Histopathological Changes In Female Albino Mice Resistant To Transplanted Mammary Adenocarcinoma Cell Line

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Abstract

The present study was carried out to observe histopathological changes in tumor mass of transplanted mammary adenocarcinoma tumor cells, spleen, liver and kidney of female albino mice successfully showed growth of tumor cells (group I) following subcutaneous transplantation with mammary gland adenocarcinoma cell line, comparison to the other group of female albino mice not exhibited any tumor growth post transplantation (group II). Group (III) of female albino mice were injected subcutaneously with phosphate buffer saline and served as normal healthy control group.

The cell line which had been used in the study was mice mammary gland adenocarcinoma (Ahmad-Majed 2003) adapted for *in vivo* transplantation supplied from Iraqi Center for Cancer and Medical Genetic Researches (*ICCMGR*).Thirty female albino mice were used in this study. Twenty female albino mice transplanted subcutaneously with mammary gland adenocarcinoma cell line. Six female albino mice showed successful growth of transplanted tumor cells as a tumor mass after 10 days post transplantation, tumor mass showed increase in their size at the end of experiment(40days) post transplantation and considered as a (group I). while fourteen female albino mice not exhibited any growth of tumor cells after 10 days post transplantation until the end of experiment (40days) post transplantation and considered as (group II).while10 female albino mice were injected subcutaneously with phosphate buffer saline and served as normal healthy control group (group III) .

At the end of the experiment (40) days post transplanted, animals of all groups were scarified and tissue of growth tumor mass in group I, spleen, liver and kidney of all groups were taken for histopathological examination.

Histopathological results of tumor mass of group (I), revealed extensive tumor masses consisted of aciner like structure, trabecular and island of tumor cells, the tumor cells are hyperchromatic, pleomorphic, increase the nuclear–cytoplasmic ratio with highly blood supply, the tumor masses also showed extensive area of coagulation necrosis. Also histopathological sections showed depletion of white pulp region of spleen, vacuolar degeneration in hepatocytes and atrophy of glomarular tuft with vacuolar degeneration of renal tubules. While histopathological sections of group (II) showed extensive hyperplasia of white pulp in the periarterial sheath (T and B cell region) of spleen, mononuclear inflammatory cells infiltration (lymphocyte and macrophage) showed around central vein, portal area of liver. In addition, the kidney revealed infiltration of mononuclear inflammatory cells in interstitial tissue and around B.V. Microscopic examination of (group III) showed no histopathological changes in spleen, liver and kidney compared with (group I (and (group II).

التغيرات المرضية النسجية لأناث الفئران البيضاء التي قاومت غرس خط سرطانة الغدة اللبنية

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الخلاصة

شملت الدراسة الحالية التغيرات المرضية النسجية في الكتلة الورمية من خلايا سرطانة الغدة اللبنية المغروسة، الطحال، الكبد والكلى في إناث الفئران البيضاء التي تم نجاح نمو خلايا سرطانة الغدة اللبنية المغروسة فيها تحت الجلد (المجموعة الأولى)، بالمقارنة مع المجموعة الأخرى من إناث الفئران البيضاء التي لم تظهر أ ي علامات للنمو السر طاني بعد الحقن فيها تحت الجلد أيضاً (المجموعة الثانية). في حين تم حقن محلول داريء الفوسفات الملحي تحت الجلد في إناث الفئران البيضاء التي اعتبرت كمجموعة سيطرة (مجموعة الثالثة). ان خط الخلايا المستعمل في هذه الدراسة هو خط سرطانة الغدة اللبنية (احمد– مجيد 2003) المهيئ للغرس داخل الجسم الحي المجهز من المركز العراقي لبحوث السرطان والوراثة الطبية. تم استعمال30انثى من الفئر ان البيضاء في هذه التجربة. حقنت 20 فأرة تحت الجلد بخط سرطان الغدة اللبنية والتي ظهر نمو الخلايا السرطانية في 6 أناث بنجاح على شكل كتلة ورمية بعد 10 ايام من الغرس والتي استمر حجم الورم بالنمو حتى نهاية فترة التجربة 40 يوم بعد الغر س واعتبرت (المجموعة الأولى) في حين لم تظهر أ ي علامات للنمو السرطاني في 14 أنثى فأر بعد 10 ايام من الغر س وحتى نهاية فترة التجربة 40 يوم من الغرس واعتبرت (المجموعة الثانية). بينما تم حقن 10 إناث فئران بيضاء تحت الجلد بمحلول داريء الفوسفات الملحي والتي اعتبرت كمجموعة سيطرة (المجموعة الثالثة). عند نهاية فترة التجربة 40 يوم بعد الغر س، تم التضحية بالحيواناتو اخذ انسجة من الكتلة الورمية من المجموعة الأولى والطحال والكبد والكلى للفحص النسجي من كل مجاميع الحيوانات.

