Basil A. Stoll, London (Editor)

Endocrine Management of Cancer

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The authors and the publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accord with current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new and/or infrequently employed drug.

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The Role of Current Agents

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Preface

The last decade has seen almost incredible advances in our knowledge of the molecular biology of the cancer cell, and this has provided vital clues as to why tumours behave in a particular way in each individual patient. The new knowledge includes evidence on how growth factors which are essential to the growth of the cancer cell may be either switched on or off. In addition, we have developed insights into the production, metabolism and interaction of steroid and peptide hormones in malignant tissue, and why they may exert variable effects on the proliferative, invasive and differentiation activities of the cancer cell in endocrine target organs.

The book is unique in that it attempts to formulate principles aiming to link endocrine therapy by similar agents in cancers of the breast, prostate and uterus. Progress in the field has been hindered in the past by poor communication between the different specialist departments – surgical, urological and gynaecological – responsible for clinical research into each of the cancers. The book also breaks fresh ground in that it reviews models of hormonally-induced regression of cancer and emphasises how they differ from the model of regression following cytotoxic therapy. Different models imply not only the need for different schedules of treatment to deal with autonomy, but also the need for different methods of assessing response which are likely to be reflected in more prolonged survival.

The book aims to provide answers to questions which are rarely addressed in the literature. Does endocrine therapy lead to a state of dormancy in tumour growth? Do different endocrine modalities act through different mechanisms in causing regression of tumour growth, and how does the mechanism differ from that of cytotoxic therapy? How far do observations on animal tumours or tissue culture studies provide information which can be extrapolated to the clinical situation? How does the tumour metabolism of hormones affect response to hormonal manipulation? Are steroid receptors markers of tumour responsiveness or are they actively involved in tumour regression? What is the mechanism of subsequent tumour reactivation and its occasional response to secondary endocrine therapy?

It has become increasingly difficult for the busy clinician to keep abreast of new advances which provide pointers to future cancer management. This book is intended to expand the perspectives of the large numbers of clinicians practising endocrine therapy as part of the overall management of patients with cancers of the breast, prostate or uterus.

London, 1988

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Contents

1 Models of Tumor Regression in Endocrine-Related Cancer

Y.S. Cho-Chung

This chapter describes the biochemical and biological bases for different models of tumor regression in endocrine-related cancers. Evidence presented shows that different types of endocrine therapy can lead to different modes of cancer regression whether by autophagic cell death (apoptosis), cell differentiation or both. Again, these modes of regression are clearly different from that due to cell death caused by cytotoxic agents, immunologic host defense reaction or avascular necrosis.

Regression due to Autophagic Cell Death (Apoptosis)

Regression of mammary cancer has been induced in man and animals by the withdrawal of hormones that are essential for the growth of the cancer. The methods used include surgical removal of endocrine organs producing sex hormones (ovariectomy, adrenalectomy and hypophysectomy) and functional ovarian ablation by the use of aromatase inhibitors or luteinizing hormone-releasing hormone (LHRH) agonists.

Other endocrine-related cancers, such as prostate and endometrial cancers, also regress after hormone withdrawal. The cancers that respond to endocrine ablation are the *more differentiated* tumors. These cancers retain hormone dependence, meaning that they shrink in size after hormone withdrawal, just as normal mammary tissue will undergo atrophy after removal of the ovaries.

Endocrine Ablation

Hormone dependence of human mammary carcinoma is mimicked by mammary carcinomas of the rat, and both 7,12-dimethylbenz[α]anthracene (DMBA)-induced and transplanted MTW9 mammary carcinomas are examples of such hormone-dependent well-differentiated tumor models. Following endocrine ablation, the tumors regress to half their initial size within a few days, mostly because of cell destruction [1]. During regression, the blood supply remains as high as during growth and cell death cannot be attributed to vascular deficiency. Since no invasion of macrophages and lymphocytes can be demonstrated in regressing tumors, an 'immunologic

type' of tissue breakdown is also excluded. These observations were interpreted to indicate that tumor regression was mainly due to an autophagic process confined to each cell without apparent danger to the survival of neighbouring cells [1].

