Effects of Various Hormones on Human Carcinoma Cell Proliferation

Y.L. GAO*, B.S. LEUNG**, A.H. POTTER***, W.C.Y. Yu****

Abstract — Cell proliferation of a mammary adenocarcinoma cell line, CAMA-1, is affected by a number of steroid hormones and prolactin in long-term cultures. Estrogenic compounds stimulate cell growth while antiestrogenic compounds inhibit it. Prolactin can synergise the estrogenic effect. Progesterone, glucocorticoid or androgen reduce cell proliferation. Dihydrotestosterone is a very potent inhibitor, effective at as low as 10 nM, and its effect is independent of estrogen action. Cortisol is a less effective inhibitor at concentrations below 10 nM; at these levels cortisol exhibits a slight inhibition which appears to be unrelated to estrogenic action. However, at higher concentrations, cortisol markedly reduces cell division and drastically diminishes the stimulatory effect of estrogen on cell growth. The precise mechanisms of hormone actions on cell proliferation remain to be explored.

Breast cancers of both humans and animals are affected by many hormones (Leung, 1978, 1981). Since the studies by Huggins and his co-workers (1962) both additive and ablative hormonal therapies have been extensively used for the treatment of disseminated breast carcinoma. A number of carcinogen-induced mammary tumor models have been developed in animals for the study of single and combined hormone effects on tumor growth and cellular events (Leung 1978, Leung, 1982). Despite the obvious merits and information generated from these studies, the exact primary or secondary effect of a particular hormone cannot be differentiated because of the complex hormonal environment in the intact animal. It is known that regulatory control mechanisms exist to govern the secretion of gonadal and pituitary hormones. These feed-back mechanisms are invariably perturbed by the alteration of serum hormone levels via exogenous administration of either gonadal or pituitary hormones or by the deletion of any one of them through endocrine gland ablation. For this reason, the removal of estrogen by ovariectomy plus adrenalectomy provides essentially the same palliative benefits for mammary carcinoma patients as those subjected to hypophysectomy. Similar observations were reported in animal models. Furthermore, it has become evident that several hormones interact to achieve a particular effect. Some of these hormones, such as prolactin, insulin or thyroid hormones, are permissive in their action; that is, only suboptimal levels need to be present to permit the full expression of another hormone, e.g. estrogen. Therefore, unequivocal demonstration of the discrete role of a particular hormone or the combined effects of several hormones is difficult to achieve. In recent years, a number of laboratories were successful in obtaining hormone-responsive cell lines for investigating the effects of different hormones in vitro. Although cell culture experiments have many limitations, the advantages are also obvious: cells can be cultured in chemically defined media so that uncertainties due to hormone feed-back conditions in vivo can be prevented.

Estrogen, progesterone and prolactin are three major hormones that affect the growth of mammary tumors induced in rodents by the administration of carcinogen (Huggins et al., 1962, Leung, 1978, and Leung, 1982). Similarly, exacerbation of mammary tumor growth in patients by low levels of estrogen has been documented. Pharmacological levels of progesterone, glucocorticoid, androgen or estrogen inhibit experimental tumor growth and are often used for hormonal therapy in disseminated breast cancer (McGuire et al., 1975). There is very little information in the literature indicating prolactin involvement in human breast cancer. For the past several years, investigations on the role of an individual hormone, and the interactions of different hormones, were conducted in this laboratory by the use of a human adenocarcinoma cell line, CAMA-1, growing in long term culture (Leung, 1978, Leung et al., 1981, 1982 and Yu et al., 1981). This communication addresses the synergistic or antagonistic effects of prolactin, progesterone, androgen and glucocorticoid with estrogen on cell growth.

Description of the cell culture

CAMA-1 originated from the malignant pleural effusion of a post-menopausal woman with adenocarcinoma of the breast (Leung et al., 1981). These cells have been maintained and cultivated as monolayers in minimum essential medium (MEM) with Earle’s salts and supplemented with 2mM L-glutamine, penicillin (100 units/ml), streptomycin (100 micrograms/ml), and 25 percent FBS. Cells were plated routinely in MEM at a density of 10^5 cells in a T-75 flask, or 5 x 10^5 cells in T-25 flask, without further change of medium for 7 days, and were incubated in humidified atmosphere of 95 percent air -5 percent CO2 at 37 degrees. At the end of 7 days, cells were lifted from the surface of the flask by trysin-EDTA (0.025 percent) in Hank’s balanced salt solution with calcium and magnesium. All
cultures were supplemented with 1 nM estradiol. For experimental media, endogenous hormones were removed from serum using dextran-coated charcoal (DC) to absorb steroids and other components from FBS (Leung, et al., 1981). This serum (DC-FBS) was used for the determination of hormonal effects on cell proliferation during culture. Hormones were added during plating as indicated in each experimental flask were plated with 5 x 10^4 cells.

