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Microscopic Examination of Biological Tissues: Study of Microscopic Changes

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Abstract

Histopathology is the microscopic examination of biological tissues to observe the appearance of diseased cells and tissues in very fine detail. Hence histopathology is the study of microscopic changes or abnormalities in tissues that are caused as a result of diseases.

Keywords: Histopathology; Diseased cells; Microscopic changes

Introduction

Histopathologists are doctors who diagnose and study disease using expert medical interpretation of cells and tissue samples. The specialty determines the cause of death by performing autopsies and is integral to cancer management through staging and grading of tumours [1]. Over the past decade, dramatic increases in computational power and improvement in image analysis algorithms have allowed the development of powerful computer-assisted analytical approaches to radiological data. With the recent advent of whole slide digital scanners, tissue histopathology slides can now be digitized. In lymphomas, lymph nodes are the tissue most commonly examined in histopathology [2]. For many types of blood cancers, a bone marrow biopsy may also be required for a definitive diagnosis. Data shows that optimal time for formalin fixation for most stains is 3-7 days. After fixation, tissue can be stored for 1 to 3 days in 70% ethanol. A colonoscopy is considered positive if the doctor finds any polyps or abnormal tissue in the colon. Most polyps aren't cancerous, but some can be precancerous. Polyps removed during colonoscopy are sent to a laboratory for analysis to determine whether they are cancerous, precancerous or noncancerous. The biopsy, or the tissue sampling itself, shouldn't hurt [3]. Most biopsy procedures are performed either with local anaesthesia or with local anaesthesia plus some sort of sedation. So the goal is that the biopsy shouldn't be painful. Medscape: Many patients have significant misconceptions about biopsies. A pathologist is a doctor who specializes in interpreting laboratory tests and evaluating cells, tissues, and organs to diagnose disease. The report gives a diagnosis based on the pathologist's examination of a sample of tissue taken from the patient's tumour.

Methods

1. Northern blotting–analyses mRNA expression in tissue
2. In situ hybridization–Visualizing gene activity (mRNA) directly in fixed cells or tissues
3. Western blotting–analyses proteins expressed in tissue
4. Immuno Histochemistry (IHC) or Immuno Cytochemistry (ICC)–labels proteins in fixed cells or tissues

Northern blotting

Purpose: Can tell us if a particular gene is being expressed in a specific cell or tissue type or if there are changes in levels of expression

General procedure:

1. Isolate mRNA from cells
2. Separate mRNA with gel electrophoresis
3. Blot (transfer) the mRNA to a membrane
4. Label mRNA you are interested in (probe with a complementary sequence that binds your mRNA of interest)

In situ hybridization

Purpose: Allows us to visualize gene activity directly in tissues or cells

General procedure:

1. Tissues may be cut into thin sections or entire embryo may be adhered to a microscope slide
2. mRNA probes are labelled with a dye or enzyme that makes a coloured precipitate
3. Tissues are imaged with a microscope

Western blotting

Purpose: Detects levels of proteins being produced in a particular cell or tissue (Figure 1)

General procedure:

1. Isolate proteins
2. Separate proteins with gel electrophoresis
3. Blot (transfer) the proteins to a membrane
4. Label protein you are interested in (antibody that binds specifically to your protein)
5. Bands can tell if protein is present and if there are different molecular forms of it

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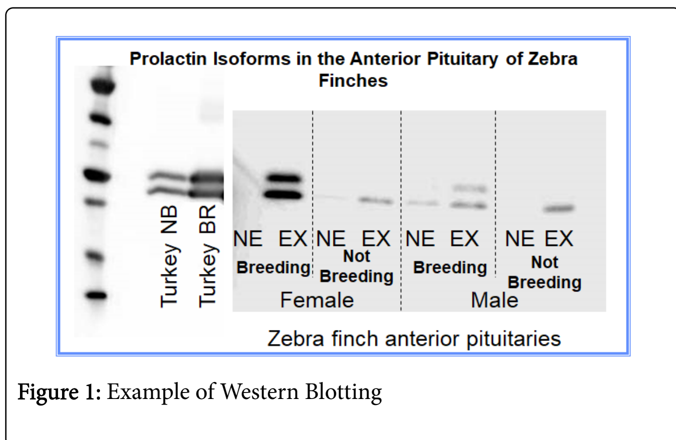

