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Stain Materials' Role in Biological Research: A Tool in Heath Care

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Abstract: Stains and staining methods significantly assist in diagnoses in medical research and health care. Certain color of a dye can identify the location of a tumor within a specimen. Applying histochemical-staining enabled morphological identification of fibrin in the lymphoid tissue during cancer progression. Staining methods in combination with the LSFM (light-sheet fluorescence microscopy) allowed tracing the drug penetration, development and spread of tumors. The Curcumin dye is in use for labelling and imaging of Aβ plaques in post-mortem brain tissue. Immunofluorescent staining methods are employed in detection of some important proteins in early diagnostic changes relevant to heart damage. The methods are developed in medical research to include stem cells and tissue engineering, cell cultures` properties and capabilities, connective tissues and extracellular matrix , nervous system , musculoskeletal system; respiratory system, liver and gastrointestinal tract, and male and female reproductive systems.

Key words: Cancer, diagnoses, dyes, histochemistry, immunohistochemistry, staining, stains.

1. Introduction

The integral theory means that all phenomena are wholes in and of themselves yet parts of other wholes. So an atom is a whole atom, but it is part of a molecule, which is part of an organelle, which is part of a cell. However, a perspective includes subject and object, as well as individual and collective [1]. The integral theory is applied in environmental studies and ecological research [2, 3]. Therefore , the reactions of the chemical structures of stain material with the tissues' constituents at their molecular/atomic structures that result in particular colors is herein representing an integral chemical-biological process. Due to the increase and spread of cancer cases on records around the world, the author, however, aimed to shed the light on the stain materials that significantly assist in developing techniques which are employed in cancer diagnosis and lead to improving treatments and care of patients in addition to other pathological diagnoses. Historically, botanists were those who retained a basic interest in the cellular chemical processes whereas, the first microscope study of tiny biological entities was invented in the 17th Century [4]. In the 19th Century the histologist Camillo Golgi won the Nobel prize for the staining technique he invented to make it possible to detect micro organelles under the microscope. The dyes that had entered into biological use included mucicarmine [5]. From 1949 to 1984 Schiff-base derivatives, colloidal suspensions of metal ions, phthalocyanines, cotton dyes (e.g., Congo red, Pagoda red), methyl and ethyl green and others were introduced. Later were the aniline dyes [6], hematoxylin and its congeners [7], and the precipitable silver solutions [8]. In this respect, histochemistry was evolved as pairing biochemistry, microscopy, molecular biology and immunology. It is worth mentioning here that the field of immunofluorescence-based histochemistry (tissues' stain reactions) was thereby established by Coons et al. in 1941 [9], who won the prestigious Albert Lasker award in 1959 for that contribution. Coons and his coworkers used FITC (Fluorescein isothiocyanate)-labeled antibodies to localize Pneumococcal antigens in infected tissues. The present article is a brief collective reviews dealing with the

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integral chemical-biomedical processes emphasizing stain materials and their application in medical research with particular focus on cancer and its diagnosis.

2. Staining and Stain Material; Description and Application

2.1 Staining

Staining biologically can be by one stain/dye, counterstaining, and differential staining, or both that include double staining and triple staining. Staining process can assist also to study the morphology of lamellar structures of semi-crystalline polymers or the domain structures of block copolymers [10]. *In vivo* staining is the process of dyeing living tissues "in life", whereas, *in vitro* staining involves coloring cells or structures that have been removed from their biological context. In this respect, histochemistry is to be considered as an art and science integral process in which chemical reactions between laboratory stain materials and coloring components within tissues take place. The use of a fluorescent stain molecule increased the ability to identify DNA and/or RNA molecules [11]. Thus, the IHC (immunohistochemistry staining technique) is that the binding reaction of antibodies conjugated with antigens can be visualized with fluorescence dyes, when exposed to light of specific wavelength under fluorescence microscope. It is performed as one-step, two-step, three-step or as a multi-step staining processes [12]. In the immunoenzymological staining, enzyme-labeled antibodies and by adding a substrate; insoluble/high-electronic density particles are generated and can be localized under light microscope or electronic microscope [13, 14].

On the other hand, colloidal gold is in use as a marker, which can bind proteins rapidly and stably [15].

