Ectopic Hormone Production by Malignant Tumors

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ABSTRACT

Malignant tumors of nonendocrine tissues may produce ectopic hormones. The most likely mechanism is depression of genes which code for hormones. Ectopic hormones are invariably peptides, and each is identical to some peptide product of an endocrine gland. However, the majority of ectopic hormones occur as biologically inactive precursors or subunits and therefore remain occult unless they are specifically sought. When appropriate assays are made for such inactive forms, it is found that ectopic production of hormone-like peptides occurs frequently. Clinical syndromes result only in the relatively rare patients in whom a biologically active form is synthesized in large quantities. Laboratory research in this area improves our understanding of genetic control mechanisms in neoplasia. Ectopic hormones may be of limited use in diagnosis of cancer, especially when multiple markers are measured simultaneously.

Introduction

To most of us, the ectopic synthesis of hormones by malignant tumors brings to mind a rare patient whose vigorous workup by an enthusiastic endocrinologist merited a case report. The phenomenon is neither new nor all that rare, but our awareness of it has increased with the advent of radioimmunoassay techniques which have improved the sensitivity and availability of hormone measurements. Recent work has greatly advanced our understanding of ectopic hormones and has indicated that they may provide a tool for exploring the molecular biology of neoplasia.

Ectopic hormone production is synthesis of a hormone by tissues which do not normally produce that hormone. This definition implies, of course, that all of the normal sites of origin of the hormone are known, but this assumption conceals complexities to which we will return later. The organ in which the ectopic synthesis occurs may or may not have an endocrine function in healthy persons. In the great majority of cases, it is tissue not considered endocrine until neoplasia endows it with that capacity.

The four glycopeptide hormones are human chorionic gonadotrophin (HCG), luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyrotropin.
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stimulating hormone (TSH). They are each composed of an alpha chain and a beta chain which lack biologic effect when separated but which, when bound as a dimer, form a biologically active molecule. The alpha chains of all four are nearly identical, and the biological and immunological specificity of these hormones is conferred by their beta chains. In addition to the peptide structures, each hormone has a carbohydrate component which, if removed, leaves a molecule which retains immunologic reactivity in assays but is of greatly reduced biological potency because of an enormous decrease in metabolic half-life.9

Until quite recently, HCG could be assayed only as the sum of HCG and LH because the beta chains of these two hormones are sufficiently similar to cause immunologic cross-reaction. The production of small amounts of HCG had escaped detection because it could not be separated from the relatively large normal levels of LH. The fairly recent development of the so-called beta-subunit assay which is highly specific for HCG was the technical advance which made possible much of the current understanding of the ectopic production of this hormone.

The Special Role of Placental Hormones

One of the first problems encountered in considering ectopic hormones is how to prove that they are synthesized ectopically by tumors rather than produced eutopically at a normal site of origin. The most elegant and definitive methods are a demonstration of an arteriovenous gradient in hormone concentration across the tumor or in vitro production of hormone by tumor explants. These studies, while optimal, are rarely done. Direct analysis of tumor tissue for hormone is somewhat more practical, but it cannot distinguish genuine synthesis of hormone by tumor from mere concentration or “trapping” of hormone in tumor tissue.

In most cases, of course, plasma hormone concentrations are the only data available. Since most hormones are subject to a negative-feedback-physiologic regulation, production of ordinary quantities of hormone ectopically succeeds only in shutting off eutopic synthesis, and the net result is a non-diagnostic blood level in the normal range. The diagnosis of ectopic production by this approach must often wait until the plasma hormone concentration clearly exceeds normal levels, which lowers the rate of detection to the point where this phenomenon becomes a medical curiosity.