Figure 1: Example of Western Blotting

The lane on the extreme left is the ladder (mol/wt marker). Tissue extracts in the remaining lanes are labelled with a prolactin antibody. Some birds demonstrate a second, slightly heavier band of prolactin. Because it is heavier, it migrates more slowly, and shows prolactin exists in two forms in these birds; the heavier prolactin has been modified by adding a sugar group.

The idea behind any type of microscopy is to see inside the tissue and/or cell.

What we see and how well we see it, depend upon:

1. Type of microscope
2. Tissue preparation
3. Tissue staining

Types of microscopes

Light microscope: Resolution of about 0.2 mm; Good for routine staining

Fluorescent microscope: Fluorescent dyes are excited by specific wavelength of light and re-emit it at higher wavelengths (lower energy). Filters eliminate stray light, so the image is sharper

(Ex: FITC–a very common fluorescent dye–is excited at 488, emits at 525 nm - looks green)

Confocal microscope:

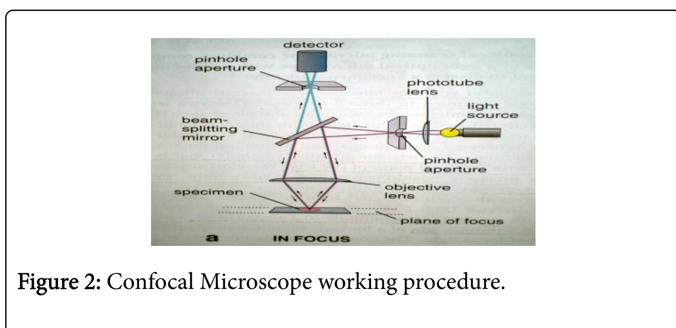

Figure 2: Confocal Microscope working procedure.

Uses a laser to excite fluorescent dye in tissue or cell one spot at a time Precisely positioned pinholes allow only in-focus light to pass through Images in XYZ planes Computer takes all images and creates a 3D reconstruction (Figure 2)

0.2-0.5 mm resolution 3D images can be obtained. Anterior pituitary of a zebra finch. Cells secreting prolactin appear red, while those secreting growth hormone appear green (Figure 3).

An individual cell in the lower right corner has both hormones and appears yellow. The dark "hole" in the middle of the cell is its nucleus.

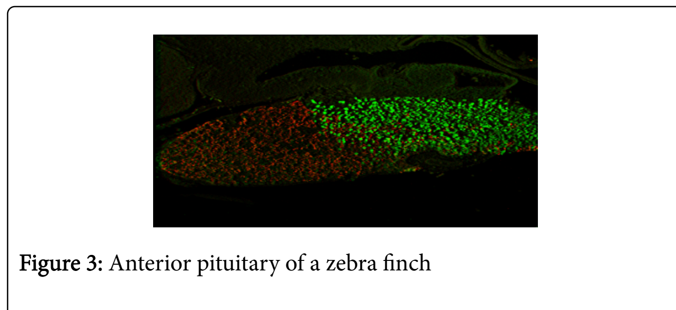

Figure 3: Anterior pituitary of a zebra finch

Tissue preparation

Fresh tissue is thick, mushy, and opaque. Also has active enzymes and bacteria that promote degradation.

3 basic steps:

1. Fixation–stabilizes structure
2. Embedding & sectioning–makes it hard so we can cut thin slices
3. Staining–provides contrast so we can see it easier

Histological techniques

Fixation: The most common fixatives cross-link tissue proteins. An example is formaldehyde.

