NEW FRONTIERS IN MAMMARY PATHOLOGY

2

Edited by K.H. Hollmann and J.M. Verley

NEW FRONTIERS IN MAMMARY PATHOLOGY

Volume 2

Edited by
K. H. Hollmann
and
J. M. Verley

Marie-Lannelongue Hospital
Le Plessis Robinson, France

PLENUM PRESS · NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Main entry under title:

New frontiers in mammary pathology, volume 2.

"Proceedings of the Second Symposium of the International Society against Breast Cancer, Contra Cancrum Mammarium, held December 7-10, 1981, in Paris, France"—T.p. verso.

Volume 1 published as: proceedings of the first Symposium on Mammary Pathology.

Bibliography: p.

Includes index.

1. Breast—Cancer—Congresses. I. Hollmann, K. H. II. Verley, J. M. III. International Society against Breast Cancer. IV. Symposium on Mammary Pathology (1st: 1979: Paris, France). New frontiers in mammary pathology. [DNLM: 1. Breast neoplasms—Pathology—Congresses. 2. Breast neoplasms—Therapy—Congresses. W3 IN887T 2nd 1981n / WP 870 I582 1981n]

RC280.B8N477 1982 616.99'449 82-18943

ISBN 0-306-41178-4

Proceedings of the second Symposium of the International Society against Breast Cancer, Contra Cancrum Mammarium, held December 7-10, 1981, in Paris, France

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

NEW FRONTIERS IN MAMMARY PATHOLOGY

Volume 2

PREFACE

The second Symposium of the International Society Against Breast Cancer, Contra Cancrum Mammarium, was held in Paris, at UNESCO, Bonvin Building, on December 7-10, 1981.

Scientists from Belgium, Brazil, France, Great Britain, Greece, Italy, Japan, Portugal, Spain, Switzerland, the USA and Yugoslavia contributed to the success of the Symposium. We would like to cordially thank them all for their participation, particularly the Speakers and the Chairmen of the sessions. Greatest thanks are also due to those who helped in the preparation and organization of the Symposium : the UNESCO officers, the interpreters and the tireless secretarial assistance of Mrs. Josiane Caillaud and Mrs. Jacqueline Garaudel.

The restricted number of participants made contacts and exchanges easy and gave the meeting a personal atmosphere. Again, a large part of the contributors were pathologists, mostly with extensive experience in experimental research. The exchange between laboratory investigation and clinical application was the most fruitful for both, fulfilling one of the goals of Contra Cancrum Mammarium : to bridge the gap between fundamental researchers and practitioners interested in mammary pathology.

It is hoped that the second volume of " New Frontiers in Mammary Pathology " will satisfy the contributors and participants of the Symposium, and provide new stimuli for future collaboration of clinical and basic research in the field.

K.H. Hollmann

CONTENTS

INTRODUCTION: TRENDS IN BREAST CANCER RESEARCH

K.H. Hollmann

Hôpital Marie-Lannelongue, 133 avenue de la Résistance
92350 Le Plessis Robinson, France

Reflecting upon the title of the Symposium " New Frontiers in Mammary Pathology " a quote from a great astronomer comes to mind: when asked by His Majesty " What is new in Astronomy? " He replied: " Does His Majesty already know what is old?

Indeed, before listening to the forum of eminent scientists assembled here, it would be tempting to review what is already known about breast cancer and its etiology. But this being impossible, we must limit remarks to a few points.

Mammary cancer is probably as old as mankind, or even older. Hippocrates was the first to describe the disease and to coin the name καρκίνωμα. But only at the turn of this century did experimental investigation of mammary cancer begin, due to availability of spontaneous, and then serially transplanted, mammary cancers in mice. The pioneer studies of Apolant (1906), Haaland (1911) and others were extended after World War I, when inbred strains of mice were developed by American geneticists such as Little, Strong, McDowell, Maud Slye and others. At that time, it became clear that a genetic and a hormonal influence was involved in mammary carcinogenesis. A third causative factor was elucidated in 1936 when Bittner detected a non-Mendelian influence, the milk factor, later called mammary tumor virus (MTV). It is an RNA-virus, the physical and biochemical properties of which have been clearly established in the past few decades. Several years ago, an increasing number of arguments suggested the presence of a similar viral influence in human breast carcinogenesis. But despite many positive findings such as the presence of viral-like particles, reverse transcriptase, 35 S or 70 S RNA in human milk and molecular hybridization between nucleic acids from the murine mammary tumor virus and those from

human breast cancer, the existence of a human mammary tumor virus could not be demonstrated.

