ORIGINAL ARTICLE

Mouse spontaneous mammary adenocarcinoma as a suitable model of breast cancer in Iran

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ABSTRACT

Background: Breast cancer is a complicated disease and has various clinical manifestations and prognosis. The aim of this study is to make a breast cancer model suitable for basic, preclinical and clinical studies.

Methods: Five virgin Bulb/c mice aged 6 weeks and spontaneous Murine Mammary Adenocarcinoma Cells (MMAC) were used. After anesthesia induction, 1-3 million of MMACs were subcutaneously injected into the mice's right flank. Four weeks after the tumor induction, tumor size in both length and width were weekly measured using caliper for 4 weeks. Histopathology studies were done 2 months later by Scraff-Boom-Richardson grading.

Results: Visible tumors were appeared after 4 weeks. Take-rate of all cells was nearly 100%. Tumor sizes reached 0.6±0.1 mm3, 1.1±0.3, 1.68±0.5 and 3±0.7 cm3 with at fifth, sixth, seventh and eighth weeks respectively. The histopathological studies using H & E method showed invasive ductal carcinoma, grade II/III.

Conclusion: Elucidation of the molecular mechanisms of breast cancer progression and metastasis has extremely gained from mouse models in which some stages of tumor progression are recapitulated.

Keywords: Mice, adenocarcinoma model, breast cancer.

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زمینه و هدف: سرطان پستان یک بیماری پیچیده با علائم کلینیکی و پیش آگهی متفاوت می باشد. هدف مطالعه حاضر، ایجاد مدل حیوانی مناسب سرطان جهت مطالعات پایه ای، پیش بالینی و بالینی در زمینه های مختلف سرطان پستان می باشد.

مواد و روشها : رده های سلولی آدنوکارسینومای پستانی موش به همراه ۵ سر موش نژاد بالب سی از انستیتو پاستور ایران خریداری شده است. بعد از بیهوشی، مقدار ۳-۱ میلیون از رده های سلولی به صورت زیرجلدی در قسمت پهلوی راست یا چپ حیوانات تزریق گردید. ٤ هفته بعد از تزریق، اندازه تومور در محور طول و عرض بطور هفتگی، بمدت ٤ هفته با کولیس ورنیکه اندازه گیری شد. مطالعات هیستوپاتولوژی با روش Scraff-Boom-Richardson grading در پایان ماه دوم انجام گردید.

یافته ها: تومورها در پایان هفته چهارم با چشم قابل رویت بود. اندازه تومورها در هفته های پنجم، ششم، هفتم و هشتم به ترتیب 0.1±0.6، 0.3±1.1، 0.5±1.68 و 0.7±3 میلی متر مکعب رسیده است. مطالعات هیستوپاتولوژی با استفاده از رنگ آمیزی روتین هماتوکسیلین-ائوزین، داکتال کارسینوما پستان با گرید ۳/۲ را نشان داد.

نتیجه گیری : مطالعه حاضر نشان داد که بیان و شناسایی مکانیسم های مولکولی سرطان پستان و متاستازهای آن می تواند توسط ایجاد مدلهای مختلف حیوانی سرطان پستان بدست آید.

واژه های کلیدی: موش، آدنوکارسینوما، سرطان پستان

Introduction

Breast cancer is the most common form of diagnosed cancer and the second leading cause of death in western women. It has different prevalence among countries; the highest prevalence is in American white women and the lowest is in Chinese and Japanese women.[1] The highest rate of the disease is in developed European countries and the North America, and the peak mortality rate, 14,000 deaths per annum, is seen between the ages of 40 to 50 years. Breast cancer is the second common cancer among Iranian women with the prevalence of 120 in 100,000.[2-4] Breast cancer is a complicated disease and has various clinical manifestations and prognosis. In the recent past, our understanding of breast cancer progression and metastasis has greatly profited from the use of genetically modified mouse models and advanced transplantation techniques. These findings highlight the importance of animal models to make new therapeutic strategies against primary tumor or its metastasis and reduce mortality rate among the patients.[5]

Due to high prevalence of breast cancer in the world and Iran, many basic studies in vitro or/and in vivo are performing in cancer institutes. These days, the xerograph and syngeneic models of tumors are accepted as the best animal models in basic or pre-clinical breast cancer studies. The aim of this study is to make breast cancer model suitable for basic, pre-clinical and clinical studies.

Materials and Methods

Five virgin Bulb/c mice aged 6 weeks purchased from Iran Pasteur institute and housed in a temperature-controlled room at 12 h light/dark cycle.