أظهر ت نتائج الفحص المرضي النسجي للكتلة الورمية في (المجموعة الاولى) كتلة من الخلايا الورمية والتي كونت اشكال شبيهة بالغدد وحواجز و جزر من الخلايا الورمية والتي تميزت هذه الخلايا بفرط الصبغين وتعدد اشكال الخلايا الورمية مع زيادة نسبة حجم النواة إلى الهيولي وزيادة التجهيز الدموي. كذلك اظهرت بعض المناطق انقسامات خيطية مع مساحات واسعة من المناطق النخرية التجلطية. كذلك أظهر الفحص المرضي النسجي نفاد خلايا منطقة اللب الأبيض في الطحال وتنكس خلوي في خلايا الكبد وظمور في المة الكبيبية مع تنكس خلوي في النبيبات الكلوية. بينما أظهر الفحص المرضي النسجي في (المجموعة الثانية) فرط التنسج في اللب الابيض في الطحال. كذلك وجد ارتشاح خلايا وحيدة النواة (لمفاويات وعدلات) حول الوريد المركزي والمنطقة البابية في الكبد، بالإضافة إلى ارتشاح هذه الخلايا في النسيج الخلالي وحول الأوعية الدموية في الكلية. في حين لم تظهر مجموعة السيطرة (المجموعة الثالثة) أ ي تغيرات مرضيةنسجية في الطحال و الكبد والكلية عند الفحص المجهري بالمقارنة مع المجموعتين السابقتين.

Introduction

Tumor is a broad group of diseases in which one or more cell lineages have escaped from regulators of cell proliferation as the result of inherited or acquired genetic changes, leading to form either benign or malignant tumor "cancer" (1). A benign tumor does not spread, or *metastasize*, to other parts of the body and so are not cancerous. They can often be removed and are rarely a threat to life. A malignant tumor, however,

can spread and is cancerous. Cancer is used for all malignant tumors when this tumor spreads, its malignant cells break off and travel through the blood and lymph system to other parts of the body resulting in a secondary tumor or metastasis(2).

A mammary tumor is a tumor originating in the mammary gland, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Cancers originating from ducts are known as ductal carcinomas; those originating from lobules are known as lobular carcinomas. There are many different types of breast cancer, with different stages (spread), aggressiveness, and genetic makeup; survival varies greatly depending on those factors (3). According to an estimates in 2002, there were 1,151,298 new cases of breast cancer diagnosed around 410, 712 deaths caused by breast cancer, and more than 4.4 million women living with breast cancer worldwide(4). According to the result of Iraqi Cancer Registry (ICR), breast cancer accounted for 19.8% of all malignancies in women and was the commonest cancer in females (5).

Breast cancer, like other cancers, occurs because of an interaction between the environment and a defective gene, normal cells divide as many times as needed and stop, they attach to other cells and stay in place in tissues, cells become cancerous when mutations destroy their ability to stop dividing to attach to other cells and to stay where they belong, then cells divide, their DNA is normally copied with many mistakes. Error-correcting proteins fix these mistakes (6).

A mammary tumor also is common in dog, cats as well as experimental animals(3). Most mammary tumors in mice are adenocarcinomas, they can be caused by viral infection (7). A well known tumor virus of the mouse is the mouse mammary tumor virus MMTV, which may be the most common cause of this tumor in mice (8).

However some transplanted mammary adenocarcinoma cell line in mice failed to grow post transplantation in mice. Therefore, there is little study about histopathological examination of internal organs in their transplanted mice with mammary gland adenocarcinoma cell line which resist tumor cell growth *in vivo*, compared with histopathological examination of internal organs in mice successfully observe growth mammary gland adenocarcinoma cell line *in vivo*.

