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Animal and In Vitro Models

Prognostic factors in MNU and DMBA-induced mammary tumors in female rats

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ABSTRACT

Chemically-induced mammary tumors in rats by the carcinogens 1-methyl-1-nitrosourea- (MNU) and 7,12-dimethylbenz[a]anthracene (DMBA) are the most widely used models for studies related with human breast cancer. This study aimed to evaluate the immunoexpression of the prognostic factors estrogen receptor α (ERα), progesterone receptor (PR) and Ki-67, in MNU and DMBA-induced rat mammary tumors, in order to know the model that best suits to woman breast cancer. Twenty-eight MNU-induced and 16 DMBA-induced mammary tumors in virgin female Sprague-Dawley rats were analyzed. The expression of the prognostic markers ERα, PR and Ki-67 proliferation index (Ki-67 PI) was assessed by immunohistochemistry. Mitotic activity index (MAI) was also evaluated. More than one histological pattern was identified in each mammary tumor. Carcinomas constituted the lesions most frequently induced by both carcinogens: 33 MNU-induced carcinomas and 23 DMBA-induced carcinomas. All MNU and DMBA-induced mammary carcinomas were ER^+/PR^+, with a higher expression of ERα when compared with PR. Tumors' weight, the expression of ERα, PR, Ki-67 PI and MAI were higher in MNU-induced mammary carcinomas when compared with the DMBA-induced ones. Statistically significant differences between groups were observed for ERα, PR and MAI ($p < 0.05$). The higher KI-67 PI and MAI in MNU-induced mammary carcinomas are suggestive of a higher aggressiveness of these carcinomas when compared with the DMBA-induced ones, and consequently a worse response to the therapy and a worse prognosis. In this way, the use of the rat model of MNU-induced mammary tumors is advised in experimental protocols aiming to study more aggressive mammary tumors within the group of double-positive mammary tumors (ER^+/PR^+).

1. Introduction

The 1-methyl-1-nitrosourea (MNU) and 7,12-dimethylbenz[a]anthracene (DMBA) are the two most widely used chemical carcinogens for the induction of mammary tumor development in female rats [1]. A single administration of MNU or DMBA induces tumor development in female rats within eight to ten weeks after the administration [2–6]. Although both carcinogenic agents induce mammary tumor development through DNA alkylation, they are different in terms of their metabolism. MNU does not require metabolic activation, being classified as a direct-acting alkylating agent [7,8]. Unlike MNU, DMBA is an indirect carcinogen that requires prior metabolic activation by liver cytochrome P450 enzymes. In this way, the carcinogenic activity of DMBA is slower when compared with MNU, which results in a longer latency period for DMBA-induced mammary tumors [9]. Both carcinogens modify the expression of several micro RNAs (miRNAs) in a short period of time, such as miRNA-21, miRNA-34a and miRNA-155, supporting their role in initial process of chemical carcinogenesis. The effect

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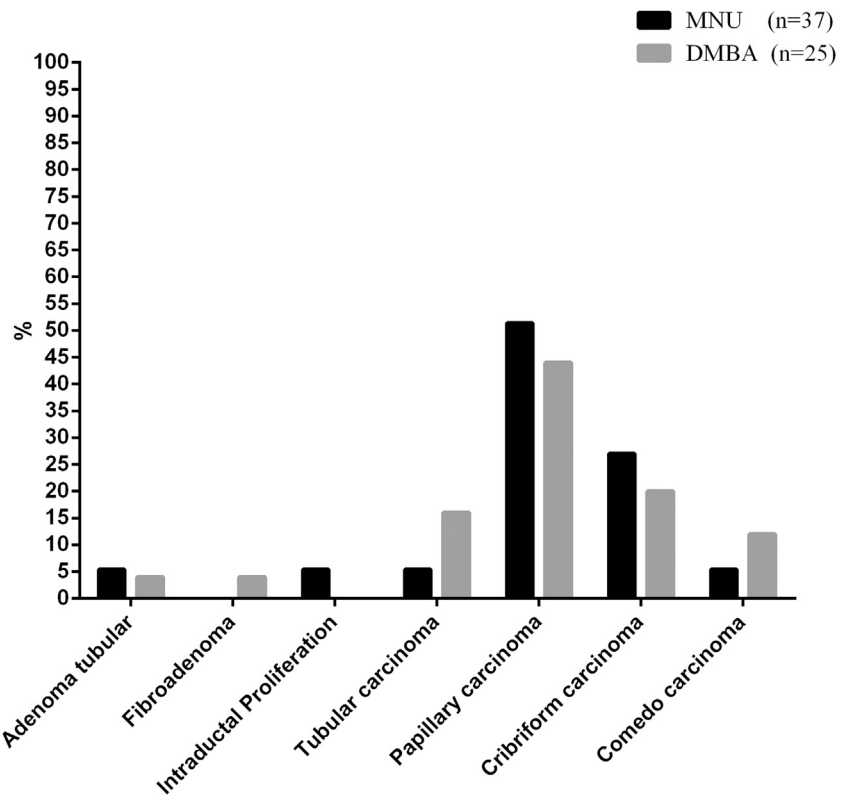

Fig. 1. Percentage of histological patterns observed in MNU and DMBA-induced mammary tumors.

