Ultrastructural Study of Peripheral Blood Mononuclear Cells in Patients with Urinary Bladder Carcinoma

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Clinical and Chemical Pathology

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Abstract

the morphologic appearance of PBMCs in the studied cases showed some signs of activity in cells of innate and adaptive immunity in superficial and invasive carcinoma respectively. Furthermore, it delineates inability of the cells of immune system to mount an effective immune response capable of preventing .

Key word

Mononuclear

Carcinoma

Pathology

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Table of Content

List of Tables

List of Figures

List of Abbreviations

APCs	Antigen presenting cells
CAD	Caspase-activated DNase
CTL	Cytotoxic T lymphocyte
DC	Dendritic cell
DISC	Death-inducing signaling complex
EM	Electron microscope
FADD	Fas associated death domain
FH	Ficoll Hypaque
GZMs	Granzymes
IFN-ϒ	Interferon ϒ
LGL	Large granular lymphocyte
LVEM	Low voltage electron microscope
M-CSF	Macrophage colony-stimulating factor
MVB	Multivesicular body
NK	Natural killer
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffer saline
PFR	Perforin
PMN	Polymorphonuclear
REM	Reflection electron microscope
RER	Rough endoplasmic reticulum
RHEED	Reflection high energy electron diffraction

RHELS	Reflection high-energy loss spectrum
SEM	Scanning electron microscope
SPLEEM	Spin-polarized low-energy electron microscopy
STEM	Scanning transmission electron microscope
TBRI	Theodor Bilharz Research Institute
TCC	Transitional cell carcinoma
TCR	T cell receptor
TEM	Transmission electron microscope
TH	T helper cell
TNF-a	Tumor necrosis factor-a
TRAIL	TNF-related apoptosis-inducing ligand
UTI	Urinary tract infection

Introduction and Aim of Work

Bladder carcinoma is the second most frequent genitourinary tumor and is a significant cause of morbidity and mortality (Jemal *et al*., 2004)

More than 90% of urothelial cancer in the bladder is transitional cell carcinoma (Lamm and Torti 1996).Other important histological types include squamous cell carcinoma and adenocarcinoma.

This neoplasm accounts for 30.3 % of the total cancer incidence and is ranked first among all types of cancer recorded in Egyptian males (El-Mawla *et al*, 2001)

The majority of urothelial tumors are superficial when the patient first present, but despite adequate resection of the primary lesion the recurrence rate is particularly high. In a small but significant group of patients the tumor is primary invasive or subsequently can progress and lead to death (Koenig *et al,* 1999).

Clinical outcome in patients with bladder cancer is determined by a variety of factors such as size, multicentricity, histologic grade, stage and the contribution of the immune system to control the tumor growth (Droller *et al*., 1998). Virtually all the effector components of the immune system have the potential to the eradication of tumor cells (Greenberg 1994). However, tumor progression, often seen in the presence of substantial lymphocytic infiltration, suggests that the cells are not capable of mounting an effective immune response to control tumor growth.

Earlier studies held to evaluate the immune system of patients with bladder carcinoma revealed presence of immunological abnormalities in respect to count, subsets and function of immune effector cells (Soygur *et al*, 1999). These abnormalities were found to correlate with the stage and grade of tumor (Kastelan and Lukac *et al*, 2003). Furthermore changes of lymphocytes

structural morphology from resting to active state after immunotherapy were detected by Theano *et al* (2002)**.**

Aim of the Study

The main aim of this work is to further evaluate the immune system by studying the ultrastructure of peripheral blood mononuclear cells (PBMCs) and to correlate the changes that might occur in these cells with tumor stages. This may be of value in understanding the pathogenesis of this lesion and in determining the approach to treatment through immunomodulation.

Chapter 1
Electron Microscopy Principles

An electron microscope is a type of microscope that produces an electronically-magnified image of a specimen for detailed observation. The electron microscope (EM) uses a particle beam of electrons to illuminate the specimen and create a magnified image of it. The microscope has a greater resolving power (magnification) than a light-powered optical microscope, because it uses electrons that have wavelengths about 100,000 times shorter than visible light (photons), and can achieve magnifications of up to 1,000,000x, whereas light microscopes are limited to 1000x magnification. The electron microscope uses electrostatic and electromagnetic "lenses" to control the electron beam and focus it to form an image. These lenses are analogous to, but different from the glass lenses of an optical microscope that forms a magnified image by focusing light on or through the specimen. For EM, the ultrastructure must be preserved as close to the in vivo situation as possible (Hall, 1991).