Cell culture

The vial of spontaneous Murine Mammary Adenocarcinoma Cells (MMAC) (derived from M05 cell line) was provided from Iran Pasteur Institute. The cells rinsed with cold PBS, minced with a scalpel, and transferred into 10 ml of 0.25% trypsin–EDTA for 10 min incubated at 37°C in 5% CO2. The cellular material was then washed four times with serum-free DMEM. The cells were then recovered by centrifugation and transferred to 1.6 mL of low calcium medium in a swine skin gelatin–coated T-25 flask to select for cell growth. To avoid the toxic effects of DMSO, serum and culture medium was diluted to 10 times. The next day, the supernatant (enriched for tumor cells) was transferred to a new flask. Once the cells adhered, 3 mL of additional medium were added. The total number of cells was approximately 50-55 million after centrifuging and concentration to 2×106 ml.

Tumor induction

Anesthesia was preformed using mice anesthesia cocktail (60.6 mg/ml ketamine and 6.06 mg/ml xylazine, i.p). 1-3 million of MMAC were subcutaneously injected into the right flank. Tumor growth was visible 4 weeks post injection.

Tumor growth measuring

Four weeks after the tumor induction, tumor size in both length and width were weekly measured using caliper for 4 weeks.

Histopathology study

After euthanasia of animals, the tumor was completely removed. Two mm thickness serial slices were made in longitudinal axis, and immediately fixed in 10% formalin, passaged and embedded in paraffin. Then the paraffin blocks were sectioned by 3-5 µm thickness for hematoxylin and eosin (H & E) staining. For each sample, 9-12 serial sections were used for H & E examination. The slides were studied by OLYMPUS-BX51 microscope and digital photos taken with OLYMPUS-DP12 camera and graded by Scraff-Boom-Richardson method.[6]

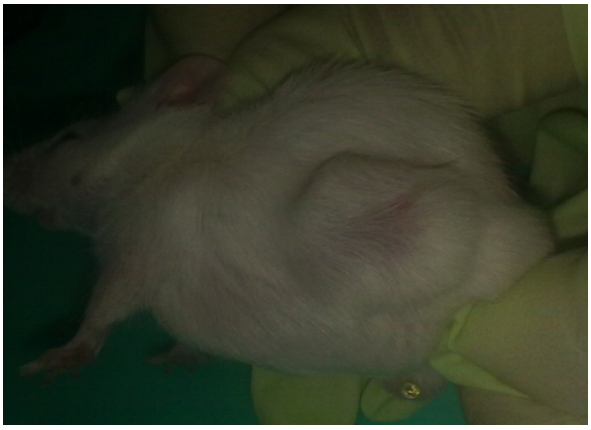
Results

We found visible tumor at weeks fifth, sixth, seventh and eighth with size 0.6 mm3, 1.1, 1.68 and 3 cm3, respectively (*Fig. 1*).

The histopathological studies using H & E method showed Invasive Ductal Carcinoma or Not Otherwise Specified (NOS), grade II/III based on Scraff-Boom-Richardson grading scheme and also with data of the following three below parameters (*Fig. 2*):

- Tubule formation 1/3 - Nuclear polymorphism 2/3
- Mitoses 3/3

A



B

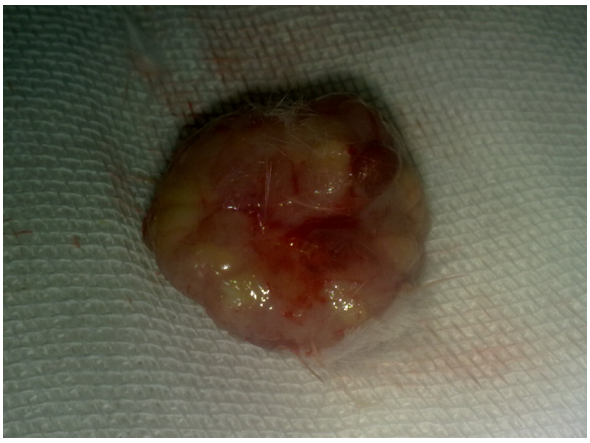
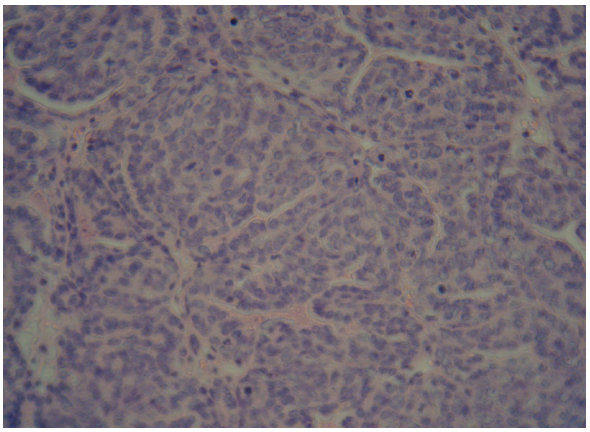