1. stabilizes structure
2. stops enzymes
3. prevents bacterial degradation (putrefaction)

Embedding: Infiltrate tissue with harder substance that is more easily cut into thin sections

1. Resin–plastic, used for very thin sections, 0.5-1 μm
2. Paraffin–used for sections >5 μm
3. Freezing–used for sections 20-100 μm

Sectioning:

1. Paraffin (frozen similar)
2. Sectioned with a rotary microtome (Figure 4)
3. Metal or glass knife or razor blade (Figure 5)
4. Sections to 1-2 μm thick

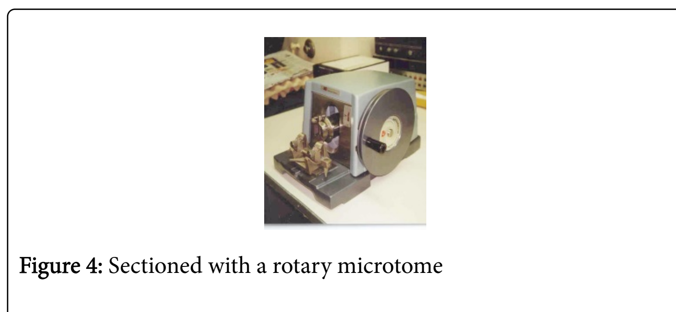

Figure 4: Sectioned with a rotary microtome

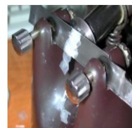

Figure 5: Metal or glass knife or razor blade

Staining: Introduces contrast to tissue which is otherwise essentially transparent

Common stains: Hematoxylin (looks blue-purple) & eosin (looks pink-red) usually Hematoxylin and Eosin are used together (H&E)

Conclusion

DNA

BRd-U - bromodeoxyuridine - analog of thymidine, incorporated when DNA replicates

1. Antibody stains labelled cells
2. Can follow time sequence of cell division

Immunohistochemistry (IHC):

Advantages:

1. High specificity for molecular species
2. Can be used for light, confocal, or EM

Disadvantages:

1. Time consuming & expensive
2. Fixation can interfere with Ab binding
3. Reproducibility - false positives - cross reactivity
4. Difficult to get Abs to small molecules

Immuno methods

Direct method: label (dye) is on the antibody (Figure 6)

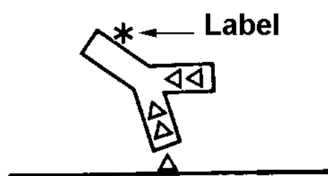

Figure 6: Specific antigen-antibody reaction with labelled primary antibody

Indirect method: label is on a secondary antibody, amplifies label

1. Primary antibody binds to molecule of interest
2. Second antibody (labelled with dye) against species in which primary was raised - e.g. goat anti-rabbit antibody (Figure 7)

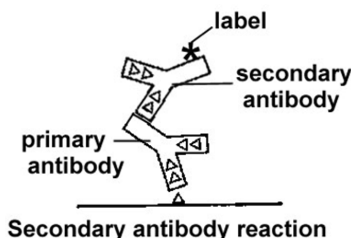

Figure 7: Secondary antibody reaction

Fluorescent dyes - FITC, rhodamine, Cy3, Cy5 - different excitation and emission spectra allows double labelling. Enzymes (Horse Radish Peroxidase (HRP) or alkaline phosphatase) give coloured precipitate (Figure 8)

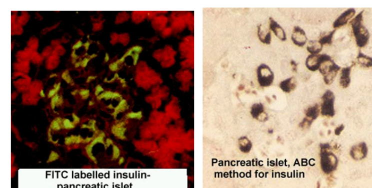

Figure 8: FTIC labelled insulin- pancreatic islet

References

1. Zhu Y, Kang G, Yu C, Poh LH (2016) Logarithmic rate based elasto-viscoplastic cyclic constitutive model for soft biological tissues. J Mech Behav Biomed Mater 61: 397-409
2. McCauley HA, Wells JM (2017) Pluripotent stem cell-derived organoids: using principles of developmental biology to grow human tissues in a dish. Development 144: 958-962
3. Maes C (2017) Signaling pathways effecting crosstalk between cartilage and adjacent tissues: Seminars in cell and developmental biology: The biology and pathology of cartilage. Semin Cell Dev Biol 62: 16-33