Tissue destruction during hormone-dependent regression follows a pattern that is more characteristic of shrinkage necrosis or apoptosis [2, 3] than of ischemic coagulative necrosis [4, 5]. The latter involves an entire sector of tissue, primarily the center, and can be found in almost every type of tumor regardless of its hormone dependency. Thus, coagulative necrosis is not a hormone-related event but reflects an inability of the vascular system to maintain an environment compatible with cell survival in the face of continuous cell proliferation. Apoptosis, on the other hand, involves a cell-limited process of self-digestion, and tumor regression appears to evolve as a programmed phenomenon.

Structural changes characteristic of apoptosis are found in regressing DMBA-induced mammary tumors after ovariectomy, and in MTW9 mammary carcinoma upon elimination of mammotropin supply. Cytoplasmic 'loosening' is one of the earliest morphological events indicating impending cell death. The cell appears to 'condense', and its association with neighboring cells is altered, probably by a process of dehydration [2]. When apoptosis is the predominant event in regression, a large number of cells become fragmented, but a characteristic is the preservation of the plasma membrane and the rapid phagocytosis by the surrounding neoplastic cells [3]. The autophagocytosis and morphological change occur in the absence of obviously toxic stimuli, whereas metabolic supplies are well preserved [1]. Tumor shrinkage occurs as a coordinated event, and a high level of RNA and protein synthesis can be observed at the beginning of regression [6].

The hypothesis that autophagocytosis and morphological evidence of tissue lysis are relatively late events in the course of tumor regression implies that specific cellular events triggering tissue lysis should occur at an earlier stage of regression. The following evidence suggests that cAMP plays the triggering role in these events of tissue lysis: cAMP level increases after ovariectomy; cAMP receptor increases as estrogen receptor decreases within a few hours of ovariectomy; translocation of cAMP-dependent protein kinase into the nucleus followed by new phosphorylation of nuclear protein occurs after ovariectomy; finally, these changes are reversed in tumors when they resume growth upon replenishment of estrogen [7, 8]. Thus, the ability of cAMP to overcome the effect of estrogen appears to be involved in triggering tumor regression after ovariectomy.

Aromatase Inhibitors

Peripheral tissues can metabolize the androgenic steroids produced in the adrenal gland so that androstenedione can be converted into estrogen not only within the breast tissue but also in the liver and fatty stores of the body. The enzyme aromatase mediates the conversion of androgens to estrogen.

The antitumor activity of aromatase inhibitors has been studied in DMBA-induced mammary carcinoma of rats [9]. Treatment with an aromatase inhibitor, 4-hydroxyandrostene-3,17-dione (4-OHA), consistently reduced estrogen secretion without increasing gonadotropin concentration, and caused tumor regression. Furthermore, 4-OHA treatment prevented the increases in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) that occur after ovariectomy, indicating direct action of the agent on the pituitary-hypothalamic axis. Since ovarian aromatase activity is under the regulation of gonadotropins, new enzyme synthesis would not occur after aromatase inactivation by 4-OHA.

In contrast, gonadotropin levels were found to be increased in rats after 4 weeks of treatment with aminoglutethimide (AG). Although ovarian aromatase and estrogen secretion were inhibited 3 hours after injection of AG, after 4 weeks estradiol levels were not consistently suppressed, and the total volume of mammary tumors was increased [9]. Both 4-OHA and AG inhibited peripheral aromatase activity. These observations suggest that aromatase inhibitors causing functional ovariectomy may produce tumor regression by the same mechanism as does ovariectomy.

LHRH Agonist

With the discovery of the structure of the decapeptide gonadotropin-releasing hormone, a number of synthetic analogs were found to have LH/FSH-releasing activities several times that of the natural hormone [10, 11]. However, at higher doses they inhibit the uterotropic and ovariogenic effects of human chorionic gonadotropin [12, 13], and cause atrophy of the ovaries and uterus, consistent with an antagonistic role, and also decrease levels of circulating ovarian steroids [14].