Types:

1- Transmission electron microscope (TEM)
2- Scanning electron microscope (SEM)
3- Reflection electron microscope (REM)
4- Scanning transmission electron microscope (STEM)
5- Low voltage electron microscope (LVEM)

Transmission electron microscope (TEM):

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on

a layer of photographic film, or to be detected by a sensor such as a CCD camera. TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small wavelength of electrons. This enables the instrument's user to examine fine detail—even as small as a single column of atoms, which is tens of thousands times smaller than the smallest resolvable object in a light microscope. TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. TEMs find application in cancer research, virology, materials science as well as pollution and semiconductor research. At smaller magnifications TEM image contrast is due to absorption of electrons in the material, due to the thickness and composition of the material. At higher magnifications complex wave interactions modulate the intensity of the image, requiring expert analysis of observed images. Alternate modes of use allow for the TEM to observe modulations in chemical identity, crystal orientation, electronic structure and sample induced electron phase shift as well as the regular absorption based imaging (Robards, 1993).

Sample Preparation:

Chemical fixation for biological specimens: aims to stabilize the specimen's mobile macromolecular structure by chemical crosslinking of proteins with aldehydes such as formaldehyde and glutaraldehyde, and lipids with osmium tetroxide.

Dehydration: freeze drying, or replacement of water with organic solvents such as ethanol or acetone, followed by critical point drying or infiltration with embedding resins

Embedding biological specimens: after dehydration, tissue for observation in the transmission electron microscope is embedded so it can be sectioned ready for viewing. To do this the tissue is passed through a 'transition solvent' such as

epoxy propane and then infiltrated with a resin such as Araldite epoxy resin; tissues may also be embedded directly in water-miscible acrylic resin. After the resin has been polymerised (hardened) the sample is thin sectioned (ultrathin sections) and stained - it is then ready for viewing.

Sectioning: produces thin slices of specimen, semitransparent to electrons. These can be cut on an ultramicrotome with a diamond knife to produce ultrathin slices about 60-90 nm thick. Disposable glass knives are also used because they can be made in the lab and are much cheaper.

Staining : uses heavy metals such as lead, uranium or tungsten to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). In biology, specimens can be stained "en bloc" before embedding and also later after sectioning. Typically thin sections are stained for several minutes with an aqueous or alcoholic solution of uranyl acetate followed by aqueous lead citrate (Glauert, 1974).

Techniques of Transmission Electron Microscopy:

1- Conventional EM

2- Immuno-EM

Electron microscopy can be used to study the detailed microarchitecture of tissues or cells. Immuno-EM allows the detection of specific proteins in ultrathin tissue sections. Antibodies labeled with heavy metal particles (e.g. gold) can be directly visualized using transmission electron microscopy. While powerful in detecting the sub-cellular localization of a protein, immuno-EM can be technically challenging, expensive, and require rigorous optimization of tissue fixation and processing methods.

Electron cryomicroscopy

(cryo-EM or sometimes cryo-electron microscopy) is a form of electron microscopy (EM) where the sample is studied at cryogenic temperatures (generally liquid nitrogen temperatures). It allows the observation of specimens that have not been stained or fixed in any way, showing them in their native environment (Harris, 1997).

Scanning electron microscope (SEM):

The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity (Roomans, 1990).

Sample Preparation:

All samples must also be of an appropriate size to fit in the specimen chamber and are generally mounted rigidly on a specimen holder called a specimen stub. Several models of SEM can examine any part of a 6-inch (15 cm) semiconductor wafer, and some can tilt an object of that size to 45° For conventional imaging in the SEM, specimens must be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge at the surface. Metal objects require little special preparation for SEM except for cleaning and mounting on a specimen stub. Nonconductive specimens tend to charge when scanned by the electron beam, and especially in secondary electron imaging mode, this causes scanning faults and other image artifacts. They are therefore usually coated with an ultrathin coating of electrically-conducting material, commonly gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation. Conductive materials in current use for specimen coating include gold,