METHODS IN CANCER RESEARCH

Edited by

HARRIS BUSCH

VOLUME VII

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Preface

The initial volumes of this treatise on "Methods in Cancer Research" dealt with a variety of topics that are basic to the problems of experimental approaches to cancer. Although a number of special areas were included the coverage in some specific areas needed to be broadened. In this volume, two major topics are included, namely, some aspects of endocrine tumors and some aspects of carcinogenesis. In the endocrine sections, three chapters deal with mammary tumors and one with ovarian tumorigenesis. I am especially grateful to Dr. Russell Hilf, Department of Biochemistry of the University of Rochester, for his help and advice in the development of this section.

In the section on carcinogenesis, specific types of carcinogenesis are dealt with, particularly with respect to stomach cancer and aflatoxins. In addition, the broad topic of interactions of chemical carcinogenesis with DNA is reviewed. Considerable attention is now being given to intermediate stages of oncogenesis, and the chapter on hyperplastic liver nodules is representative of current interest in this field.

The increasing interest in cancer research has made it essential that the methods involved be as soundly based as possible. It is hoped that in the extension of this treatise the topics covered in this volume are both timely and useful to workers in experimental oncology.

HARRIS BUSCH

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ENDOCRINE TUMORS



CHAPTER I

PRENEOPLASTIC LESIONS IN MOUSE MAMMARY TUMORIGENESIS

DANIEL MEDINA

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I. Introduction*

The process of neoplastic development is a multistage phenomenon. Upon exposure to a carcinogen, a cell(s) undergoes a series of qualitative and quantitative changes which ultimately are expressed as a neoplasm. Neoplasms also undergo a series of qualitative changes which determine their behavior patterns. The concept that explains the qualitative changes which determine a neoplasm's behavior has been termed "tumor progression" (Foulds 1956, 1964). The qualitative and quantitative changes undergone by a normal cell population exposed to a carcinogen can be conceived as a preneoplastic progression. This phenomenon of progressive stages in development of neoplasms was recognized at the turn of the century. Haaland (1911) recognized the existence and stressed the biological significance of hyperplastic changes which preceded neoplasia. The concept of multistage development of neoplasia gained general acceptance after the studies by Rous. Mottram. Berenblum. Boutwell, and others (Rous and Kidd, 1941; MacKenzie and Rous, 1941; Friedewald and Rous, 1944, 1950; Mottram, 1944a,b; Berenblum and Shubik, 1947, 1949; Berenblum, 1954, 1958, 1964; Boutwell, 1964), on experimental skin tumorigenesis. They demonstrated that the neopletic transformation was a multistage phenomenon rather than a single-stage phenomenon. The multistage nature of carcinogenesis has been demonstrated subsequently for liver (Farber, 1963, 1968; Epstein et al., 1967; Merkow et al., 1967), mammary gland (DeOme et al., 1959a; Blair et al., 1962), pituitary (Furth, 1963), and for a variety of human cancers as evidenced by the terms "carcinoma in situ," "precancerous cystic hyperplasia," etc., commonly used by pathologists (Willis, 1967).

The development of model systems to study the multistage process of the neoplastic transformation has been hampered by the inability to define, observe, and/or manipulate the intermediate cell populations. This has been particularly true for skin carcinogenesis where intermediate stages are postulated because neoplasms arise as a consequence of one agent which initiates alterations in cell populations and a separate agent(s) which subsequently promotes the initiated alterations. Intermediate stages in the skin are difficult to visualize. To analyze alterations at the cellular and molecular levels, however, it is important to use model systems in which the intermediate populations can be visualized, defined, and manipulated. By gaining an understanding of the biological properties of the intermediate populations

^{*}Abbreviations used in this chapter: HAN, hyperplastic alveolar nodule; MTV, mammary tumor virus; NIV, nodule-inducing virus; GR, GR virus; MCA, 3-methylcholanthrene; DMBA, 7,12-dimethylbenzanthracene; MER, methanol-extracted residue of tubercle bacillus; ALS, antilymphocyte serum.

and analyzing their cellular and molecular properties, one can obtain insight into the essential alteration(s) of neoplasms. The mammary gland and liver are the two major systems in which intermediate populations exist. The ability to transplant normal, preneoplastic, and neoplastic mammary tissues into their natural site, i.e., the mammary fat pad, offers the investigator the advantage of studing tumorigenesis under a variety of experimental conditions for a variety of different cell populations.

It is the purpose of this article to acquaint graduate students and researchers inexperienced in this field with (1) the simple methodology involved in the induction, observation, and transplantation of mammary preneoplastic nodules, (2) the biological properties of mammary nodules, and (3) some experiments demonstrating the effects of carcinogens on different nodule cell populations.

