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Cell Culture Established from Warts of Bovine Papilloma

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Summary

This study is considered the first in the country on growing of bovine papilloma in cell culture from skin warts of cattle in order to detect papilloma virus, and to establish a cell line of transformed cells for further studies. In this study the warts were surgically removed from cows showing lump lesions on skin of abdomen, neck and udder, and transferred aseptically to laboratory by transport media. Trypsin and collagenase enzyme were used to dispersed papilloma cells from fragments of warts, Dulbecco's modified Eagle's medium (DMEM) and Rosswell Park Memorial Institute (RPMI)-1640 Medium (Gibco, were used with 10% fetal calf serum for culturing of papilloma cells**.** Cultured cell were first noticed after 3-4 days post incubation(PI) and appeared as epithelial and fibroblastic cells. After 7days post inoculation clones of transformed epithelial cells start to appear and after (20-25) days it became a complete monolayer. Successful secondary culture was achieved by using trypsin-versene solution(TV). Further studies is needed to detect the virus from cultured cell by PCR and ELISA techniques beside preparation of vaccine from cell culture for treatment of papillomas in infected cattle.

إثبات الزرع الخلوي من الأورام الحلمية البقرية

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الخلاصة

تعتبر هذه الدراسة الأولى في القطر حول إثبات نمو الورم الحليمي البقري papilloma (bovine) في المزارع الخلوية من ثآليل جلد الأبقار، بالإضافة إلى تشخيص فايروس البابلوما papilloma virus وكذلك إنشاء خط خلوي للخلايا المتحولة transformed cell في الدراسات اللاحقة. في هذه الدراسة تمإزالة الثآليل جراحيا من الأبقار المصابة والتي تحمل تلك الآفة في منطقة الجلدفي العنق و البطن والضرع، وبعد ذلك تم نقلها إلى المختبر بواسطة الأوساط الزرعية الناقلة. أجريت هذه الدراسة باستخدام نوعين من الأنزيمات الأول هو إنزيم التربسين Trypsin والثاني هو إنزيم الكولاجينيز collagenase enzyme وذلك لغرض هرس وتفكيك خلايا البابلوما من قطع الثآليل. استخدم نوعين من الأوساط الزرعية المنمية للخلايا هي MEM و RPMI مع نسبة 10% من مصل جنين العجل لغرض تنمية خلايا البابلوما. لوحظت الخلايا الزرعية النامية أولا بعد 3–4 أيام بعد الحضن ظهرت الخلايا على شكل خلايا ظهارية ليفية، بعد 7 أيام من الحضن لوحظ تجمعات الخلايا الظهارية الليفية المتحولة، وبعد 20– 25 يوم أصبحت خلايا المزرعة النسيجية طبقة أحادية متكاملة شملت كل أجزاء الطبق. بعد ذلك تحققت المزرعة الثانوية لخلايا البابلوما بواسطة استخدام محلول التربسين – فرسين(TV). هنالك حاجة لإجراء

مزيد من الدراسات لغرض تشخيص الفايروس من الخلايا المزروعة بواسطة تقنية الـ PCR و ELISA بالإضافة إلى ذلك تحضير لقاح من الخلايا المزروعة لغرض علاج البابلوما في الأبقار المصابة.

Introduction

Bovine papillomatosis is a common viral disease of the skin, mostly of young cattle, manifested as benign tumours or warts, caused by bovine papillomavirus (BPV)(1). Bovine papillomavirus (BPV)-associated diseases are important in veterinary medicine and can also be considered as possible important models for the study of human papillomavirus (HPV). Often, immunosuppression in cattle results from exposure to bracken fern: some animals become unable to reject the infection and succumb to widespread cutaneous or mucosal involvement (2). These stages of papillomatosis are problematic leading to economic losses (3, 4). Six types of BPV have been described (1-6) (5), and recently, four other types were reported. BPV-1 causes teat and penile fibropapillomas; BPV-2 is associated with cutaneous warts, alimentary fibropapillomas and urinary bladder tumors; BPV-3 causes cutaneous papillomas; BPV-4 is associated with pure epithelial papillomas of the upper gastrointestinal tract; BPV-5 induces fibropapillomas of the udder; BPV-6 causes papillomas of the teats; BPV-8 causes cutaneous papillomas; BPV-9/10 are associated with epithelial squamous papillomas of the udder (2). BPV is described as epithelium-specific (6). However, the presence of viral DNA sequences in different cells such as those of peripheral blood and gametes has been reported (7). The papilloma virus Belong to family papillomaviridae produce in their hosts benign skin tumors (papillomas);which contain variable amount of infectious virus (1). Different methods have been used to treat bovine papillomas. A formalinized suspension of bovine warts with inactivated virus provides a vaccine for effective treatment and prophylaxis of bovine papillomatosis. In Iraq, Al-Bana and Khazeil (8) prepared the bovine interferon and used it for treatment of bovine papilloma for the first time in country. Our aim of this study was to establish a cell culture system for the bovine papilloma in order to specifically identify the virus and to prepare a vaccine from the cultured papilloma cells.

