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Induction of plasmacytoma in mice and establishment of continuous cell line that lack Retinoblastoma gene expression

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Abstract :

The pristane oil induced mouse plasmacytoma (MPC) model is the most widely used and accepted model and has provided the most data on plasmacytomagenesis so far. Plasmacytoma were induced in Swiss mice by the intraperitoneal injection of pristane (2,6,10,14-tetra-methylpentadecane) after a latent period of 5-6 months where the mice developed ascites tumors.

Aspirated cells were cultured in tissue culture flasks and kept under observation and further passaged to obtain continues cell line. Peritoneal exudate cells were prepared from pristane-treated and control uninjected mice during the course of a 6-month period, and these cell suspensions were studied by means of cytology, histology and Immunohistochemistry methods to identify the plasmacytoma specific markers and the genetic changes.

Results showed induction of the tumor in 50% of injected mice and the new cultured cell line were negative for Retinoblastoma (RB) and epidermal growth factor receptor (EGFR)

Introduction:

The pristane oil (2,6,10,12-tetramethylpentadecane)-induced mouse plasmacytoma (MPC) model is the most widely used and accepted model and has provided the most data on plasmacytoma genesis so far. This model gives the opportunity to study the role of c-myc dysregulations, the mechanisms leading to cytogenetic changes involving immunoglobulin (Ig) genes, the role of chronic inflammatory factors, the role of interleukin-6 (IL-6), insulin-like growth factor-I, prostaglandins, as well as signal transduction pathways in the neoplastic process (1).

Human multiple myeloma (MM) is a currently incurable B-cell malignancy that accounts for approximately 10% of human hematopoietic malignancies and 1% of all human cancers (2). Multiple myeloma often originates from a common premalignant condition called monoclonal gammopathy of undetermined significance that affects 1% of the adult population. The clonal B-cell abnormality in MM affects precursors of terminally differentiated B-cells. MM is characterized by long-lived plasma cells that, as a rule, have suffered somatic hyper-mutation, antigen selection and Ig switching in the germinal center (3).

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Materials and methods

Mice

Fifty (6-8 week-old) white Swiss female mice were obtained from Iraqi Centre for Cancer and Medical Genetic Research (ICCMGR), Baghdad, Iraq, They were placed as six per a cage, and housed in animal house facility.

Plasmacytoma induction

Pristane was purchased from Sigma-Aldrich (USA). To induce plasmacytoma the mice were injected with three doses (0.5 ml/mouse) each given i.p. on days 0, 60 and 120 performed under Animal Use Protocol of ICCMGR. After appearance of ascites or peritoneal exudate it was aspirated with 25 gauge needle.

Plasmacytoma primary culture

Aspirated fluids washed 2-3 times with PBS by centrifugation (1000 rpm for 10 minutes at 4 °C) to pellet the cells. The pelleted cells were resuspended in RPMI-1640 growth medium (20% FCS) at concentration 1ml of packed cells /200 ml of medium, cells were seeded in 25 cm2 plastic flasks (Nunc, Denmark).

After 24-48hrs 50% of the media will replaced with fresh

prepared one, and the flask was put under observation for the following days to recorded cells growth and changing the media when needed, when the cells reach to confluent monolayer they subcultured into 2 flasks.

Cytological study

Cyto-smear preparations were made and the cells were stained with Giemsa stain. Plasmacytoma were diagnosed by finding atypical plasma cells. In most cases two diagnoses per mouse were made as we did fine needle aspiration.

Histopathological study

When the mice were autopsied, the internal organs (liver, kidney and spleen) were excised. These were then cut after fixation in 10% neutral buffer formalin into 10 mm fragments. All of the sections were stained with haematoxylin and eosin and scanned for the presence of plasmacytic foci.

Immunohistochemistry Study:

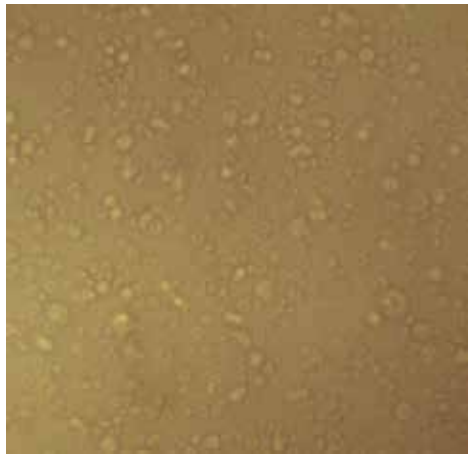
Selected cases were immuno-stained for the following markers, anti-mouse RB and anti-mouse EGFR (USbiological, USA) and we followed the manufacturer protocol.