Materials and Methods

1. The cell lines: The cell line used in the study was mice mammary gland adenocarcinoma (Ahmed-Majeed 2003) adapted for *in vivo* transplantation supplied from Iraqi center for cancer and medical genetic researches (*ICCMGR*).
2. Thirty healthy female albino adult mice (BALB/ C mice) were obtained from the animals house of College of Veterinary Medicine–University of Baghdad at 8-10 wks of age and 25-30 gm B.W. The animals were kept in well air-conditioned rooms and given pellets of balanced specially prepared animal feed and water *ad libitum*.
3. Transplantation of tumor cells in mice: Single tumor mammary adenocarcinoma bearing mouse (AM3) was supplied from ICCMGR. This mouse was used to obtain tumor cells which later transplanted subcutaneously into twenty adult female albino mice, while ten residual mice injected subcutaneously with phosphate buffer saline. The protocol was followed to perform the transplantation process according to Al-Shamery (9).
4. Study protocol: After10 days post transplanted tumor cells in mice, thirty female mice were divided as following:

Group I: Six female albino mice showed successful growth of transplanted tumor cells as a tumor mass after 10 days post transplantation.

Group II: Fourteen female albino mice not exhibited any growth of tumor cells after 10 days post transplantation.

Group III: Ten female albino mice were injected subcutaneously with phosphate buffer saline and served as normal healthy control group.

At the end of the experiment 40 days post transplanted, animals of each group were scarified and tissues of tumor mass, spleen, liver and kidney were taken for histopathological examination.

Results

Group I: Histopathological section of female albino mice were inoculated subcutaneously with mammary gland adenocarcinoma cell line and growth of transplanted tumor cells observed as a tumor mass successfully after 10 days post transplantation and tumor mass showed increasing in their size at the end of experiment (40 days post transplantation), as following:

Tumor Mass: revealed extensive tumor masses consisted of aciner like structure, trabecular and island of tumor cells, the tumor cells are hyperchromatic, pleomorphic, increase the nuclear- cytoplasmic rati with highly blood supply, also tumor masses showed extensive coagulation necrosis Fig (1, 2).

Spleen: Histopathologically showed depletion of white pulp region Fig (3).

Liver: Histopathologically showed vacuolar degeneration in hepatocytes Fig (4).

Kidney: Histopathologically showed atrophy of glomarular tuft with vacuolar degeneration of renal tubules Fig (5).

Group II: Histopathological sections of female albino mice inoculated subcutaneously with mammary gland adenocarcinoma cell line and not exhibition any growth of tumor cells after 10 days post transplantation until the end of experiment (40 days post transplantation).

Spleen: Histopathologically showed extensive hyperplasia of white pulp in the periarterial sheath (T and B cell region) Fig (6).

Liver: Histopathologically showed infiltration of mononuclear inflammatory cells (lymphocyte and macrophage) around central vein and portal area Fig (7).

Kidney: Histopathologically showed infiltration of mononuclear inflammatory cells (lymphocyte and macrophage) in the interstitial tissue and around B.V. Fig (8).

Group III: Histopathological examination of ten female albino mice were injected subcutaneously with phosphate buffer saline and served as normal healthy control group showed no histopathological changes in spleen, liver and kidney.

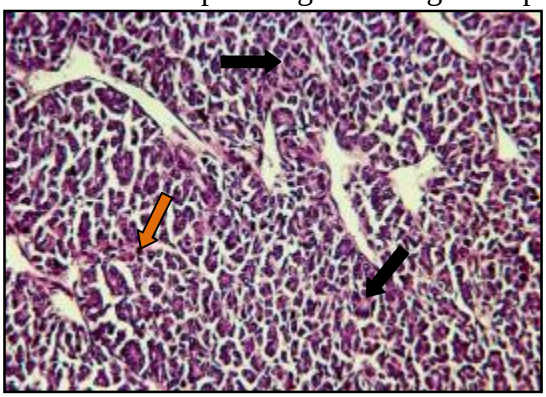

Fig. (1) Tumor cells of group (I) showing extensive tumor masses consisted of aciner like structure (➡) and island of tumor cells, the tumor cells are hyperchromatic, pleomorphic, increase the nuclear – cytoplasmic ratio (⇨) (H& E X 400).

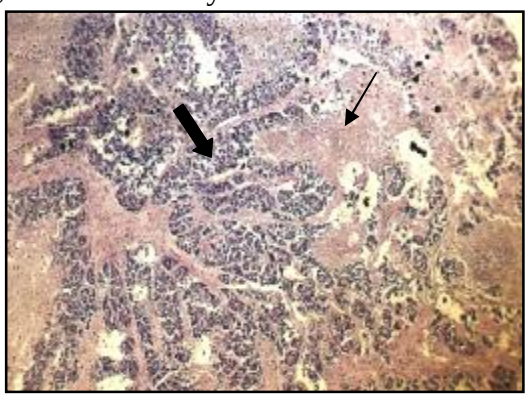

Fig. (2) Tumor cells of group (I) showing large area of necrosis (⟶) and invasive tumor cells (➡) (H&E X100).

