and the cystic ovary syndrome in young women. That these same factors were operative in the development of adenomatous hyperplasia suggested that the proliferative mitogenic property of estradiol could escape from the more usual differentiating effect of progesterone.

Studies into estradiol and progesterone receptors allowed scientists to realize the capacity for proliferation of the postmenopausal endometrium and a means to arrest the growth with progestins. This knowledge translated into therapy for adenomatous hyperplasia and certain types of endometrial cancer. Double isotope dilution methods permitted better understanding of steroid metabolism in target cells; the cofactor status of obesity as a high risk factor was clarified by observations concerning the peripheral aromatization of the adrenal androgens androstenedione to estrone in postmenopausal women. The correlation of the level of conversion to estrone with the degree of obesity demonstrated that the production rate of estrogen in these grossly obese subjects was significant.

In the 1970s, more sophisticated epidemiology methods examined this tumor and rediscovered its hormonal milieu in approximately 50-60% of cases. Case-control studies, frequently in retirement communities in the United States where postmenopausal subjects on long-term estrogen medication were readily found, revealed that these women on such a regimen suffered a 4-14-fold relative risk of developing endometrial cancer over age-matched controls. The corollary to this picture of hormone relatedness lies in the more virulent, readily invasive, metastasizing, more aneuploid tumors, fortunately a minority, that seem to be autonomous.

Steroid Hormone Metabolism Correlates. Erlio Gurbide, Ph.D. (Mt. Sinai School of Medicine), discussed estradiol metabolism in target cells, with hormonally regulated cyclicity of estrogen receptor levels and estradiol-metabolizing enzymes in endometrial cells. He indicated that estradiol 17β-dehydrogenase and sulfotransferase, progesterone-regulated enzymes, are involved in human endometrial metabolism; the dehydrogenase partially inactivates estradiol by formation of estrone, a steroid with low affinity for the estrogen receptor. The sulfokinase also
leads to inactivation of estradiol by formation of 3-sulfate which does not bind to the estrogen receptor.

In another type of metabolism of estradiol in endometrial cancer cells, the C-17 position becomes esterified with a fatty acid; the latter can accumulate in the cells forming an intracellular reservoir, from which hormone can be released when hormone production is reduced. Aryl sulfatase in target cells can also regulate the hormonal milieu by reversing sulfation and forming estrone from circulating estrone sulfate. In addition, antiestrogens, aromatase inhibitors, and progestins used in breast and endometrial cancer therapy alter the milieu.

Endocrine Factors in Regulation of Growth and Differentiation of Endometrial Tissue. John McLachlan, Ph.D. (National Institute of Environmental Health Studies), noted that while estrogens as chemical agents have been associated with the induction of neoplasia in their target sites, the mechanisms underlying this action are not known. It has been demonstrated that immature murine endometrium is a sensitive estrogen target in that neonatal mice treated with diethylstilbestrol develop uterine adenocarcinoma in almost 100% of the animals. This response is not seen, however, when such treatment is given after puberty. The epithelium of the newborn mouse is heterogeneous with respect to the presence of the estrogen receptor but homogeneous for EGF receptors. The additional presence of EGF receptor, proEGF peptide, and pre-proEGF mRNA in the uterus suggests that these target tissues may utilize this class of growth factors as a substitute for estrogenic stimuli. In accord with this view, Dr. McLachlan demonstrated that EGF implants can stimulate multiple estrogen-like end points in the murine reproductive tract.

Protooncogenes in Endometrial Growth. George Stancel, Ph.D. (University of Texas Medical School), discussed protooncogene expression in endometrial growth. After administration of a physiological dose of estradiol to immature female rats, a large increase in c-fos mRNA occurs; this response was hormone specific and dose dependent. A 5'-flanking sequence of c-fos confers estrogen responsiveness to reporter gene constructs transfected into GH4 cells. In addition to c-fos, estrogen treatment in vivo causes a rapid increase in c-jun and junB transcript levels. Following the initial increase in the transcription of uterine c-fos after estrogen, the level falls to baseline in 6–12 h and remains refractory to further treatment with an estrogen until the uterine cells undergo a round of DNA replication. This finding suggests that uterine cells have an estrogen receptor-mediated mechanism to both initiate and then limit c-fos expression to the G1 interval of the cell cycle.