During recent years a flood of publications has appeared concerning factors associated with breast carcinogenesis (see Guerin, Moore, Pinotti, this volume), but no single causative factor could be clearly demonstrated. It appears that mammary carcinogenesis is of multifactorial origin and that inherited factors increasing the susceptibility to breast cancer play an important part. The question of the existence of cancer genes is now under experimental study (see Lasfargues, this volume).

Mammary carcinogenesis is not a single step process but rather a cascade of events. One of the earliest visible lesions is the hyperplastic alveolar nodule, already described by Apolant (1906) and Haaland (1911) in the mammary gland of the mouse. Similar structures were observed in the mammary tissues of other species, including man (Wellings, 1981, New Frontiers in Mammary Pathology, Vol. I). It appears likely that hyperplastic nodules are premalignant lesions and represent an important step toward in situ carcinoma and infiltrating carcinoma (Squartini, Wellings, this volume). From the practical point of view, the pathologist's interpretation of borderline hyperplastic lesions in ducts and lobules of otherwise non-cancerous breasts is extremely important. The therapeutic decision and ultimately the fate of the patient depend on this appraisal (Azzopardi, Koss, Ozzello, this volume).

Other recent research efforts have focused on the detection of tumor cell markers and hormone receptors in histological sections. Such new methods should provide more accurate characterization of tumor cell populations (see Bussolati, Berger, this volume).

Neovascularization of the developing tumor is also related to the multistep process of mammary carcinogenesis. It could be shown that neoplastic cells produce substances which are released into the extracellular space and induce angiogenesis. A prostaglandin and copper ions are two of the components involved in this process (Gullino, this volume). The understanding of angiogenesis may help predict the neoplastic potential of a cell population and may allow development of procedures to block tumor growth.

For clinical routine it is important that pathologic vascularization and abnormal heat transfer in a breast can be visualized by thermography using infrared scanners or liquid crystal films. The superficial thermal pattern of the breast is useful in the detection of early cancers, all the more so that an unequivocal relationship between heat production and the doubling time of tumors has been demonstrated (Gautherie, this volume).

In the clinical diagnosis of breast cancer, needle puncture has now been solidly established, but the practitioner as well as the pathologist must know the pitfalls of the method (see De Brux, this volume). Systematic correlation of cytologic smears and histological sections has shown that aspiration cytology gives reliable results and can provide valuable prognostic information (see Adnet, Bogomoletz, this volume).

Immunity to breast cancer consists of complex interactions which are far from being completely understood. Tumor surface antigens induce immune responses in the host which can be morphologically detected at the tumor border and in the draining lymph nodes. The modifications observed in the latter have a prognostic value. Attempts to relate the reaction in the primary tumor to survival have rarely been made and the results are difficult to evaluate. A better understanding of the immune mechanisms involved in breast cancer would be of great help in designing a rational immunotherapy. Attempts to boost non-specific effector mechanisms through the injection of immunostimulators such as BCG, Corynebacterium parvum or P 40, an extract of Corynebacterium granulosum, give striking morphological results, but clinical efficacy appears limited (see Kraft, Zimmermann, Parodi, Pompidou, Verley and Hollmann, this volume).

The few points stressed here will be developed in the following chapters. The reports will no doubt serve as illustrations for what can be expected in the next future.

ANGIOGENESIS AND RISK OF NEOPLASTIC TRANSFORMATION

Pietro M. Gullino

Laboratory of Pathophysiology, National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205 USA

The parenchyma of human mammary glands bearing a carcinoma shows, in the majority of cases, a large number of hyperplastic outgrowths involving the epithelium of regions distant from the tumor (Sandison, 1962 ; Wellings et al., 1975 ; Wellings and Rice, 1978). These outgrowths are probably the result of an altered turnover of the epithelium (Meyer, 1977 ; Ferguson and Anderson, 1981 ; Masters et al., 1977), and the accumulation of cells with partial distortion of the glandular structure is the morphological expression of this event. Although predominant in the tumor bearing gland, the epithelial outgrowths are not due to the presence of the tumor, instead they precede it. In most women approaching menopause the number of mammary epithelial outgrowths found in the glands probably depends only upon the accuracy of the search (Jensen et al., 1976 ; Jensen and Wellings, 1976). The relationship, if any, between the presence of these outgrowths as indicators of high risk conditions and the onset of a carcinoma is still unclear. Morphological criteria are presently utilized to distinguish "benign" outgrowths from neoplastic proliferation. As one would expect, in a number of cases this distinction is doubtful. In fact, some morphological images are interpreted to represent intermediate stages of progression from benign to malignant proliferation. This condition is not exclusively that of the human gland.