Materials and Methods

- **Collection of wart samples:** Cauliflower like wart were collected from cow suffering from papilloma skin lesion on the udder, teat, abdomen and the back of affected animals. Before surgical removal of the warts the animal were given 1 ml of xylazine 2% (0.25-0.5ml/100kg.b.w), as relaxant intramuscularly and lidocaine 2% as local anesthesia infiltrated around the lesion, followed by cleaning with normal saline. Aseptically the warts were removed by surgical procedure. Pieces of the lesion were added to transport solution containing MEM medium supplemented with a mixture of Antibiotics (amikasin, streptomycin and amphotricin) in sterile containers. Other small pieces were added to 10% formalin in plastic containers for histopathological examination.
- **Dispersion of papilloma cells:** The papilloma pieces were first washed thoroughly with sterile phosphate saline (pH 7.2) and MEM medium to remove the dirt and extraneous material covering the lesions. The papilloma were cut into small pieces by sterile scissors and washed again then divided into two parts: The first part was added to trypsin 0.25% solution and second part was added to collagenase enzyme 10 mg/ml, was added (100 µl),at 37°C. Trypsin part was allowed to disperse the papilloma cell for 30 minutes by continuous stirring, while, collaginase part was allowed for 40 minutes by continuous shaking. The dispersed cells in both methods were filtered by using sterile gauze followed by centrifugation at 1000 rpm for 15

minutes at 4 °C. The sedimented cells were mixed with two kinds of media RPMI, MEM supplemented with 10% fetal calf serum, then cells were seeded in a disposable plastic flask (25 cm^2) and incubated at 37 °C in Co_2 incubator.

- **Subculture of the Primary Papilloma Cells:** Trypsin-Versene Solution (US Biological, USA) was prepared by dissolving 10g of trypsin-Versene (TV) powder (USbiological, USA), in one liter PBS, then sterilized by Nalgen filter (0.22µm). The primary monolayer papilloma cell culture were dispersed from the surface of the cell culture flask by using TV solution (at 37°C) and then seeded into 2 new flask after mixing the cells with growth MEM medium and incubated at 37°C in CO_2 incubator.

Results and Discussion

- **Growth of cultured of papilloma cells:** Trypsin dispersed papilloma cells attached to the bottom surface of cultured flasks were first noticed after 48-72 hr. post incubation (PI) at 37°C. These cells started to multiply after 4 days (PI) and appeared as epithelial and fibroblastic cells. After seven days (PI) clones of transformed epithelial cells started to appear with new fibro plastic cells as the periphery of these clones (Fig. 1). Other part of the cultured flask showed fibroblastic cells only. The clones of transformed cells becoming larger in size with multi layer cells center and these fibroblastic cells at the periphery focus (Fig. 2, A and B). Our results are in agreements with (6) which indicate the culturing of fibroblastic cells only from papilloma lesion without presence of clones of transformed epithelial cells (Fig. 3).

After 20-25 days post culturing of papilloma cells the whole surface area of flask were filled with cultured cells and look like multilayer of transformed cells (Fig. 4). Results of using collagenase enzyme for dispersion of papilloma lesion showed fibroblastic type of cells only started at 7-9 days post incubation without epithelial cells (Fig. 5). After confluent cell cultured reached papilloma cells were sub cultured by using (TV) solution and successfully a second passage of the cells result in duplicate culture flasks and after 3 days both type of epithelial clone cells and fibroblastic cells were noticed.