Placental hormones such as HCG and human placental lactogen (HPL) assume special importance as ectopic products because they are not found in the sera of healthy non-pregnant persons and are not subject to any feedback-loop control, so that they are almost specific for cancer when present in serum in any measurable quantity. With the great sensitivity allowed by this low level of significant production, the placental hormones are found early and relatively frequently, and much of the most interesting and important recent research in ectopic hormone production has centered about these hormones.12 These placental hormones are not to be confused with fetal proteins, such as alpha fetoprotein or carcino-embryonic antigen, which are normally present in sera of healthy adults in small quantities.

Genetic Derepression

The mechanism of polypeptide production by nonendocrine tumors has been the subject of controversy ever since the phenomenon was first described. One theory has been that it is part of the chaos of malignancy, in which a peptide corresponding to the active site of a hormone is fortuitously synthesized by an occasional tumor. There is now much evidence, however, that ectopic synthesis is not ran-
dom, and that it is of very basic significance in tumor biology.

An hypothesis termed "genetic derepression" explains the observations in a much more satisfactory way than does any previous theory. Essentially, it states that all tissues contain the same genetic information in the nuclei of all cells, but much of it is repressed as cellular and tissue differentiation advances. In the neoplastic process, some of these repressed genes may be activated, leading to production of normal peptides by tissues other than their natural sources.

If this theory is true, then ectopic hormones should all be polypeptides and never steroids or thyroid hormones, and this is in fact what has been found. All ectopic hormones which have been studied are peptides. In those patients who have appeared to produce steroid or thyroid hormones ectopically, the tumor makes only the tropic hormone, which is a peptide, while the steroid or thyroid hormones are secondary products of eutopic origin. To believe otherwise would be to ignore what is known from ultrastructure and biochemistry, since all nucleated cells possess the organelles and enzymes for making peptides, but only certain cells in the body have the long and complex pathways necessary for steroid synthesis.

Additional support for this theory comes from the finding that those ectopic hormones which have been isolated and characterized are structurally identical to eutopic hormones and their precursors and subunits. Not only the active site but an entire polypeptide is faithfully duplicated by the tumor. Such fidelity would be most unlikely if ectopic hormones arise from chaos, but it is the expected result if their origin lies in genetic derepression.

The association of certain tumors with ectopic production of one hormone much more frequently than others, such as squamous cell carcinoma and parathormone, suggests that derepression of deoxyribonucleic acid (DNA) in malignancy may have an element of selectivity. This has fostered an additional hypothesis in which there is a hierarchy of availability for derepression of DNA, ranging from DNA which is inaccessible through various degrees of reversible repression to DNA which is actively coding messenger ribonucleic acid (RNA). In this theory, the synthesis of ectopic hormones by tumors is governed by DNA which is in those middle levels of reversible repression, and the frequency with which a peptide is synthesized ectopically should correlate with the ease of derepression of the DNA which codes for it.

Research using tissue cultures of malignant tumors has made significant contributions to our understanding of the mechanism of ectopic hormone production. The well-known HeLa cell line was derived from a carcinoma of the uterine cervix and has been maintained in cell culture for over 25 years. HeLa cells have been demonstrated to secrete the alpha subunit of HCG ectopically in significant amounts, and the fact that alpha subunit was synthesized by all of the HeLa cell strains studied by one laboratory suggests that transcription of this peptide may be relatively easily derepressed.

Variability in expression of this derepressed genetic information has been elegantly demonstrated in another cell culture system. In 1971 a subcutaneous metastasis was excised from a 45-year-old man with an HCG-producing bronchogenic carcinoma. A number of clonal strains from single cells of this tumor, designated ChaGo, were established in long-term tissue cultures. Secretion of HCG and its subunits into the culture media of three of these clones has been measured and was found to be unbalanced in all three but in very different ratios of alpha to beta to complete HCG in each. The remarkable aspect of these data is that the three hormone-producing clones were all derived from that single lesion, so that even in one man's tumor the
individual cells express their repressed genetic information in heterogeneous fashion.\textsuperscript{15}