Results:

A total of 25 of 50 mice (50%) developed pristane-induced peritoneal (figure-1, 2). Cells were aspirated and cultured in tissue culture flasks, they were floating (figure-3) then showed attachment after 24-48hrs of seeding (figure-4, 5). Cells were polygonal in shape and need 3-5 days to achieve confluency.

Cytosmear for each aspirated fluid showed presence of high number of inflammatory exudate especially plasma cells with atypical cells that diagnosed to be plasmacytoma cells (figure- 6). Immunohistochemistry study showed that cultured cells were negative for EGFR and RB (figures-7, 8).

Figure-1,2 Mice developed pristane-induced peritoneal plasmacytoma ascites.



Figures-3 Floating plasmacytoma in primary culture after 12hrs

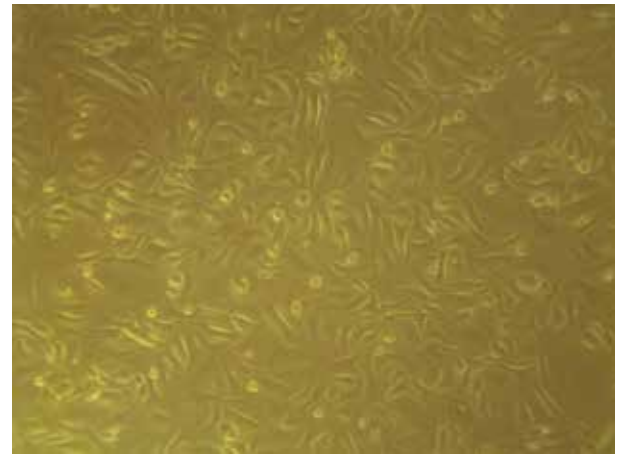


Figure-4 Cultured plasmacytoma cells from female mice after 180 days of induction and 24hrs of culture, 20x

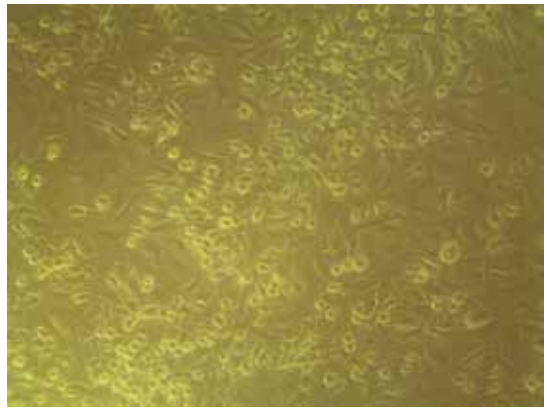


Figure-5 Cultured plasmacytoma cells from mice after 180 days of induction and 48hrs of culture, 20x