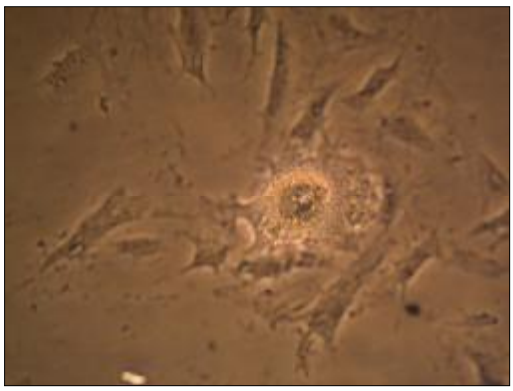

Fig. (1) Showing clone of epithelial cells surrounded by fibroblastic cells 7 days post incubation (40x)

A B

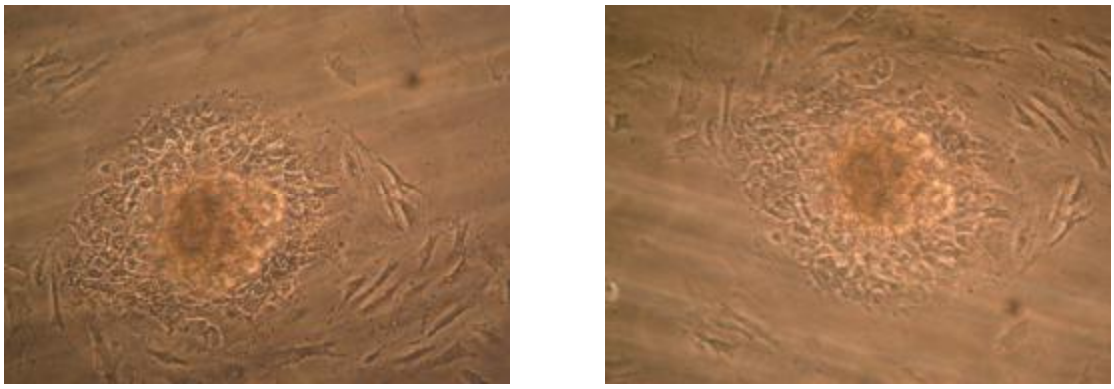

Fig. (2 A, B) Showing clone of transformed multilayer cells surrounded by fibroblastic cells-20X

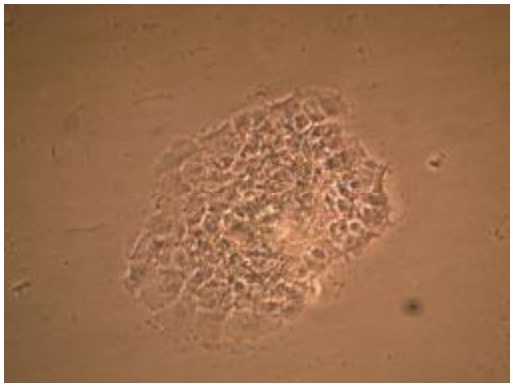

Fig. (3) Showing clone of transformed epithelial cells-20X

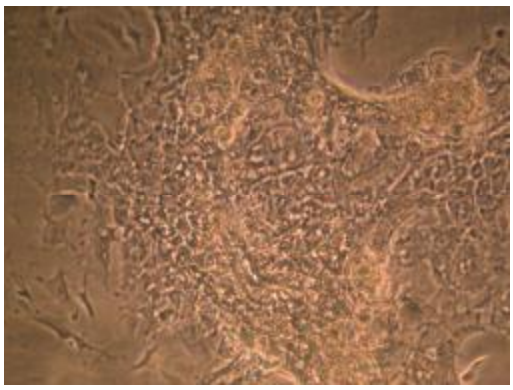

Fig (4) Showing complete monolayer of papilloma cells After 20-25 days post culturing 20x

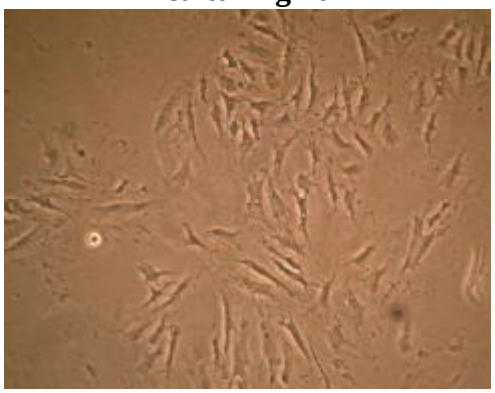

Fig. (5) Showing fibroblastic cells by using collagenase enzyme-20X

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