Such results mimic the effect of ovariectomy and it is found that chronic daily administration of LHRH agonists causes regression of hormone-dependent mammary tumors in experimental animals [15–17]. High doses of LHRH or lower doses of LHRH agonists have also demonstrated *direct* growth inhibitory effect on the human breast cancer line MCF-7 [18]. Presently, trials with LHRH agonists are being conducted in premenopausal

women as a form of medical ovariectomy [19, 20], but treatment with LHRH agonist can also cause objective responses in postmenopausal patients with breast cancer and inactive ovaries [21]. The latter response may reflect a direct antitumor effect by LHRH agonists.

To summarise this section. We have seen that in the apoptosis model, highly hormone-dependent cancer cells in the host die as a result of hormonal deprivation. But such cell death may represent a prelude to tumor differentiation, just as in the collapse of tadpole tails, the undifferentiated cells die before evolving the more differentiated progeny cell. Tumor regression resulting from endocrine ablation is usually an acute process not accompanied by morphological or phenotypic change. At a nuclear level, antagonism between the effects of cAMP and estrogen is demonstrated in mammary tumors regressing after ovariectomy but its mechanism is not clear.

Regression due to Cell Differentiation

Until recently, high dose estrogen therapy was widely used to cause regression of mammary cancer in postmenopausal women. Its use has been replaced by that of the antiestrogen, tamoxifen, and by high-dose progestogen therapy. In experimental mammary cancer, cAMP derivatives may cause growth arrest. High-dose progestogen therapy can cause regression of endometrial cancer, and antiandrogen therapy can cause regression of prostate cancer. It is possible that the mechanisms involved in all these cases may be linked to tumor cell differentiation.

High-Dose Estrogen

The transplantable R323OAC mammary adenocarcinoma of the Fisher rat is autonomous and does not regress after hormone withdrawal. Nevertheless, it is hormone sensitive and treatment of tumor-bearing animals with high-dose estrogen (17β-estradiol, 0.1 mg/kg rat/day) retarded tumor growth and induced lactation [22]. Androgen treatment also decreased tumor growth rate. A correlation was found between morphological changes and tumor biochemistry [22], and a striking relationship was found between lactation and glucose-6-phosphate dehydrogenase and malic enzyme activities. Thus, R323OAC mammary carcinoma is hormone sensitive, even though it is not hormone dependent. The induction of lactation by hormone treatment indicates that the epithelial cells of mammary carcinomas can be functionally differentiated by the hormone as they are in normal mammary gland.

High-Dose Progestogen

High-dose progestogens have been shown effective in the growth inhibition of human endometrial adenocarcinoma, and histochemical and histological findings suggest functional differentiation in the epithelial cells [23]. In response to hormone therapy, the endometrial cancers have been shown to increase glycogen accumulation and markedly accumulate mucopolysaccharide, and also manifest a decrease in mitotic index and evidence of atrophy in the cancer tissue. The histological pathway leading to the disappearance of the adenocarcinoma includes differentiation, secretion, epithelial metaplasia, decidualization and fibrosis of the stroma. High-dose progestogens have also been shown effective in causing regression of human mammary cancer.

Antiestrogen

Estrogen binds to a specific binding protein in the cytoplasm and the complex formed is then activated and translocated into the nucleus where it triggers the biological response [24]. Hormone-dependent tumors contain the same specific estrogen binding protein (estrogen receptor) as estrogen-responsive normal tissues [25–27]. The antiestrogen, tamoxifen, competes with estrogen for the estrogen receptor [28]. Tamoxifen binds to estrogen receptor, the complex moves into a nuclear compartment, and its prolonged retention in the nucleus causes the cells to be refractory to subsequent estrogen stimulation [28]. In the treatment of breast cancer, its antitumor effect has been related to its binding to estrogen receptors in tumor tissues but not to its effect on the secretion of pituitary hormones [29].

The histochemical and histological response of endometrial adenocarcinomas to tamoxifen demonstrates some analogies to, but also some striking differences from, that of high-dose progestogens [23]. The response to tamoxifen includes glycogen accumulation, reduction in the mitotic index and atrophy in the adenocarcinoma. The histological change during the disappearance of the adenocarcinoma is characterized by monostratification and even necrosis.