Due to a significant level of peptide hormones present in serum, a serum substitute composed of vitamins, phospholipids and various defined nutrients (Leung, et al., 1981) was prepared to study the effects of prolactin and other peptide hormones on cell proliferation. Uptake of thymidine by 2-hr pulse labeling has been detailed in a previous publication (Yu et al., 1981).

**Effect of estrogen on cell proliferation**

The effects of estrogen in stimulating cell proliferation in DC-FBS have been studied extensively in this laboratory (Leung, et al., 1981, 1982; Yu et al., 1981). The addition of estradiol to culture medium accelerated cell growth as compared with controls (no estradiol). A subline of CAMA-1 which was continuously passaged in FBS in the absence of added estradiol exhibited an apparent partial loss of response to estradiol-induced thymidine (TDR) uptake. Thus, the CAMA-1IR subline which was supplemented with 1nM estradiol retained a mean of an approximately two-fold increase in TDR uptake in response to estradiol stimulation while the IN subline, without added estradiol in culture, was refractory. These two sublines exhibit different growth rates and levels of steroid receptors, several researchers have indicated. However, both sublines retained their ability to respond to estradiol in the induction of progesterone receptor. These effects of estradiol on cell proliferation and cellular events are dose-dependent and are reproducible.

The effects of other estrogens and antagonists on cell proliferation were investigated. As shown in Fig. 1, the classical estrogens, such as estradiol, estrone, 17-beta estradiol and synthetic estrogen-like compounds such as diethylstilbestrol were all effective in stimulating cell growth. Nevertheless, 17-alpha estradiol, which is known to be antagonistic to many estrogen-initiated cellular events, markedly inhibited cell growth.

**Effect of other steroids noted**

Both progesterone and dihydrotestosterone (DHT) were very effective in inhibiting cell proliferation (Fig. 1). These results are consistent with clinical observations where patients with hormone responsive mammary tumors respond objectively to adjuvant therapy with progestin or androgenic drugs. At dosages as low as 10 nM, DHT can exert a marked inhibition of cell growth in comparison with controls (Fig. 2). In the presence of estradiol, cell numbers during harvest were consistently higher than cells plated in its absence. The degree of inhibition by DHT in estradiol-treated cultures is not different from that in estradiol-free controls. These data suggest that DHT may be acting through a mechanism different from that of estradiol, which accelerates cell proliferation. DHT levels higher than 10 nM did not cause greater inhibition, regardless of whether estradiol was added to the culture medium.

Producing responses similar to the clinical effects of progesterone and DHT, glucocorticoids are effective drugs for palliating hormone-sensitive mammary tumors. Cortisol showed little inhibition at low levels (10 pM to 1 nM) but was very effective in reducing cell growth at levels higher than 10 nM (Fig. 3). Cell proliferation was accelerated when both estradiol and cortisol were added to the cultures. High levels of cortisol induced a more pronounced inhibitory effect on estradiol-treated cells. Therefore, it appears that the slight inhibition induced by cortisol is not estradiol mediated. However, at higher levels, cortisol may also turn off the estradiol stimulatory mechanism.

**Effects of prolactin**

When cultured in the absence of serum, CAMA-1 cells do not proliferate and cellular uptake of TDR is very low, presumably due to the lack of adequate nutrients and growth-promoting factors. The effect of estradiol on TDR uptake is dependent on the proportion of serum added to the medium; in 10 percent DC-FBS, estradiol stimulates a two-fold or more TDR uptake above the level of controls without estradiol. This stimulation is not observed in a 1 percent serum substitute (SS), or at best, is minimally observed with 10 percent SS plus 1 percent DC-FBS (Leung, et al., 1981). The addition of prolactin to estradiol-treated cultures in the 10 percent SS alone or 10 percent SS plus 1 percent DC-FBS markedly increases TDR uptake over controls (without hormone) or with either estradiol or prolactin alone. However, in 10 percent DC-FBS, prolactin suppresses estradiol stimulation.