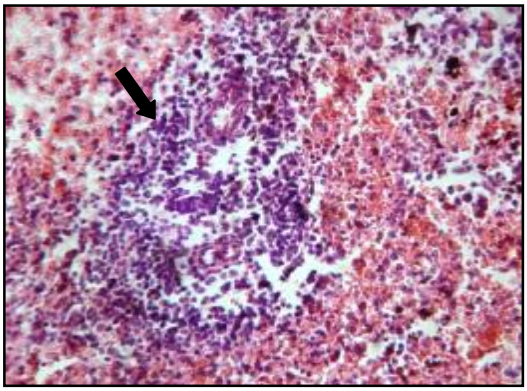

Fig (3) Spleen of group (I) showing depletion of white pulp region (➡). (H & E X40)

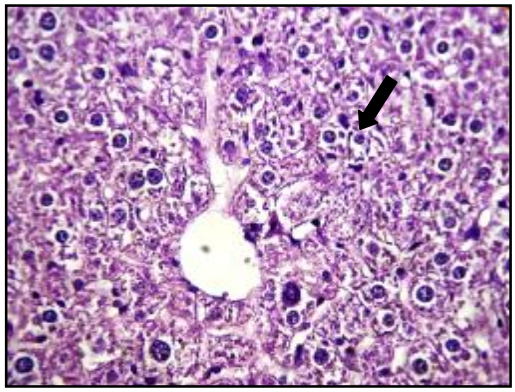

Fig. (4) Liver of group (I), showing vacuolar degeneration in hepatocytes (➡). (H & E X40)

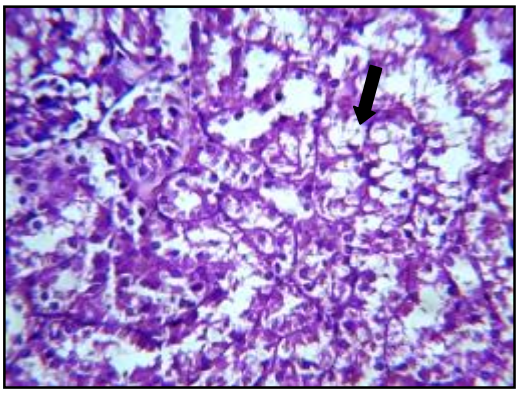

Fig. (5) Kidney of group (I) showing atrophy of glomarular tuft (⟶) with vacuolar degeneration of renal tubules (➡). (H & E X40)

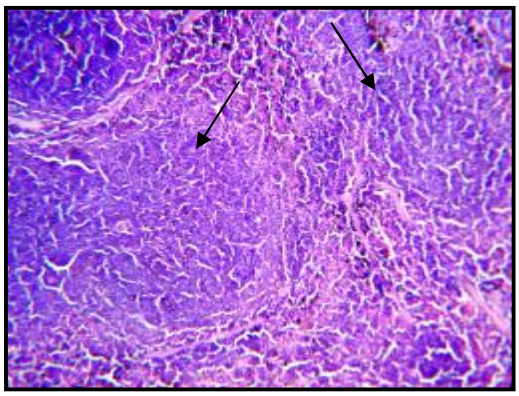

Fig. (6) Spleen of group (II) showing extensive hyperplasia of white pulp in the periarterial sheath (T and B cell region) (⟶) (H & E X 40)

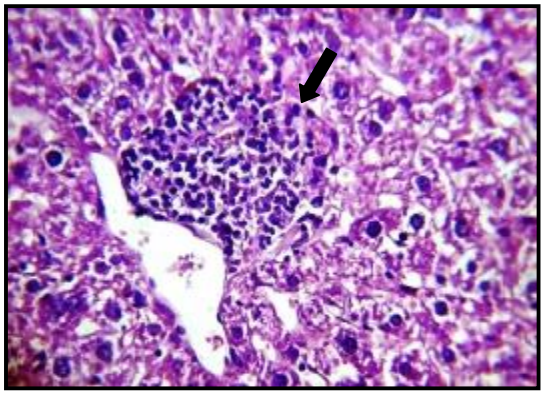

Fig. (7) Liver of group (II) showing monicellular cells infiltration around central vein (lymphocyte and macrophage) (➡) (H & E X 40)