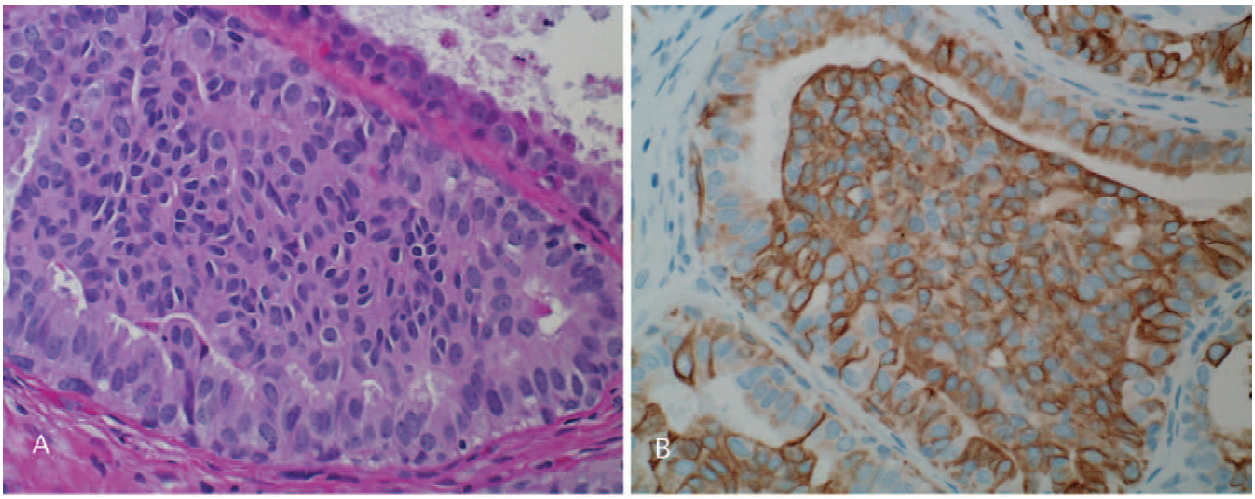
3. Stain Material Application in Cancer Diagnostic Research

3.1 Hematoxylin and Eusin

Hematoxylin is a compound extracted from the heartwood of the logwood tree (Haematoxylum campechianum). It is a positively charged (cationic) basic dye [16]. Eosin dye is a negatively charged (anionic) acid one. Eosin is a name of several fluorescent acidic compounds which bind to and form salts with basic, or eosinophilic, compounds like proteins containing amino acid residues and stains them dark red or pink. In addition it can be used to stain collagen and muscle fibers [17, 18].

Hematoxylin and eosin is a complex in predicting the breast origin, and is a combined stain material [19].

This stain complex is a permanent stain as opposed to temporary stains. In the H&E staining patterns, the basophilic affinity for the basic hematoxylin refers to blue color. The acidophilic affinity for eosin refers to red/pink color. The amphophilic affinity for both acid and basic dyes refers to a purple color. Eosin Y (eosin yellowish) is the mostly used, whereas eosin B is eosin bluish or imperial red which has a very faint bluish cast. The H&E stain is useful in predicting the breast origin for adenocarcinoma of unknown primary origin in ductal carcinoma of the breast, adenocarcinoma of stomach and clear cell carcinoma of the ovary. Sequential application of the dyes to histologic sections results in nuclei being stained blue, and cytoplasm and extracellular matrix pink [19]. However, in a tumor of unknown primary origin in a woman (Figs. 1a and 1b) the use of immunohistochemical markers is coupled with standard hematoxylin-eosin histology and panels of markers are used for estimating prognosis and predicting therapy response [20]. In this respect, the AJCC (American Joint Committee on Cancer) staging guidelines incorporate keratin IHC staining results in breast cancer staging [20].



(a) (b)

Fig. 1 Comparison of UDH (usual ductal hyperplasia) with ADH (atypical ductal hyperplasia) with immunostains. (a): UDH (hematoxylineosin, original magnification_200). (b): UDH, keratin 903, note strong cytoplasmic staining of cells (original magnification_200) [20].

3.2 Deep Tissue Imaging

Interestingly, later a technique that depends on staining methods in combination with LSFM (light-sheet fluorescence microscopy) was developed. Its objective is to understand the factors that influence drug penetration during medical treatments in respect to preclinical cancer research and drug development. The LSFM allows an extremely fast optical sectioning for three-dimensional reconstruction of centimeter-sized tissue samples at cellular resolution. However, application of tissue auto-fluorescence, *in vivo* fluorescence labeling, endogenous fluorescence or *ex vivo* whole-mount immune-labeling reached a level of three-dimensional in situ visualization of morphological and functional features of un-sectioned whole-mount tissue samples. This imaging technique had operated via fluorescent-labeled antibody in breast cancer tumor detection on a cellular level, which thus enabled tracing the formation, development and spread of tumors closer to the clinical situation providing more accurate therapy response in cancer research [21].

3.3 Immunohistochemical Method

In an earlier study, the immunohistochemical method was applied as an *in vitro* staining on frozen and paraffin-embedded sections of the lymphoid tissue of lymph nodes' samples taken from oncological patients. This allowed tracing disorders of blood rheological properties, such as a reduction in vascular wall functional activity and an increase in wall permeability, which results in large deposits of intra- and extra-vascular fibrin during cancer progression. The study revealed that the fibrin content in lymph nodes may promote the fixation of metastasis in them, as well as blockade of the cytotoxic effect of immunocomponent cells against tumor cells [22]. Simultaneously, a comparative immunohistochemical analysis of expression of oncoproteins revealed that the metastases of squamous cell carcinoma and adenocarcinoma of uterine cervix were shown to retain the main immunohistochemical attributes of cells of the primary cancer node. The results are of importance for the diagnosis of metastases in the unrevealed primary site and for detection of micrometastases [23]. More recently, the modern histochemistry and cell biology experimental techniques on tissue microarrays from cases of invasive breast carcinoma succeeded in detection of a strong correlation between lymph node stage and expression of estrogen receptor by immunohistochemical analysis of breast tumor microarrays [24].