The effect of prolactin and estradiol on cell proliferation was studied in 10 percent DC-FBS and in 10 percent SS plus 1 percent DC-FBS (Leung, et al., 1981). Without exogenous hormones, CAMA-1 cells proliferate better in 10 percent SS plus 1 percent DC-FBS than in 10 percent DC-FBS. While estradiol
Mechanism of steroid hormone action

Not all breast cancers in humans and animals are hormone-sensitive. Although extensive studies have been undertaken in the past three decades regarding the roles of steroid hormones in proliferation, little is known concerning the mechanism of action of an individual hormone, let alone the mechanism of their interactions. Recently, abundant evidence (Clark and Peck, 1979) has accumulated to show that the initial action of a steroid hormone in its target tissues is to bind to a specific protein molecule, termed a receptor. The steroid-receptor complex in the cytoplasm undergoes transformation or activation immediately following interaction with the specific steroid ligand. This complex then moves into the nucleus and interacts with chromatin materials resulting in the initiation of gene transcription and ultimately, protein synthesis, cellular functions and differentiation of the tissue. Receptors for estrogen, progesterone, androgen and glucocorticoids are present in some mammary cells. Recently, it has been documented (McGuire et al., 1978 and Cancer Supplement, 1980) that the presence of estrogen receptors and progesterone receptors in mammary carcinoma is the best single index, independent of any clinical information, for predicting the objective response of a patient to hormone therapy. Furthermore, a significantly higher survival rate of breast cancer patients with receptor-positive tumors than receptor-negative tumors was noted. CAMA-1 cells contain estrogen receptors (Leung, 1978, Leung et al., 1982), progesterone receptors (Yu et al., 1981), also glucocorticoid and androgen receptors. These results are in agreement with findings of other investigators who observed these four classical steroid receptors in mammary tumor specimens and other mammary carcinoma cell lines in long-term culture. The clinical importance of androgen and glucocorticoid receptors in mammary carcinoma is uncertain.

Although it is well documented that some steroid-induced cellular events are mediated by these receptor molecules, there is no direct evidence indicating that cell proliferation is receptor-mediated. Therefore, whether the presence of these four classes of steroid receptor protein in CAMA-1 cells is associated with the observed stimulatory or inhibitory action of these steroid hormones must await further experimentation. There is evidence demonstrating that in the absence of receptor proteins in cells, the probability of the tissue responding to hormonal manipulation is poor (McGuire et al., 1975; Cancer Supplement 1980, Leung, 1978). Nevertheless, the mere presence of its receptor is not the sole factor for responsiveness of the tissue to a particular hormone. The cellular mechanism of action for the particular hormone must be operational without any defect or impairment. That is, the steroid receptor complex must be able to be activated, to translocate to the nuclei, and to interact with the acceptor protein located in the chromatin.

Interestingly, androgen is markedly inhibitory to CAMA-1 cells as it is for many clinical mammary carcinomas but is stimulatory to an androgen-sensitive mouse mammary cell line, S115 (King et al., 1976) which also contains androgen receptors. If proliferation is mediated by androgen receptors, these diametrically opposite results of the two cell lines indicate that other factor(s) must also be present to determine the stimulatory
It is possible that the androgen receptors in CAMA-1 cells are quite different from those of the S115 cell line so that it becomes associated with a different gene; one gene is responsible for up-regulating, and the other for down-regulating of cell proliferation. A similar situation may be true for the effect of glucocorticoid in CAMA-1 cells and other glucocorticoid-responsive tumors.

Results from this and previous reports (Leung et al., 1981, 1982, Yu et al., 1981) show that estrogen stimulation of proliferation requires the presence of serum. Such findings are consistent with other reports (Sirbasku, 1978, Sonnenschein and Sato, 1980) that serum growth factors have stimulatory effects upon mammary carcinoma cell proliferation in vitro. Prolactin appears to serve as a growth factor in the presence of estrogen. The synergistic effect exhibited by prolactin in this study is intriguing because the role of prolactin in human carcinoma cells is unknown. Previous reports (Leung et al., 1981) also demonstrate the synergistic effect of prolactin on estrogen-induced thymidine uptake and cellular incorporation of uridine and estradiol (Leung, unreported results). The exact nature of prolactin and estradiol interaction remains to be investigated.

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