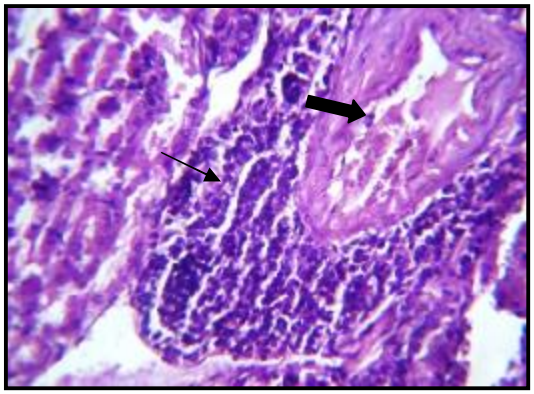

Fig. (8) Kidney of group (II) showing infiltration of monicellular cells (lymphocyte and macrophage) (⟶) around B.V. (➡)(H & E X 40)

Discussion

The experiment was carried out to observe histopathological changes in internal organs of animals transplanted with mammary adenocarcinoma tumor cells S/C, in some of them the tumor cells successfully grow (group I) and other animals tumor cells failed to grow (group II). This growth of mammary adenocarcinoma tumor cells subcutaneously in mice (group I) agreed with the result which mentioned that successful transplantation of tumor cells belong to species-specific adaptation property as a result of recurrent transplantation in the same species of mice, and the inability of host immune system to recognize these transplanted cells as foreign cells, even without uses of any immunosuppressive agents (10). Also other workers mentioned tumor cells

causing suppression of T cells by tumor derived factors e.g. (TGF-B .IL10 and VEGF)(12). All these factors can induce a state of immune unresponsiveness that allow progressive tumor growth (13). In contrast to the group (II) showed failure of transplantation of tumor cells in transplanted mice, this our result supported by previous idea that mentioned by Kumar et al.,(14) that due to direct recognition of host T cells as allogeneic foreign MHC molecules that expressed on tumor cells, or indirect recognition when CD4 T cells recognition MHC of tumor cells after being picked up, processed and presented by host`s own antigen presenting cells.

In group (I), extensive tumor masses consisted of aciner like structure, trabecular and island of tumor cells, the tumor cells are hyperchromatic, pleomorphic, increase the nuclear- cytoplasmic ratio and certain section showed extensive mitotic figure, extensive coagulation necrosis with karyolysis, karyorrhexis and pyknotic nuclei. Our interpretation of these aggressive features of tumor cells could be due to rapid proliferation of tumor cells which outstrip the capacity of new vessels to supply adequate oxygen and nutrient. This our result agreed with (15) who mentioned that patchy necrosis is characteristic of rapidly growing malignant tumor.

The causes of depletion of white pulp of spleen in group I it may be caused by growth of tumor cell and its secretion. This evidence agreed with result that mentioned mammary adenocarcinoma cell line secretion had ability to secrete immunesuppression factors and decreased T cell proliferation when incubation with these cells *in vitro* (9) and mammary tumor suppressed the population of T-Lymphocyte and cytotoxic T cells in lymphoid follicles of white pulp of spleen *in vivo* compared with mice not bearing tumor (16). Vacuolar degeneration of hepatocytes and atrophy of glomarular tuft with vacuolar degeneration of renal tubules in the same group, these tissue damage also caused by tumor cells. Some researchers reported that tumor cells secrete reactive oxygen species in large amount that cause oxidative stress and with these immunosuppressive substance induced reversible cell injury in liver and kidney due to direct damage to the cells (16), and failure of energy dependent ion pump in the plasma membrane and nutritional deficiency remain a major cause of cell injury (14). Group(II), showed extensive hyperplasia of white pulp in the periarterial sheath (T and B cell region) of spleen, mononuclear cell infiltration (lymphocyte and macrophage) in liver and renal parenchyma. These changes in these organs resulted from immune response of these lymphoid tissue mainly spleen against tumor cell proliferation (17), which made transplanted tumor disappear from beginning in this present study, the immune response characterized by hyperplasia of white pulp and inflammtory cells infiltrate kidney and liver which considered be resorviour of $CD4^{+}$ memmory T-cells in mice in immune state (18).

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