Cellular Pathology Assignment

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1)Write explicitly on 5 diagnostic techniques use in cellular pathology, relevant illustrations and examples required.

Special stains

Pathologists use the chemical properties of components of the tissues being studied in their choice of different stains. The stain(s) are applied to the thin sections on glass slides to allow the pathologist to see the cells under the microscope. The most widely used stain is haematoxylin and eosin. This stain is a combination of a basic stain (haematoxylin) and an acidic stain (eosin), which react with acidic and basic cell components in the tissue on the slide to give purple and pink colours to the tissues. Other stains available highlight fats, different tissue fibres, different types of mucus, microorganisms, proteins etc.

Immunohistochemistry

A major change in histopathology in recent times has been the development of immunohistochemistry. Where special stains are a relatively crude and, in most cases, relatively non-specific way of staining tissue components, immunohistochemical stains are by comparison far more specific in what they stain.

This technique involves attaching a dye to an antibody that will only bind to a certain protein type on or within a cell. Antibodies are like keys that can only open a certain lock (cell protein or antigen). Hundreds of antibodies are available which allow labelling of hundreds or even thousands of different protein types. Where a special stain may allow the pathologist to identify, for example, a cell as being cancerous, immunohistochemistry can identify which organ in the body that cancerous cell came from and how aggressively it may behave.

The dyes that attach to the labelling antibody can be also altered, including using different coloured dyes or even fluorescent dyes that are easier to see on microscopy. Some laboratories can use fluorescence-labelled antibodies to allow for computerised slide analysis, reducing the time taken to examine large numbers of slides and identifying which slides need to be reviewed by a pathologist and which are within the normal range.

Electron microscopy

The usual microscopes used by pathologists are not powerful enough to see the smallest parts that make up a cell. This is not usually a problem, but some diseases can only be diagnosed at this subcellular level. Examples include types of kidney disease (glomerulonephritis) or aggressive cancers which lose their normal proteins, making immunohistochemistry less useful in their identification.

In these cases a very powerful type of microscope is used called the electron microscope. This utilises beams of electrons rather than visible light to magnify the cells in a tissue sample. It can magnify up to 2 million times, whereas the maximum power of a conventional light microscope is only 1 to 2 thousand times.

Flow cytometry

This technique is used most commonly as an adjunct in the diagnosis of cancers of the blood cells (leukaemias and myelomas). Cells are suspended in a liquid and passed through a laser beam (single wave length light beam). A detector measures how the beam is scattered and if fluorescent light is emitted from excited particles on the cells. This is interpreted by a computer as a number of cells/ particles/ proteins (whatever substance is being examined for) and is shown on a graph. This can be used to give the quantities and relative proportions of different types of cells in the blood and identify any abnormal cells (e.g. leukaemias).

Molecular Pathology and Cytogenetics

With the explosion of information about cell DNA (the genetic coding material) and genes that has resulted since the completion of the Human Genome Project, increasing numbers of genes are being recognised that, if faulty, may be involved in the development of disease including cancers. This is shaping up to change the way that disease is thought of, diagnosed and treated.

Molecular pathology is an umbrella term for the analysis of the genetic material (chromosomes and their DNA) of cells, and is becoming an increasingly widely requested component of the pathology workup of a submitted tissue. One of the subdivisions of molecular pathology is cytogenetics, which is the analysis of chromosomes (the form in which DNA is found in the cell nucleus). The two most commonly used techniques in molecular pathology and cytogenetics are fluorescence in situ hybridisation (FISH) and direct sequencing of DNA.

FISH is a technique used to stain chromosomes to reveal areas where genes may have been deleted, duplicated or broken. Fluorescent labels are attached to specific DNA sequences (parts of specific genes) which allow faulty genes to be seen when examining the cells under a special type of microscope.

Direct sequencing of cell DNA is a way of looking at individual genes or groups of genes, to detect and characterise which mutation is present in a particular patient’s tumour. This can be done in the traditional manner (Sanger sequencing, capillary electrophoresis), or by the newer and much faster method of Next Generation Sequencing.

As an example of the usefulness of cytogenetics one can look at breast cancer. Anatomical pathology can give a diagnosis of what type of breast cancer a patient may have, how far it has spread, whether or not it is likely to be an aggressive tumour and whether it will respond to hormone and targeted therapies. Cytogenetics can add to this information by identifying whether the patient has a faulty gene(s) which predisposed them to the development of breast cancer. If present, this would mean that they have an increased chance of developing cancer in the opposite breast and of developing other specific cancer types (e.g. ovarian cancer). It also has implications for the patient’s direct relatives and offspring. Did they inherit the faulty gene(s) and what are the chances that they will develop cancer in the future? By direct sequencing of the faulty gene, the close relatives of the patient can be screened for the mutation, after appropriate consent, allowing for preventative steps to be taken to minimise their chances of developing a similar cancer in the future.  There are also treatments being developed which will target the products of specific gene mutations in a patient.

2.)Cellular adaptation precedes cell death discuss!!!

The diagram below explains this sequence. Cellular adaptation is the ability of cells respond to various types of stimuli and adverse environmental changes. These adaptations include Hypertrophy (enlargement of individual cells), Hyperplasia (increase in the number of cells), Atrophy (reduction in the number and size of cells), Metaplasia (transformation of one epithelium to another) and Dysplasia (disordered growth of cells). Tissues adapt differently depending on the replicative characteristics of the cells that make up the tissue. For example, labile tissue such as the skin can rapidly replicate and therefore can also regenerate after injury whereas permanent tissue such as neural and cardiac tissue cannot regenerate after injury. Its cells are not able to adapt to the adverse environmental changes. Cellular adaptation could be normal (physiological) or abnormal (pathological).

When cells are injured, one or two patterns will gradually occur; reversible cell injury leading to adaptation of the cells and tissues, or irreversible cell injury leading to cell death and tissue damage. Injured cells may accumulate materials including fat, cholesterol, protein, glycogen or pigment. When cells are irreversibly injured and dying, specific nuclear changes may be visible including pyknosis, karyrrhexis and karyolysis. If large number of cells dies, tissue necrosis may occur. Observable patterns of necrosis include; coagulative, liquefactive, fibrinous, gummatous, fat, gangrene and caseous necrosis.