The mammary gland of mice, during natural as well as experimental carcinogenesis, presents a pattern similar to the human gland (Medina, 1973). The mammary gland of rats is much less prone to produce hyperplastic outgrowths, but Rivera et al. (1981) showed that they can be induced with appropriate treatments and she could follow their neoplastic transformation. The three species in which mammary carcinogenesis was most extensively studied show a similar

pathological history. Before the unrestrained growth becomes an established property of the neoplastic epithelium in a limited area of the gland, diffuse changes occur in the parenchyma and the hyperplastic outgrowths are probably one of the more evident manifestations.

Work of the past decade has shown that neoplastic cells produce angiogenic factor(s) (Folkman, 1974 ; Gullino, 1981) which are released in the pericellular fluid, both in vitro (Klagbrun et al., 1976) and in vivo (Ziche et al., 1982). Angiogenesis may be counteracted by inhibitory factors obtained from avascular organs such as cartilage and corpus vitreous (Brem et al., 1977 ; Langer et al., 1980). Since adult mammary epithelium is not angiogenic but the neoplastic epithelium is (Brem et al, 1978 ; Gimbrone and Gullino, 1976) the angiogenic capacity should be acquired in the course of neoplastic transformation. If angiogenesis precedes the appearance of unrestrained growth, i.e., the symptom pathognomonic of neoplasia, detection of angiogenic activity in a tissue normally devoid of it should suggest that progression toward neoplastic transformation is occurring. Acquisition of angiogenic capacity could, therefore, be a marker of populations at high risk. The validity of this hypothesis has been tested in experiments to be summarized next.

Angiogenic response of human mammary tissue : The material for these experiments was obtained from mammary glands removed for carcinomas or from mammary biopsies of benign lesions. Angiogenic capacity was evaluated with the iris test as previously described (Gullino 1981). Briefly, female albino rabbits, 2-3kg, were anesthetized and a few drops of 0.5 % tetracaine were deposited on the cornea. A 2 mm incision was made close to the limbus and the aqueous fluid was drained. A $1mm^3$ fragment of tissue was deposited on the iris through the corneal incision. By gentle pressure on the cornea the fragment adheres to the iris. Post-surgical irritation was usually resolved within 2 days. In positive tests, a discrete ring of capillaries, tortuous, radially oriented around the implant and often forming loops is visible by the 5th or 6th day. Since the iris vessels are oriented in a spoke-like formation, the newly formed vessels have an irregular orientation and form an image quite distinct from the surroundings. The neovascularization process can be followed continuously through the transparent cornea with a slit lamp stereomicroscope. Photographic documentation can be obtained at any desired interval and it is an easy task since the rabbit does not need anesthesia during observation. Strict asepsis must be followed because the onset of an inflammatory process makes the preparation useless. In expert hands the assay is very reliable and permits comparison between a negative and a positive specimen placed in opposite eyes of the same rabbit.

In our experiments the results were scored at day 7 after implantation because by the 8th day the immunological rejection of the

implant was starting. The iris was then removed and several histologic sections were prepared through the implant to ascertain its identity.

Since the cells must be alive for angiogenesis to be induced, the assay was performed immediately after surgery. On the fresh mammary tissue the finding of lobules and ducts is difficult, particularly in the resting gland where they are sparse. To facilitate the task, the specimen was cut in slices about 3 mm thick and places in a Petri dish containing methylene blue chloride (Chroma 11045) in L-15 medium (1 mg/dl) sterilized by filtration. The manipulation was carried on in a laminar flow hood and the dish was left at room temperature for 1-2 hr protected from the effects of direct light. The epithelial cells concentrated the blue color and under the dissecting microscope clusters of alveoli appeared as spots darker than the background. This procedure, applied under strict asepsis, preserved viability of the tissue, did not alter the angiogenic response and did not cause an inflammatory reaction.