The regulatory mechanisms for hormone production may differ between ectopic and eutopic sites. Sodium butyrate induces HCG and HCG-alpha synthesis in the two nontrophoblastic cell lines HeLa and ChaGo but represses synthesis in several cell cultures of trophoblastic tumors.\textsuperscript{3}

It has been generally assumed that ectopic protein production is intrinsically less efficient than eutopic synthesis, and the occasional patient in whom high serum levels of hormone cause clinical symptoms if sustained long enough is accounted for by the sheer enormity of his kilograms of tumor relative to the size of endocrine glands. This belief has been questioned by work in which one particular clone of ChaGo has been shown to secrete alpha subunit at a rate exceeding that of the most active known choriocarcinoma clone, representing ectopic production more efficient than eutopic synthesis under comparable in vitro conditions.\textsuperscript{6}

**Universal Ectopic Hormones**

Ectopic synthesis of prohormones or of subunits should not be viewed as exceptions to the apparent rule that ectopic hormones are identical to the eutopic product. These precursors and fragments are normally present in the glands which synthesize the hormones and are equally as “natural” as the circulating active hormone. The related enzyme systems necessary to complete the synthesis of the active form of the hormone (e.g., hydrolysis of prohormone, assembly of subunits, addition of carbohydrate) are not likely to be well developed in tumor tissue, leading to production of peptides which are replicas of biologically inactive forms of hormones.\textsuperscript{17}

In fact, ectopic synthesis of these inactive precursors and subunits appears to be much more common than production of complete active hormones. The great majority of these cases ordinarily go undetected because no symptoms are produced and the assays used clinically show nothing amiss. However, research workers using appropriate methodology have detected this kind of ectopic hormone production so frequently that one group has stated boldly that “ectopic protein synthesis is a universal concomitant of neoplasia.”\textsuperscript{10}

In their well-known study, Gewirtz and Yalow\textsuperscript{5} found “big ACTH” (adrenocorticotrophic hormone, a prohormone) in tissue extracts of almost all lung cancers they studied, regardless of histologic type. Normal tissues contained no immunoreactive “big ACTH” in their assay system. Carrying this idea much further, Odell\textsuperscript{10} and his group have reported the presence of measurable quantities of ACTH, beta-lipotropin (beta-MSH, melanocyte stimulating hormone), HCG and alpha glycopeptide even in normal lung, colon and liver as well as in neoplasms of these and other organs. The ACTH and beta-MSH activities were greater in tumors than in normal tissues, while the quantities of the other hormones were similar in both. This work suggests that the genetic material coding for these hormones is not completely suppressed even in normal nonendocrine tissue and is relatively less suppressed in neoplastic tissue.

There is other evidence that ectopic hormone production, at least of HCG, is not limited to malignancy. The presence of HCG has been demonstrated in tissue extracts of normal testis\textsuperscript{2} and in extracts of colon and liver in male patients who died of non-neoplastic disease.\textsuperscript{19} Occasional laboratories have reported HCG in serum of patients with non-neoplastic gastrointestinal diseases\textsuperscript{17} and benign nontrophoblastic gynecologic conditions,\textsuperscript{13} although these findings in serum have not been widely confirmed. A likely interpre-
tation is that the peptide portion of HCG is produced in most or all human cells, normal and neoplastic, but it is either not secreted into blood or it is secreted but degraded very quickly, since HCG of nontrophoblastic origin has much less carbohydrate component than placental HCG.9 The unanswered question is whether it is the neoplastic process per se or merely the increased cell replication rate found in neoplasms which correlate with the quantity of peptide secreted.10

This greatly complicates the basic definition of ectopic synthesis, since eutopic production of HCG, and possibly of other hormones, is not truly restricted to what we regard as the normal sites. It goes on at a very low level in many tissues, perhaps in all. It may be more realistic to think of ectopic synthesis as an occult process becoming manifest rather than as a function acquired de novo. This concept is the correlate, on the tissue level, of the theory of genetic derepression.