II. The Preneoplastic Nature of Hyperplastic Alveolar Nodules (Methodology of Noduligenesis)

A. EARLY OBSERVATIONS BEFORE 1955

The principal preneoplastic lesion in mouse mammary glands is the hyperplastic alveolar nodule (HAN) which is a focus of hyperplastic lobulo-alveolar development in an area of nonstimulated mammary gland (i.e., ductal). A number of investigators have reported the presence of hyperplastic lesions in the mouse mammary gland, and have described the correlation between the occurrence of these lesions and mammary carcinoma. Apolant (1906) first described these lesions as local hypertrophies of the mammary gland which resembled adenomas. Haaland (1911), in an extensive description of the pathological changes associated with mammary cancer in mice, pointed out the increased frequency of hyperplastic lesions in mice with cancer and with age, and emphasized that gradations existed between hyperplastic nodules and mammary cancer.

The development and widespread use of inbred strains of mice allowed the comparison of mammary gland structures found in high and low mammary cancer strains. Fekete (1938) described persistently growing areas of mammary parenchyma cells (hyperplasias) that eventually transformed into mammary cancers. Gardner et al. (1939) described hyperplastic ductal lesions as well as hyperplastic alveolar lesions in three high mammary tumor strains, and suggested that these lesions progressed through a series of changes into malignant neoplasia. Hyperplastic alveolar nodules also have been noted and described histologically by Taylor and Waltman (1940), van Gulik and Korteweg (1940), Huseby and Bittner (1946), Kirschbaum et al.

(1946), Pullinger (1947, 1949), Jones (1951, 1956), Mühlbock and Tengbergen (1952), Foulds (1956), Bern et al. (1957), Squartini (1959), DeOme et al. (1959a), Nandi (1963a), and Medina and DeOme (1968) in a variety of mouse strains which include A, Balb/c, Balb/cfC3H, C3H, C3Hf, DBA, I, R III, and their hybrids. Similar nodules have also been described in rats (Tuba et al., 1953; Middleton, 1965; Beuving et al., 1967a,b; Faulkin et al., 1967).

mexperienced in this field with (1) the simple freeholders with the B. Occurrence and Induction of HAN

Hyperplastic alveolar nodules can be induced in mice by the mammary tumor viruses (DeOme et al., 1959a; Nandi, 1963a), chemical carcinogens (Faulkin, 1966), X-irradiation (Faulkin, 1964), and prolonged hormone stimulation (Medina and DeOme, 1968, 1970a), and in the rat by chemical carcinogens (Beuving et al., 1967a,b) and X-irradiation (Faulkín et al., 1967).

Hyperplastic alveolar nodules are found in high mammary tumor strains

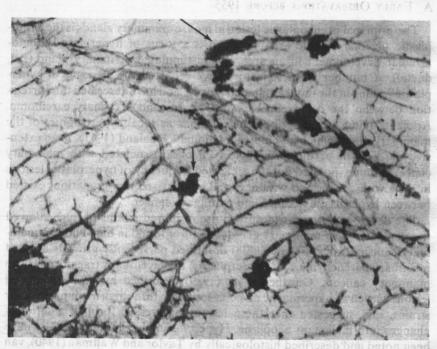


FIG. 1. Whole mount of mammary gland from a MTV-positive C3H mouse. Several HAN (arrows) are seen in addition to a mammary tumor in lower left corner. × 13.

(i.e., C3H, A) which carry the mammary tumor virus (MTV) and the nodule-inducing virus (NIV) (Pitelka, et al., 1960; DeOme, 1963), in high mammary tumor strains which carry GR virus (i.e., GR/A) (Mühbock and Bentvelzen, 1969), in low mammary tumor strains which carry just NIV (i.e., C3Hf) (Pitelka et al., 1960, 1964), and in MTV-free, NIV-free mice (Medina and DeOme, 1968, 1970a). Hyperplastic alveolar nodules can be induced in susceptible strains by MTV if MTV is introduced by foster-nursing (Nandi, 1963a,b), intraperitoneal injection of cell-free extracts of virus-containing normal mammary tissues and tumors (Nandi, 1963a,b), or intraperitoneal injection of blood from virus-positive mice (Nandi et al., 1965, 1966). HAN are induced by NIV in susceptible strains only be mating the susceptible strain with an NIV-positive strain. So far, NIV induction of HAN has not been successful via the foster-nursing or injection routes (DeOme, 1963; DeOme and Nandi, 1966; Nandi, 1967). The induction of HAN by the GR virus has not been systematically examined.