Table 1 summarizes the results. Induction of angiogenesis by normal lobules and ducts was an exceptional event but carcinomas were highly angiogenic. A second important observation concerned

TABLE 1

Angiogenic response of normal and neoplastic human breast (iris assay)

	No. of patients	Implants (Positive/Total)	%
Normal lobules and ducts	33	8/200	4
Hyperplastic outgrowths	8	13/50	26
Invasive carcinomas	10	41/63	65
Fibrocystic disease	16	0/96	0
Fibroadenoma	5	0/28	0
Fibrous tissue	22	0/172	0
Adipose tissue	17	1/41	2.4

the hyperplastic lesions. Regardless of the morphological classifications, about one third of the hyperplastic lesions elicited angiogenesis while fibrocystic disease ; i.e., microcysts or walls of macrocysts, fibroadenomas and mammary stroma were not angiogenic. We have been unable to find any difference, morphological or otherwise, between hyperplastic outgrowths that induced or failed to induce angiogenesis.

Although the mechanism of angiogenesis in unknown, the data of Table 1 suggest that angiogenic capacity is a property that may distinguish normal from neoplastic tissues. If this criteria is accepted, a good number of epithelial outgrowths with no morphological indication of being carcinomas have already acquired a property peculiar to carcinomas, i.e., angiogenic capacity. One interpretation of this finding is that epithelial outgrowths with angiogenic capacity are a higher risk of neoplastic transformation, although a carcinoma does not have to develop. Partial support of this interpretation was obtained from the study of angiogenic activity in rodent mammary glands.

Angiogenesis by mammary tissue of rodents : The material for these experiments was obtained from inguinal mammary glands removed from adult C3HA^{vy} and Balb/c mice or Sprague Dawley, Lewis and Fisher 344 rats. Comparison of angiogenic activity was done between the resting gland, i.e., at least one month after the last pregnancy, and primary carcinomas. In the C3HA^{vy} mice the carcinomas were of "spontaneous" origin. They are common in 8-10 month old females (Heston and Vlahakis, 1971). In the rat the carcinomas were produced by DMBA or NMU in females receiving the carcinogen at 50 days of age (puberal period) (Shimkin et al., 1969 ; Gullino et al., 1975).

As shown for the humans, the resting gland of both mice and rats had a negligible angiogenic capacity in the iris assay while the primary tumors were strongly angiogenic (Table 2).

Angiogenesis induced by the hyperplastic outgrowths was examined under two separate conditions. First, the naturally occurring hyperplastic outgrowths were tested. In the C3HA^{vy} mice as in humans, about one third of the outgrowth showed angiogenic activity without any morphological sign of neoplastic transformation (Table 2). Note that almost all of the C3HA^{vy} females have a breast carcinoma before they reach 20 months of age (Heston and Vlahakis, 1971) and morphologically it is common to see tumors originating from the hyperplastic outgrowth.

In Sprague-Dawley rats naturally occurring hyperplastic outgrowths are rare. We detected them by injecting into the lactiferous ducts the methylene blue solution used to reveal the human outgrowth. The angiogenic capacity of the hyperplastic outgrowths was very low

TABLE 2

Angiogenesis by mammary tissue of rodents (iris assay)

	C3HA^{vy} mice Implants positive/total	%	Sprague Dawley rats implants positive/total	%
Resting gland	4/63	6	3/108 3/94	3(a) 3(b)
Carcinomas primary	89/98	90		
NMU induced			98/125	78 (c)
DMBA induced			85/114	75
Hyperplastic outgrowths:				
Primary	7/23	30	7/161	4
Transplanted				
D_1	19/59	32(d)		
D_2	83/109	76(d)		
T18			33/159	21(e)

(a) Virgin females 50 days of age.
(b) Multiparous females about 800 days old.
(c) Fisher 344 females. Rats treated with either carcinogens at 50 days of age. Tumors present in females during their 4th month of life.
(d) Balb/c female mice bearing the transplant in the gland-free fat pad.
(e) Lewis female rats bearing the transplant in the gland-free fat pad.

and not different from that of the resting mammary gland. Note also that natural occurrence of breast carcinomas is very low in Sprague-Dawley rats during the first year of age, and that during treatment