4. Role of Stain Material in Other Biomedical Research Aspects

Alkali injury of the cornea and massive calcium overload in central nervous system. Staining process with haematoxylin and eosin provided a significant knowledge to demonstrate the role of severe alkali injury on the activities of enzymes of pericellular proteolysis, i.e. plasminogen activator or urokinase type (u-PA), plasmin. The study carried out on the rabbit cornea revealed that corneal ulcers occurred frequently and associated with the presence of many inflammatory cells in the injured corneal stroma in large and very large injuries. In contrast, in small injury, healing took place within a month and corneal ulcers were not observed. The results while on a mammal but certainly will be of significant help in dealing with such pathologic diagnostic cases and contribute to research on cases of the corneal melting in human if exposed to alkaline conditions [25]. Also, in rat cells where stain material was used via histochemical application enabled the diagnosis of massive calcium overload in the central nervous system. This occurred in neuron irreversibly injured by ischaemia and then reperfused with arterial blood. Calcium overload is also traced as a feature of catecholamine cardiotoxicity, the calcium paradox phenomenon, and prolonged high-flow substrate-free anoxia *in vitro*. In each of these instances, Ca^{2+} enters the cell, where it is massively accumulated by mitochondria. From the results it was reported that potentially lethal changes occur because of unrestricted entry of calcium into the cells [26].

4.1 Detection of the NOS (Nitric Oxide Synthase)

Experiments on the grey matter of the spinal cord of rabbits [27], histochemical methods are used as a good marker for detection of the NOS (nitric oxide synthase). The NADPH-d (nicotinamide adenine dinucleotide hydrogen phosphate-diaphorase) is the enzyme responsible for the synthesis of newly discovered gas NO (nitric oxide), which mediates communication between neurons and adjacent cells and signals inside cells. It is worth noting here that the NADPH-d staining suggested the possibility that NO might be utilized within the thymus [28]. In addition, by using the rat olfactory bulb, a comparative study on the role of NO as a novel type of messenger molecule revealed that in the nervous system, NO is formed by the neuronal isoform of the enzyme, NOS (nitric oxide synthase). In this study, the NADPH-d activity was characterized histochemically with the intention of elaborating on the optimal staining conditions with regard to intensity and specificity of NOS marker reaction [29]. In this respect, the addition of triton or pre-incubation of the tissue in an acid buffer showed an increase in the specificity of NADPH-d staining as a marker for nitric oxide synthase [29].

4.2 Detection of Molecular Malformations

The immunofluorescent microscopy helped in the detection of molecular malformations. In such a study, the effects of the integrated genes on embryonal morphogenesis and gene expression were investigated in tissues of different stages of embryonal development. This enabled the detection of cytoskeletal architecture and intercellular binding protein distribution that were taken as a marker of the molecular malformations. Therefore, the problem of intercellular interactions in morphogenes and interactions of especially cell-surface proteins can be emphasized [30].

4.3 Proteins in Heart Research

The unique characteristics of the immunofluorescent staining methods were therefore exploited as a tool to detect some intracellular and/or extracellular proteins, as well as endothelial cells and lymphocytes leading to an improvement in heart research. This allows the early diagnosis of pathophysiological changes in the myocardium, which is important for the recognition of the stage of heart damage. The staining of certain vital proteins that

shows the structure of these proteins in normal as well as in damaged tissue and the presentation of leucocytes, macrophages or monophages in the cardiomyopathic heart reached a complete monitoring of changes in cardiomyopathic cases [31].

4.4 Labeling and Imaging of Brain Tissues in the Alzheimer Disease

While the stain material alum-hematoxylin was used for nuclear staining process [32]; a fast, low cost, and highly efficient fluorescent DNA labeling method using methyl green staining was introduced recently [11]. In correspondence, histochemical labelling by Curcumin and Nano-curcumin dye is used in labelling and imaging of brain tissue.

In addition, a probe derived from boro-fluoro-Cur has been shown to have several times higher fluorescence properties than natural Cur upon binding to certain proteins. In this respect, a comparative study of dietary curcumin, nanocurcumin, and other classical amyloid-binding dyes were applied for labeling and imaging of amyloid plaques in brain tissue in the Alzheimer's diseased mice. Curcumin dye (Cur) is a bright yellow-colored pigment, derived from the root of the herb, Curcuma longa [33].

Recently, MDs from the Department of Pathology and Laboratory Medicine, and Microscopy Imaging Center, in the University of Vermont College of Medicine of USA and in University of Zurich of Switzerland had emphasized the comprehensive categories covered the advancement in stain materials and histochemical applications in research. These categories included: advances in methodologies; molecules in health and disease; organelles; subcellular structures; and compartments; the nucleus; stem cells and tissue engineering; cell cultures: properties and capabilities; connective tissues and extracellular matrix; developmental biology; nervous system; musculoskeletal system; respiratory and cardiovascular system; liver and gastrointestinal tract and male and female reproductive systems [34].

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