Hyperplastic alveolar nodules can be induced by a variety of chemical carcinogens which include 3-methylcholanthrene (Faulkin, 1966), 7.12dimethylbenzanthracene, urethane, 1,2-benz[a] pyrene, and 2,7-fluorenediamine (Faulkin, personal communication). The polycyclic hydrocarbons (3-MCA, 7,12-DMBA, BP) are dissolved in cottonseed oil and administered by gastric intubation. The carcinogen is administered to unanesthetized mice by means of a blunted 16-gage needle ending in a silver soldered bulb. Watersoluble carcinogens (urethan) are dissolved in distilled water and injected intraperitoneally (Haran-Ghera, 1963, Medina and DeOme, 1970b). The ability of the above chemical carcinogens to induce HAN is greatly facilitated by the addition of exogenous hormone stimulation (Table 1). Viralinduced noduligenesis is also greatly facilitated by hormone stimulation. especially in MTV-positive A (Blair et al., 1960) and Balb/cfC3H mice (Medina et al., 1970), and in NIV-positive C3Hf/Crgl mice (Medina et al., 1970). Prolonged hormone stimulation of the mammary gland is achieved by the transplantation of pituitary glands taken from 3- to 5-month-old isogenic male mice. One or two pituitary glands are transplanted into the inguinal mammary fat pads or under the kidney capsule. In both cases, pituitary glands, free from hypothalamic control, secrete primarily luteotropic hormone. Pituitary isografts in intact mice induce recurrent pseudopregnancy and lobuloalveolar development of the mammary glands (Loeb and Kirtz, 1939; Mühlbock and Boot, 1959; Bardin et al., 1966). The pituitary isograft under the kidney affords a more uniform and rapid hormone stimulation and therefore is the site of choice for rapid noduligenesis. However, tumorigenesis in (C3H × O20) F₁ mice is not significantly affected by the site of pituitary graft (Dux and Mühlbock, 1969).

Prolonged hormone stimulation by pituitary isografts or forced breeding

TABLE 1
EFFECT OF HORMONE STIMULATION OF NODULIGENESIS IN MICE

	;		No. of mice with HAN/total No. of		Average No. HAN in HAN-bearing	
Strain	Carcinogen	Hormone	mice	Percent	mice	Reference
Balb/cfC3H a	MTV	1	6/270	0	0	Medina et al. (1970)
Balb/cfC3Ha	MTV	+	13/15	87	25.0	Medina et al. (1970)
C3Hf*	NIV		0/35	0	0	Medina et al. (1970)
C3Hf*	NIV	+	15/18	83	30.0	Medina et al. (1970)
Balb/c ^a	MCA	. 1	1/15	6.5	1.0	Faulkin (1966)
Balb/ca	MCA	+	11/11	<u>8</u>	4.0	Faulkin (1966)
Balb/ca	i	+	0/13	0	0	Faulkin (1966)
^a 20-week-old mice. ^b 48-week-old mice.		·			·	

will induce nodules in MTV-free, NIV-free Balb/cCrgl (Medina and DeOme, 1968, 1970a). In these cases, nodules are rare and appear late in the second year of life.

Hyperplastic alveolar nodules in Sprague-Dawley and Fischer rats can be induced by 7,12-dimethylbenzanthracene (Beuving et al., 1967a,b), and by X-irradiation (Faulkin et al., 1967). Exogenous hormone stimulation is not necessary for rapid noduligenesis in the rat as it is in mice. The formation of hyperplastic alveolar nodules can be inhibited by prolonged lactation (Zeilmaker, 1968), by dietary restriction of phenylalanine (Hui et al., 1971a,b), by immunological activators (Weiss et al., 1966), by thymectomy (Sakakura and Nishizuka, 1967; Heppner, 1970; Squartini, 1971), splenectomy (Squartini, 1971), and by administration of antilymphocyte serum (Lappé and Blair, 1970).

C. VISUALIZATION OF HAN IN SITU

Hyperplastic alveolar nodules are recognizable within the mammary fat pads of live, anesthetized mice because of their yellowish coloration (color due to content of hemosiderin; Mühlbock and Tengbergen, 1952). The mammary glands of live, anesthetized mice are observed under a dissecting microscope at 8 to 12 × magnification with reflected light. The observation of mammary glands for HAN in live, anesthetized mice requires some experience and generally is not adequate in albino mice or in thick mammary fat pads (i.e., No. 4 and 5 mammary fat pads in mice). A more satisfactory procedure is to use a combination of hormones to stimulate secretory activity in nodules present in the mammary gland (Bern et al., 1959). The hormones somatotropin, luteotropin, and hydrocortisone acetate are injected subcutaneously for 3 consecutive days prior to the time of observation of the mammary glands (see appendix B for concentrations and procedure). Hyperplastic alveolar nodules, if present, will appear as compact alveolar foci of milky white-filled material. Lesions of scattered alveoli filled with chalkywhite material are to be avoided since there are not HAN and will give rise only to ductal outgrowths upon transplantation (Medina, unpublished observation).

The induction of selective milk secretion in nodules in rat mammary glands has not been successful (Beuving et al., 1967b). In rats, HAN can be identified with intraductal injections of whole blood (Beuving et al., 1967b). The hyperplastic nodules become filled with blood and stand out in contrast to the creamy color of the adipose tissue. This method works for HAN in rats, but fibrous nodules and ductal hyperplasias do not become filled with blood (Beuving et al., 1967b).