Investigating protooncogene expression in two animal models for hormonal carcinogenesis, he also found c-fos and c-myc expression to be elevated 7–9 months after chronic estrogen treatment in the Syrian hamster and c-fos to be elevated 4–5-fold at 30 days of age after neonatal diethylstilbestrol treatment in female rats. He further noted that progesterone diminished c-fos expression after induction with estradiol but did not block JUN expression.

Müllerian Inhibiting Substance. Patricia Donahoe, M.D. (Massachusetts General Hospital), presented data concerning the gene structure, regulation, and mechanism of action of MIS. This substance, which is a fetal regressor or negative growth regulator, appears to block the action of growth factors, acting constitutively at certain developmental levels. MIS action is inhibited in turn by estrogen and stimulated by progesterone and androgens. Furthermore, MIS is inhibited by EGF, apparently limiting the tyrosine phosphorylation by this system. MIS is to be compared with other members of this gene family such as TGF-β and the newly discovered homologous protein that functions as an insulin tyrosine kinase inhibitor.

The observation that MIS can inhibit tumors arising embryologically from müllerian tissue prompted Dr. Donahoe to suggest the possible use of MIS as a chemotherapeutic agent in such cancers as ovarian carcinomas.

Role of Growth Factors in the Prostatic Cancer Problem (Donald Coffey, Ph.D., Johns Hopkins University, discussion leader)

The prostate is an important gland in the male body, but it is susceptible to disease and infection. For example, benign prostatic hypertrophy, with its urinary obstructive effects, occurs in 30–50% of men over 50 years of age. In addition, clinically diagnosed prostate cancer is the primary cancer diagnosed in men in the United States and the second cause of cancer death in males. In spite of its prevalence, however, prostate cancer is not well understood. Interestingly, prostate cancer occurs only in humans and dogs (the London zoo, which has kept autopsies reports on many animals since the 1700s, shows no record of this disease in other animals).

Dr. Coffey, Ph.D. in his presentation on prostate cancer, noted that the driving force in the development of prostate tumor cell heterogeneity appears to be an inherent genetic instability in this tissue. However, it is unknown as to where this instability rises: is it an instability of the DNA/chromatin itself or some unique metabolic hazard present in this tissue? He noted that cancer cells appear characteristically unstable, as reflected in their DNA, the function of their genes, and their cytological structure. He raised the intriguing point that cancer is usually diagnosed by looking at the cells and seeing their abnormal shapes, i.e., a reflection of the instability in structure and a product of genetic instability. Can it possibly be the other way around, that structural instability gives rise to genetic instability?

This linking of structural instability to genetic instability is based on the concept of “tensegrity,” a word which architect/designer Buckminster Fuller coined to show how 3-dimensional structure can be achieved by the linking of compression-resistant elements by tension cables. In his view, humans are examples of such tensegrity; i.e., bones, which are discontinuous compression-resistant elements, are linked together by tension cables (ligaments and muscles). Cells are also examples of such tensegrity because of their different formed elements linked together by microfilament units. This can be seen during metaphase, where the microtubules are the compression elements and the actin and myosin, which anchor the microtubules, provide the tension cables or elements.

Applying this concept of tensegrity to the cancer cell could show that any tweaking of the structure would relay effects throughout the cell. Perturbations to the structure, for example, could be turned on with growth factors and other oncogene products. For example, nuclear oncogenes, such as myc, acting at the nuclear matrix, could trigger a progression of events leading to structural alterations, DNA instability, tumor cell heterogeneity, and finally, immortalization. Other oncogene products acting at the cytoskeleton and plasma membrane, such as ras, could alter cell motility and give rise to metastases.

Steroid-sensitive and -insensitive Proliferation of Tumors. Progression from the steroid-sensitive to -insensitive state of tumors occurs by multiple pathways, but a general feature of that
progression is the accompanying increased growth rate. The question of how increased growth might be achieved was discussed by Roger King, Ph.D. (Imperial Cancer Research Fund Laboratories), in relation to steroid receptor function and growth factor activity. Loss of steroid receptor function as a causal event in progression could most easily be explained by a derepression model in which basal inhibitory pathways are relieved by a ligand deficit in responsive cells or receptor loss in unresponsive cells. Alternatively, progression could be a consequence of receptor mutation resulting in a constitutively inactive protein. In either situation, the actual molecular events and their biological effects remain to be elucidated.

