

Role of Hormones in Mammary Neoplasia¹

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Abstract

A testable hypothesis for the role of hormones in mammary carcinogenesis with implications for other endocrine-related carcinogenesis is presented. The hypothesis is based on these observations: (a) hormones are involved, directly or indirectly, in regulating cell division in normal mammary cells; (b) emergence of overt mammary tumors requires hormonal stimulation of cells receiving carcinogenic stimulus; (c) normal mammary cells are of finite divisional capabilities, whereas neoplastic cells appear to have infinite divisional life; and (d) normal cells, when present in large quantities relative to the neoplastic cells, inhibit the growth of the latter cells. According to the hypothesis hormones play at least two roles in mammary carcinogenesis induced by diverse agents, such as viruses, chemicals, and radiation. First, hormones are necessary for DNA synthesis and mitosis of initial transformed cells for their conversion into fixed transformed cells with heritable characteristics. Second, hormones, by increasing the rate of cell division, shorten the reproductive life span of normal cells, eventually causing a reduction in the normal to transformed cell ratio in the population—a condition that allows the emergence of tumor cells by overriding the inhibitory influence of normal cells.

Introduction

That hormones are involved in the development of mammary neoplasia in animals and humans is no longer a debatable question. The nature of this involvement, and whether or not hormones have a direct influence in one or more parts of the sequence of events leading to the emergence of overt tumors, still requires elucidation. Although the effects of hormones on tumorigenesis and tumor growth have been subjected to intensive investigation during the last 40 years and summarized in a number of excellent reviews (3, 9, 11, 19-22, 25, 28, 33, 34, 36-38, 44), the debate still continues as to whether hormones act directly or indirectly in the carcinogenic process and whether some of them (e.g., estrogens) should properly be classified as carcinogens. A great deal of confusion in this area stems from the way a carcinogen is defined. If carcinogen is defined as an agent, the application of which results in the development of cancer that otherwise would not have appeared, a hormone, such as estrogen, has to be considered a carcinogen, since its application results in mammary tumorigenesis in male mice that normally lack such tumors. However, if carcinogen is defined as an agent that initiates neoplastic transformation in normal cells, it becomes, at

the least, extremely difficult to classify any hormone as a carcinogen.

In this presentation a hypothesis will be discussed that I recently postulated (35) in an attempt to explain the possible role of hormones in mammary neoplasia as well as neoplasia in other endocrine organs and target tissues. In the postulation of this hypothesis, carcinogen is defined as an agent that initiates neoplastic transformation in normal cells. Alternate explanations or alternate theories and hypotheses that are already existent in the literature (9, 19-22, 41) will not be surveyed.

Events Associated with Tumorigenesis

Tumorigenesis, *i.e.*, the emergence of overt tumor, is the end result of a sequence of events that begins as a result of successful carcinogenic insult of a cell or group of cells in a host. The sequence of events in tumorigenesis, although poorly understood, could be separated into 4 broad phases: initiation phase → fixation phase → latency phase → emergence phase. Initiation phase is the period when carcinogenic stimulus results in the conversion of normal cells to initial transformed cells. During the fixation phase a mitogenic stimulus resulting in DNA synthesis and mitosis of initial transformed cells is necessary for their conversion into fixed transformed cells with heritable characteristics. This is followed by a latency phase in which the transformed cells remain dormant for a prolonged period, before passing to the emergence phase in which overt tumor appears in a host. Although many hypotheses have been postulated to explain the latency and emergence phases, none has been proven conclusively. Some of the suggested possible explanations have been: (a) transformed cells must go through a series of progressions before emergence as overt tumors (2, 18, 29, 32); (b) the doubling time of tumor cells determine their latency period and emergence (23); and (c) transformed cells are under host immunological or other inhibitory controls, and a release from such inhibition is necessary for emergence of overt tumors (8, 40, 41).

Role of Hormones in Tumorigenesis

Two concepts have been used in explaining the role of hormones in carcinogenesis (9-11, 19, 21). First, some hormones act as mutagens and presumably as carcinogens when present for a prolonged period in an animal. These hormones exert their effects by increasing the frequency of cell replication in target tissues, which increases the probability of error in DNA copying (19), resulting in somatic mutants including random neoplastic variants. Second, hormones may not be carcinogenic by themselves but may modify the host and/or the target tissues during one or more phase of the carcinogenic events initiated by carcinogens, such as viruses, chemicals, or ionizing radiation.