Table 1
Immunoexpression of ERα and PR, Ki-67 PI and MAI for each MNU or DMBA-induced mammary carcinoma (mean ± S.D).

Histological pattern	MNU					DMBA				
	n	ERα(%)	PR(%)	Ki-67 PI	MAI	n	ERα(%)	PR(%)	Ki-67 PI	MAI
Tubular carcinoma	2	57.00 ± 19.80	44.10 ± 3.25	1.05 ± 1.48	0.70 ± 0.42	4	35.35 ± 14.50	13.23 ± 9.45	3.54 ± 1.45	0.05 ± 0.06
Papillary carcinoma	19	47.08 ± 9.77	46.27 ± 10.17*	3.86 ± 4.55	1.05 ± 1.08	11	37.29 ± 7.69	18.26 ± 10.48	3.81 ± 0.84	0.43 ± 0.35
Cribriform carcinoma	10	54.97 ± 11.57	33.64 ± 8.62	11.88 ± 13.04	2.04 ± 1.33‡	5	44.10 ± 12.18	33.68 ± 10.97	4.41 ± 1.40	0.34 ± 0.35
Comedo carcinoma	2	51.75 ± 36.98	16.10 ± 16.12	2.65 ± 2.62	5.35 ± 0.64**	3	48.47 ± 10.92	24.53 ± 12.82	4.84 ± 1.09	0.97 ± 0.025

* Statistically different from MNU-induced cribriform and comedo carcinomas, and DMBA-induced tubular, papillary and comedo carcinomas ($p < 0.05$).
** Statistically different from all MNU and DMBA-induced carcinomas ($p < 0.05$).
‡ Statistically different from DMBA-induced tubular, papillary and cribriform carcinomas ($p < 0.05$).

of MNU on miRNAs is dominant when compared with DMBA due to the direct acting effect of this carcinogen in contrast with DMBA [10,11].

Estrogen and progesterone are steroid hormones that play an important role in sexual differentiation and fertility [12,13]. They act by binding to specific nuclear receptors commonly colocalized within the same cell: estrogen receptor (ER) (isoforms α and β) and progesterone receptor (PR) (isoforms A and B) [14,15]. Similarly to that observed in woman and mouse mammary gland, the rat PR is regulated by ER that sustains its high expression. These receptors are considered prognostic factors for mammary cancer. The simultaneous expression of both receptors (ER^+/PR^+) is suggestive of less aggressiveness of mammary tumors and better response to hormone therapy, when compared with those mammary tumors that only express one of these receptors (ER^-/PR^+, ER^+/PR^-) or none of them (ER^-/PR^-). Approximately 70% of human mammary tumors express both hormone receptors [16].

Beyond the hormone receptors, the Ki-67 is involved in the cellular proliferation and despite the fact its role in breast cancer management is uncertain, nowadays it is considered in conjunction with the hormone receptors as an important prognostic marker in breast cancer [17,18].

Although the histological characteristics of MNU and DMBA-induced mammary tumors have been well-described, there are no previous studies comparing the immunoexpression of the prognostic factors (ERα, PR and Ki-67) between them. So, this study aimed to evaluate the immunoexpression of these prognostic factors in MNU and DMBA-induced rat mammary tumors, in order to know the model that best suits to woman breast cancer.

2. Materials and methods

2.1. Animals and mammary tumors

Forty-four chemically-induced mammary tumors developed in Sprague-Dawley female rats (28 MNU-induced mammary tumors in 11 animals and 16 DMBA-induced mammary tumors in 12 animals) were used. Briefly, female rats were obtained from Harlan Interfauna Inc. (Barcelona, Spain) and maintained in polycarbonate cages (1500U Eurostandard Type IV S, Tecniplast, Buguggiate, Italy) with corncob for bedding (Mucedola, Italy), at a temperature of 23 ± 2 °C and a humidity of 50 ± 10%, with light/dark cycle (12 h:12 h). Water and food (Standard diet 4RF21®, Mucedola, Italy) were supplied *ad libitum*. MNU or DMBA were administered at