Ectopic Hormones as Tumor Markers

It is time to turn from theoretical to practical matters and to consider putting ectopic hormones to work as blood tests for cancer. As mentioned previously, because the ectopic and eutopic products are indistinguishable, the only hormones of diagnostic promise are those which are entirely absent from serum of healthy persons, namely, the placental hormones. For purposes of clinical diagnosis, their presence can be ignored in extracts of normal tissues and in sera of some patients with certain non-malignant disorders, since mention is made now only of practical serum markers and the great majority of people have no detectable circulating placental hormones.

For HCG, the one most extensively studied in this context, only a tantalizing minority of cancer patients have hormone in their sera, so that a positive test is significant but a negative one is not. Cancers of the liver, stomach and pancreas have relatively high incidences of ectopic synthesis of HCG detectable in serum (21 percent, 22 percent and 33 percent, respectively, in one large series).17 In another series of patients with bronchogenic and gastrointestinal carcinomas, only 17 percent had detectable plasma HCG, and the great majority of those had only modest quantities.1 In breast cancer, 36 percent of pre-operative patients and 48 percent of patients with metastatic disease had HCG in their sera, and these women may have a less favorable prognosis than women without HCG. In patients with HCG, the level generally rose and fell with clinical relapse or remission. However, the quantitative range of HCG levels was small, so that clinical utility for therapeutic monitoring or for estimation of prognosis is limited.16

One hope for increasing the diagnostic sensitivity of ectopic hormones is to screen for a battery of selected markers. In one series of patients, simultaneous determinations were made of HCG and two other placental polypeptides known to be produced ectopically by nontrophoblastic tumors, human placental lactogen and the placental isoenzyme of alkaline phosphatase. It was found that these processes were discordant, that is, that the marker peptides were secreted independently without a discernible pattern. On a theoretical level, this implies that ectopic protein synthesis is not controlled by a single mechanism. In a more practical vein, it means that diagnostic yield rises when multiple markers are measured.14 This series was very small and was intended only to establish the discordance of marker production. It appears that larger and more definitive studies along these lines have not yet been reported.

Ectopic Hormones and Tumor Immunology

Having considered the theoretical and the practical, a brieffling with speculation is allowed. Could an ectopic hormone
have a function to perform? One avenue that has been explored is the relation of HCG to lymphocyte activity. It has been shown in lymphocyte culture that HCG inhibits the response of lymphocytes to phytohemagglutinin in a reversible and noncytotoxic manner. This work was done in reference to the failure of maternal lymphocytes to reject fetal trophoblast, and the hormone concentrations required for this effect, while attained locally in trophoblast, are above the range associated with ectopic HCG. However, a possible relevance to nontrophoblastic malignancy is provided by work in which immunoperoxidase technique was used to demonstrate and localize HCG in tissue sections of a broad variety of cancers. There was a tendency for accumulation of HCG at the tumor cell surface, which would yield a high local concentration in a critical site in which it might be potentially effective in inhibiting T-cell action. By this technique, HCG was demonstrable in 25 of 28 nontrophoblastic malignancies, and the percentage of tumor cells staining for it ranged from 10 to 15 percent up to 90 to 95 percent. At this point there is not enough data to accept this as the means by which tumor cells are sheltered from immune surveillance, but it is an intriguing possibility.

Conclusion

The production of ectopic hormones by malignant tumors is detected frequently if appropriate methods are employed. The capacity to synthesize hormones extends beyond endocrine organs. It exists in partially repressed form in many tissues and is expressed more fully when malignancy develops. The peptides secreted by tumors are normal products of normal genes, but they are more often hormonally inactive precursors or subunits than complete active hormones. At the present time, the use of ectopic hormones in the clinical diagnosis of cancer appears limited. In the research setting, however, this phenomenon has an important role in improving our understanding of neoplasia.

References