¹ Presented at the John E. Fogarty International Center Conference on Hormones and Cancer, March 29 to 31, 1978, Bethesda, Md. This investigation was supported by Grant CA05388 awarded by the National Cancer Institute, Department of Health, Education, and Welfare.

Modification by hormones might involve (a) modification of the host immune system, (b) activation of viruses, (c) induction of receptors for carcinogens and/or metabolic conversion of a procarcinogen into a proximal carcinogen in the target cells, and (d) regulation of the growth of tumor cells and/or the initiation of DNA synthesis following carcinogenic stimulus, which appears to be essential for the conversion of the initial transformed cells to fixed transformed cells. Of these 2 concepts, no evidence exists in support of the thesis that some hormones are mutagens and hence may be acting as direct carcinogens (35). However, hormonal influence in some of the modifying factors mentioned previously is well known and might indeed represent pathways operating in mammary carcinogenesis.

Assuming that hormones have a modifying influence in the carcinogenic process, we have tried to unravel the minimum essential role of hormones in the sequence of events associated with mammary carcinogenesis initiated by a variety of diverse agents. My working hypothesis is that hormones need to have at least 2 functions in mammary carcinogenesis initiated by all direct-acting carcinogens. First, hormones are necessary, directly or indirectly, for DNA synthesis and mitosis of the initial transformed cells and consequently for their conversion into fixed transformed cells. Second, hormones play a role in the emergence of overt tumors by controlling the latency phase.

Explanation of the Hypothesis

The postulated dual role of hormones in mammary carcinogenesis is not intended to imply that hormones never play any role prior to, or at the time of, insult by a carcinogen. Indeed, it is likely that modification of the host and/or target organs by hormones is essential for oncogenic viruses and for most of the chemical carcinogens to act on the initiation phase. This, however, may not be a prerequisite for radiation carcinogenesis, which appears to effect mammary tissues of adult rats at all physiological states (43).

The first of the dual functions of hormones in carcinogenesis is that hormones, through their mitogenic action on mammary cells, are necessary for the conversion of initial transformed cells into fixed transformed cells with their heritable characteristics. The requirement of cell division within a short period after exposure to a carcinogen has been a remarkably constant feature in several *in vitro* transformation studies involving X-ray (7), chemicals (5, 24, 26, 27), and viruses (46, 47). Hormones are known to cause DNA synthesis and mitosis in mammary cells, and removal of mammogenic hormone sources prior to, or soon after, carcinogenic insult results in significant reduction of mammary cancer in animals (14, 15, 43, 48). These results suggest that hormone-induced DNA synthesis and mitosis is a necessary event in the conversion of an initial transformed cell into fixed transformed cells.

The second major role of hormones appears to be the one involved in determining the length of the latent period for the appearance of overt tumors. Again, hormones are considered to play a direct or indirect role in this process by controlling the growth rate of the normal cells. Hormones promote cell division in normal mammary cells that

have finite divisional life. Neoplastic cells on the other hand may or may not be under hormonal growth control and are known to possess infinite divisional capability. If in a cell population the proportion of normal cells surrounding the fixed transformed cells is very high, the former will inhibit the growth of the latter cells. A shift in the ratio of normal to transformed cells with a higher relative ratio of transformed cells locally or tissue wide will favor the emergence of tumor cells. Stimulation by hormones causing rapid growth of normal cells with finite divisional life will ultimately lead to a reduction in the number of these cells in localized areas surrounding the transformed cells or in the tissue as a whole. This will release the transformed cells from the growth inhibitory influence of normal cells, allowing the emergence of overt tumors. This thesis thus postulates that hormones control the latent period and emergence of tumors by controlling primarily the growth rate and ultimate exhaustion of normal cells rather than by controlling their influence primarily on neoplastic cells especially in the case of independent variants.

The previous hypothesis is experimentally testable and, if substantially correct, will provide a rational explanation for the role of hormones in mammary carcinogenesis and perhaps for carcinogenesis in endocrine organs and their target tissues in general.

Analysis of the Hypothesis

Is there any evidence in support of such a hypothesis? The answer is that there is some, but more is required before the hypothesis can be accepted or rejected.

Evidence in support of the first part of the hypothesis, that hormones are necessary for the conversion of the initial transformed cells to fixed transformed cells, has been provided previously. The following experimental evidence supports the second part of this hypothesis; *i.e.*, (a) that normal cells have finite divisional life, whereas the neoplastic cells are immortal, and (b) that normal mammary cells might inhibit the growth of small numbers of preneoplastic and neoplastic cells.