Figure 1- A Murine Mammary Adenocarcinoma Tumor model

A



B

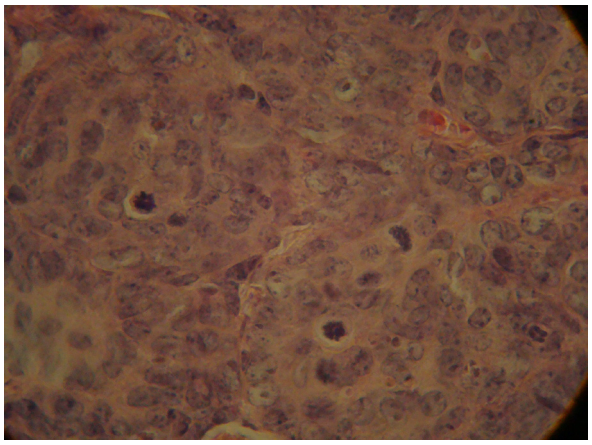


Figure 2- Histo-pathological findings based on Scraff-Bloom-Richardson scheme.
A: The tumor shows more than 75% tubule formation (1/3) (x10).
B: The nuclear polymorphism is moderate visible in size and shape (2/3). Also abundant mitotic (>11 mitoses per 10 field of x40) are seen (score 3/3) (x40).

Discussion

The focus of our study was on the receptor dependent of mice breast cancer through the use of steroid dependent mouse breast cancer models. For this, we have developed models for steroid dependent-induced breast cancer associated 100% take-rate of tumor. We can use these models to (i) investigate cell line relations in mammary tumorigenesis; (ii) identify malignant changes underlying breast tumorigenesis; (iii) study the role of steroid dependent in breast cancer development.

In the recent past, our understanding of breast cancer headway and metastasis has really profited from the use of genetically modified mouse models and highly developed transplantation experiments. A serious step towards the generation of mouse models of breast cancer is the understanding of the molecular pathways underlying mammary carcinogenesis. The understanding gained on the several mechanisms causative to tumor progression can be used to propose and create well again mouse models. There are various ways to imitate breast cancer growth and metastasis in tumor transplantation techniques.

Animal models of breast cancer have been generally used to study a variety of aspects of breast cancer biology. A number of articles describe the characteristics of these models and their importance to the human disease. Images of each of the main models, human breast cancer

cell line xenografts, also are included. Animal models of cancer, mostly mouse models of cancer, are frequently used to study tumor biology and grow new approaches to successful human cancer. Previous investigations in modeling cancer in animals' laboratory, particularly experimental mice, have highly developed our insights into the biology of cancer. Scientists are repetitively upward new animal technology to well again model human cancers. As the most commonly used systems in cancer drug development, mouse cancer models have helped us circumvent lots of ethical and economical problems for human cancer experiments. As more and younger scientists begin to be involved in animal experiments of cancer research, there is an increasing demand to have a different models to show them the basics about mouse techniques and cancer models. Our aim was to make this new model of in vivo experiments for mouse new users, which will, optimistically, keep their animals and time.

These models enable researchers to study the onset and progression of the disease, and understand, in profundity, the molecular events that contribute to the development and extend of breast cancer. They also provide a helpful biological method, to simulate human physiological conditions, proper for trying therapeutics that can potentially profit patients. An important example is the development of the spontaneous mouse model of breast cancer and the succeeding examination that cyclo-oxygenase expression is an early occurrence in breast carcinogenesis. Genetic disorder of the cyclo-oxygenase-2 (COX-2) gene or inhibition of the activity of COX-2 with chemical inhibitors reduced the polyp burden in mice.[7-8] In the future, it will be crucial to produce mouse models that more completely replicate human breast carcinogenesis. The quest for such improved models has just begun.

Conclusion

Elucidation of the molecular mechanisms underlying breast cancer development and metastasis has gained a great deal from mouse models in which the numerous stages of tumor progression are recapitulated.

Conflict of interest

The Author(s) declare(s) that they have no conflict of interest to disclose.

Acknowledgments

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