Antiandrogens

Some prostate cancer cells have been found to depend on androgens for growth [30, 31] and competitive compounds, which by themselves have no biologic action, can act as antiandrogens by binding to the receptor. Included are compounds such as cyproterone acetate, progesterone, flutamide, and megestrol acetate. Flutamide, which has been the most useful drug,

impairs nuclear binding to dihydrotestosterone and interferes with the stimulation of RNA polymerase by testosterone. Prostate cancer regression caused by flutamide is probably by a mechanism similar to that of tamoxifen in causing breast cancer regression.

Cyclic Nucleotides

It has been shown that the content of cAMP in rat mammary gland is related to the growth, development, and differentiation of the tissue [32–34]. The effect of cAMP observed in malignant cells often involves striking redifferentiation, amounting to apparent renormalization of a number of properties including morphology, adhesive properties, lectin agglutination, cell movement, and biochemical functions [35, 36].

Administration of cAMP derivatives, such as dibutyryl cAMP (DBcAMP; 10 mg/day/200 g rat s.c.), caused growth arrest of hormone-dependent mammary tumors in intact animals within a few days [37]. The presence of specific cytoplasmic receptor proteins for estrogen and cAMP has been shown in hormone-dependent mammary tumors [38]. During growth, high estrogen binding and low cAMP binding activities are found in DMBA-induced mammary tumors and when the tumors undergo growth arrest after DBcAMP treatment, cAMP binding markedly increases as estrogen binding decreases. The same decrease in estrogen binding with increase in cAMP binding also occurred during tumor regression due to ovariectomy or tamoxifen treatment [38, 39].

In a cell-free system of hormone-dependent tumors, the nuclear uptake of estrogen receptor was inhibited by the presence of cAMP receptor, whereas the presence of estrogen receptor inhibited the nuclear uptake of cAMP receptor [40]. No competition was found between cAMP and estrogen for each other's cytoplasmic binding proteins or the nuclear acceptor sites. Thus, a mutual antagonism exists between the cAMP receptor and estrogen receptor during their nuclear translocation. It was proposed that the action of cAMP (like that of antiestrogen) is antagonistic to estrogen action in the growth control of hormone-dependent tumors, probably occurring at the nuclear level [40].

An effect of cAMP on cell differentiation and growth was reported in the hormone-responsive R3230AC rat mammary carcinoma [41]. After daily s.c. injections of DBcAMP beginning on day 1 after tumor implantation, the growth rate increased appreciably. R3230AC tumor is composed primarily of epithelial cells with a histological organization resembling mammary gland alveoli [22], and increase in tumor size after DBcAMP

treatment is attributed to an increase in cell size rather than an increase in cell number.

Growth arrest which has been observed with cAMP derivatives may well be linked to cell differentiation, because cAMP-induced cell differentiation has been observed in both normal and transformed cells [35, 36]. cAMP may switch active growth into gradual cell loss through promoting differentiation of cancer cells. The tumor regression due to cell differentiation is accompanied by a morphological or phenotypic change, as shown in R3230AC mammary carcinoma in experimental animals [22] and in human endometrial cancer [23].

To summarise this section. It has been suggested that high doses of estrogens or progestogens may promote regression of well-differentiated tumors by causing their cells to differentiate terminally or mature. This process is characterized by the stimulation of enzyme pathways normally associated with cell development or differentiation. In mammary carcinomas in experimental animals, the expression of enzymes associated with a lactational pathway has been demonstrated along with tumor regression after exposure to high doses of estrogen [22].

Antihormones, such as tamoxifen, also could promote cell differentiation by interfering with the action of estrogen. Tamoxifen stimulates a number of enzymes associated with cell development, nuclear RNA polymerase activity, accumulation of polysomes, and general protein synthesis. The antitumor activity may be partly derived from its agonist (estrogen-like) hypertropic activity. It has been suggested that tamoxifen causes tumor regression by promoting limited hypertrophy of differentiated cells, which in turn down-regulates the sensitivity of tumor cells to other growth factors [42].

Regression due to Cell Differentiation and Apoptosis

Cancer regression included in this category may be brought about either by a combination of two hormones having different mechanisms of action via different receptors or by DBcAMP in combination with arginine or by site-selective cAMP analogs that are manyfold more active than DBcAMP. This model of regression appears to be due to a potent agonistic or antagonistic effect toward hormones or growth factors that are normally involved in cell development or differentiation.