Transplantation studies by Daniel *et al.* (12, 13), Aidells and Daniel (1), Blair *et al.* (6) have clearly shown that normal mammary cells appear to have finite divisional capability, whereas both preneoplastic and neoplastic mammary cells are of infinite divisional capability. Since hormones are necessary for the growth of normal cells, it appears axiomatic that hormone-induced cell division could enhance the rate of growth and ultimate exhaustion of these cells.

The evidence in support of the thesis, that normal cells inhibit growth of preneoplastic and neoplastic cells, comes primarily from *in vivo* studies in our laboratory and that of Dr. Dan Medina, Baylor University, Houston, Texas. The following evidence indicates that normal cells can inhibit the growth of small numbers of both preneoplastic and neoplastic mammary cells transplanted into cleared and uncleared fat pads of mice and rats.

(a) Upon transplantation, preneoplastic nodules grow in cleared but not in uncleared mammary fat pads containing normal mammary parenchyma (16, 17).

(b) An inoculum of 1000 primary rat mammary tumor cells gives rise to a considerably higher proportion of tumors in

cleared (7 of 9) compared to uncleared (2 of 9) fat pads within a period of 8 weeks (42).

(c) In a parallel experiment, Richards (42) using 2 different sets of rat mammary tumors compared the growth of 10^5 tumor cells with or without 10^5 normal mammary cells following their transplantation into cleared fat pads. The pooled data show a tumor incidence, during a period of 17 weeks, of 8 of 10 with tumor cell transplants, compared to 1 of 10 with a tumor and normal cell combination.

(d) Medina *et al.*² have recently reported that the D2 line of mouse mammary nodule cells gives rise to a high incidence of tumors upon transplantation into cleared fat pads. However, deliberate addition of dissociated normal mammary cells from virgin, pregnant, and lactating females to the nodule cells significantly decreased their tumorigenic potential following similar transplantation.

(e) Finally, in a recent study DeOme *et al.* (K. B. DeOme, R. Osborn, R. C. Guzman, and M. Miyamoto, unpublished data) have observed that transplantation of dissociated nodule cells (5×10^3 cells/cleared fat pad) alone gave a nodule outgrowth incidence of 85%. When these inocula of nodule cells were mixed with 95,000 normal cells, the incidence of nodule outgrowth dropped to 42%.

The experiments mentioned previously thus provide strong support for the thesis that normal mammary cells, by some as yet unknown mechanism, inhibit the growth of preneoplastic and neoplastic mammary cells. Additional support for such a thesis comes from the reports from different laboratories, which have shown that normal cells can inhibit growth of transformed cells *in vitro*. For example, normal liver cell extract can inhibit DNA synthesis in hepatoma cells in culture (30, 39), or contact or close proximity to normal fibroblasts can inhibit multiplication of polyoma-transformed BHK21 cells in culture (45). Suppression of normal cell growth in a mixed population by lowering serum concentration enhances the emergence of transformed foci in chemical or X-ray-treated cells in culture (4, 31).

In summary, there is preliminary evidence in support of all parts of the thesis presented herein to explain the role of hormones in mammary carcinogenesis. Further studies will be necessary to substantiate the validity of this hypothesis. Undoubtedly, the model is a crude one and needs to be refined in many aspects, and it is hoped that this presentation will stimulate other investigators to take a serious look at this important problem.

Concluding Remarks

The role of hormones in the carcinogenic processes has been and continues to be a difficult one to assess. The current hypothesis provides an explanation for some of the major questions in mammary carcinogenesis: (a) why carcinogenic stimulation needs to be followed by hormonal stimulation for successful transformation; (b) why a long latent period is associated with carcinogenesis; (c) why spontaneous tumors are usually found in atrophic organs, late in life; and (d) why hormonal stimulation reduces the

latency phase and causes the emergence of tumors. In fact the long latency period between carcinogenic stimulus and the emergence of overt tumors has remained an enigma in all cases of carcinogenesis in humans and animals. My hypothesis on the role of hormones in carcinogenesis suggests that normal cells, when present in high ratios, inhibit the growth of transformed cells and that hormones are involved in the reduction of the latency period and emergence of overt tumors by stimulating the growth and ultimately the reproductive death of the normal cells that have finite divisional capability. The model has allowed us to look at the problem of mammary carcinogenesis from an entirely fresh approach.

Acknowledgments

I am indebted to Dr. K. B. DeOme, Dr. J. J. Elias, Dr. R. C. Guzman, Dr. J. Yang, Dr. L. Jenkins, and Dr. J. Nandi for their comments and to S. Castillo and J. Underhill for their excellent technical assistance.

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Cancer Res 1978;38:4046-4049.

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