Oncogene Interaction in Prostatic Carcinoma. Previous studies utilizing the mouse prostate reconstitution model system have shown that the ras and myc oncogenes synergize to induce poorly differentiated prostatic adenocarcinomas. Proof of this view has been achieved through the introduction of these two cooperating oncogenes via a nonreplicating recombinant retrovirus vector (Zipras/myc 9) into both the epithelial and mesenchymal cells, respectively, of the mouse urogenital sinus and following their subsequent growth in vivo for 4 weeks as renal subcapsular grafts. In this system, the virus produces only stable integrations of the proviral DNA in the genome of the primarily infected cells and their progeny, without giving rise to subsequent spread of the virus to neighboring cells. Using this system, Timothy Thompson, Ph.D. (Veteran’s Medical Center, Houston, TX), reported experimental results which suggest that the induction of carcinogenesis by this protocol is strain specific and, further, that other tissue components contribute as well to the final normal versus malignant state.

With clean separations of the urogenital sinus epithelium from the urogenital sinus mesenchyme, Dr. Thompson’s group was able to introduce ras and myc alternatively into the epithelial or mesenchymal compartments exclusively.

Dr. Thompson’s preliminary data suggest that cancer induction is subject to differences in both the epithelial and mesenchymal components of the urogenital sinus. Whereas BALB/c mice are prone to respond to this protocol by producing only epithelial hyperplasia, C57BL/6 mice exhibit progressive malignant prostatic adenocarcinomas. Interestingly, these genetic differences leading to prostate cancer can be overridden when the two oncogenes are selectively introduced into only the urogenital sinus epithelium. Under these conditions, both strains produce epithelial hyperplasia. Dr. Thompson’s group plans to identify what it is in each tissue type that is essential for the malignant response.

Cell Proliferation versus Cell Death in Prostatic Cancer. Metastatic prostatic cancer is an androgen-promoted fatal disease for which no therapy is available which effectively increases survival. The major reason for the inability of androgen ablation monotherapy to increase survival in men with metastatic prostatic cancer is that the cancer within an individual patient is always heterogeneously composed of clones of both androgen-dependent and androgen-independent prostatic cancer cells, even before therapy is initiated. Thus, androgen ablation, while resulting in a transitory slowing of growth, does not affect the final outcome because the preexisting androgen-independent cancer cells are already present and rapidly overgrow the scene.

To overcome this dilemma, new therapeutic approaches to control androgen-independent prostatic cancer cells are required.

Because the growth of a cancer is determined not only by the rate of cell proliferation but also by the rate of cell death, John Isaacs, M.D. (Johns Hopkins), has focused upon developing methods of increasing the rate of cell death, strategies that work specifically in G0 prostatic cancer cells. To investigate this area, advantage is taken of the fact that in the normal prostate, large numbers of G0 prostatic cells can be induced reproducibly to die by androgen ablation. Studies of the mechanisms involved in these induced cell death responses, especially those occurring in G0 in the normal rat prostate following castration, are under study. The data thus far point to a cascade of specific biochemical progression of events which lead to the programmed death of the androgen-dependent cells.

Further studies have demonstrated that human prostatic cancer cells, even though oncogenically modified, retain the ability to respond to androgen ablation by activating the programmed cell death pathway. In addition, it has been demonstrated that even androgen-independent prostatic cancer cells retain the striking potential for activating this pathway leading to programmed cell death. A major difference exists, however, in that the pathway is no longer activated by androgen ablation. The long term goal of Dr. Isaacs’ work is to develop some type of non-androgen ablative protocol that can superactivate this programmed cell death cascade in androgen-independent prostatic cancer cells. In order to have any realistic chance of success, a great deal of additional basic information will have to be obtained.

Gerald C. Mueller
McArdle Laboratory for Cancer Research
University of Wisconsin
Madison, WI 53706

Saul B. Gusberg
American Cancer Society
New York, NY 10036

Amy Stone
American Cancer Society
Atlanta, GA 30329
Cancer Research

The Mary Lasker Conference, on Growth Factors in Hormone-related Tumors
Gerald C. Mueller, Saul B. Gusberg and Amy Stone


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/51/15/4114.citation

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.