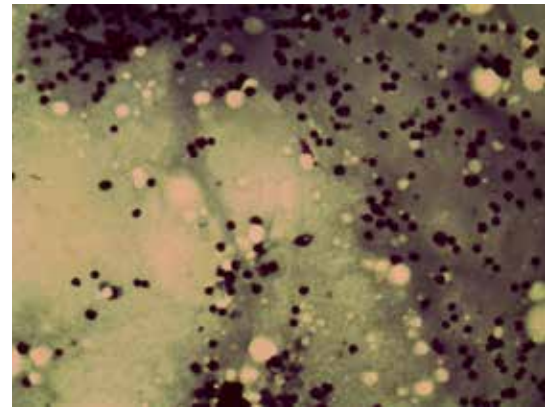
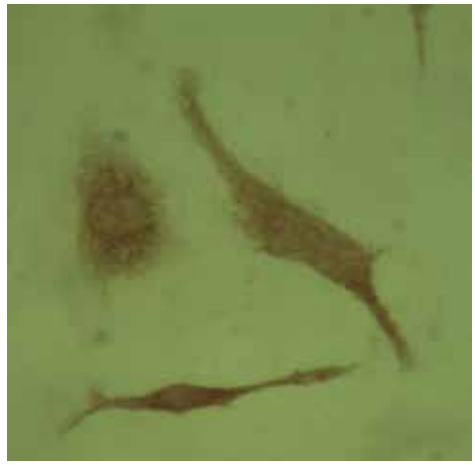
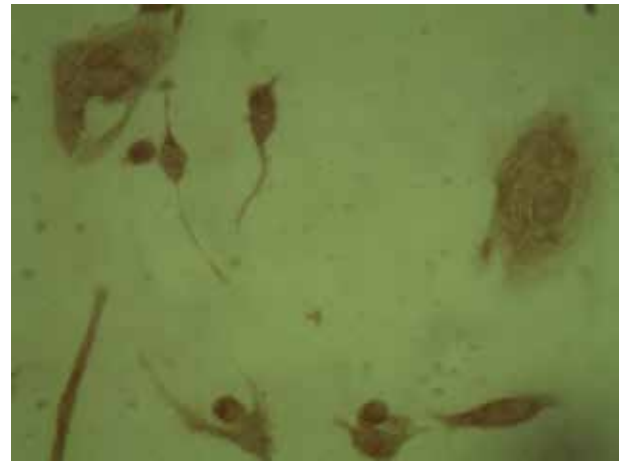


Figure-6 Cyto-smear for aspirated fluid showing presence of high number of inflammatory exudate, 20x



Firgure-7, Immunohistochemical staining for EGFR in cultured plasmacytoma cells showing negative expression.



Firgure-8, Immunohistochemical staining for RB in cultured plasmacytoma cells showing negative expression.

Discussion:

In the study presented here, we were interested to produce animal tumor model for human multiple myeloma and to produce plasmacytoma tumor cell line secrete monoclonal antibodies to be used later in monoclonal antibody (mAb) production.

Inflammation-induced peritoneal plasmacytomagenesis in mice provides a model system for studying many aspects of malignant B-lymphocyte development, including the role of chromosomal translocations that deregulate the proto-oncogene c-myc (4).

Morphological findings suggest that cultured plasmacytoma cells have polygonal shapes as these cells were the stem cells of the plasmacytoma which were originated from defected B cells as Ohno and co-workers (5) refer and the murine plasmacytomas develop from immature/mature B cells not from differentiated plasma cells.

The present study showed loss of RB which may be the main cause for plasma cell tumor development where RB gene have very important role as tumor suppresser gene as described by ZHANG (6) study, BALB/c mice exhibit partially impaired p16INK4a function but appear to have an intact p19ARF-mdm2-p53 pathway and from inactivation of both p16INK4a and p19ARF, which disrupt the RB and p53 pathways and this may lead for short latency period for plasmacytoma formation. Present study showing these cultured plasmacytoma cells as a model for human myeloma which is very fatal disease (7), and need suitable model in experimental animals to study and development of new therapeutic agents using our model for both in vitro and in vivo studies.

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استحداث ورم سرطانة الخلية البلازمية في الفئران وانشاء خط خلوي مستمر منه فاقد للتعبير للمورث Retinoblastoma

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الخلاصة:

نموذج ورم الخلية البلازمية المستحث بواسطة زيت البريستان هو من اوسع النماذج استخداما وقبولا, واكثر نموذج وفر معلومات عن نشوء سرطانة الخلية البلازمية لحد الان. تم استحداث سرطانة الخلية البلازمية في الفئران السويسرية عن طريق حقن البريستان بالخلب لمدة ستة اشهر حيث طورت الفئران اورام حبنية. تم زراعة الخلايا المرشوفة من موقع الورم في اوعية الزرع النسيجي وبقيت تحت المراقبة ومررت عدة مرات للحصول على خط خلوي مستمر منها. تم سحب رشفات خلوية من الفئران المحقونة وغير المحقونة خلال فترة حقن زيت البريستان التي امتدت لفترة ستة اشهر, وتم عمل مسحات خلوية وتم دراسة الورم نسيجيا وبالكيمياء المناعية النسيجية للمستضدات السطحية حيث اضهرت النتائج ان الورم كان سالبا لمستقبل عامل النمو الادمي EGFRو العامل الكابح للورم RB كما ضهر الورم في 50% من الفئران المحقونة بزيت البريستان.