DOWNLOAD PDF IMMUNOHISTOCHEMISTRY IN THE DETERMINATION OF PRIMARY SITE

Chapter 1 : Immunohistochemistry as an Important Tool in Biomarkers Detection and Clinical Practice

Immunohistochemistry is an umbrella term that encompasses many methods used to determine tissue constituents (the antigens) with the employment of specific antibodies that can be visualized through staining. 1, 3 When used in cell preparations it is called immunocytochemistry, a term that some authors use for all methods entailing the.

This technique uses the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell, and therefore allows visualisation of the distribution of the target molecule through the sample. Immunofluorescence is a widely used example of immunostaining and is a specific example of immunohistochemistry that makes use of fluorophores to visualise the location of the antibodies. Immunofluorescence can be used on tissue sections, cultured cell lines, or individual cells, and may be used to analyse the distribution of proteins, glycans, and small biological and non-biological molecules. Immunofluoresence can be used in combination with other, non-antibody methods of fluorescent staining, for example, use of DAPI to label DNA. Several microscope designs can be used for analysis of immunofluorescence samples; the simplest is the epifluorescence microscope, and the confocal microscope is also widely used. Various super-resolution microscope designs that are capable of much higher resolution can also be used. Types of immunofluorescence There are two classes of immunofluorescence techniques, primary or direct and secondary or indirect. Primary direct Primary, or direct, immunofluorescence uses a single antibody that is chemically linked to a fluorophore. The antibody recognizes the target molecule and binds to it, and the fluorophore it carries can be detected via microscopy. This technique has several advantages over the secondary or indirect protocol below because of the direct conjugation of the antibody to the fluorophore. This reduces the number of steps in the staining procedure making the process faster and can reduce background signal by avoiding some issues with antibody cross-reactivity or non-specificity. However, since the number of fluorescent molecules that can be bound to the primary antibody is limited, direct immunofluorescence is less sensitive than indirect immunofluorescence. Secondary indirect Secondary, or indirect, immunofluorescence uses two antibodies; the unlabeled first primary antibody specifically binds the target molecule, and the secondary antibody, which carries the fluorophore, recognises the primary antibody and binds to it. Multiple secondary antibodies can bind a single primary antibody. This provides signal amplification by increasing the number of fluorophore molecules per antigen. This protocol is more complex and time consuming than the primary or direct protocol above, but it allows more flexibility because a variety of different secondary antibodies and detection techniques can be used for a given primary antibody. This protocol is possible because an antibody consists of two parts, a variable region which recognizes the antigen and constant region which makes up the structure of the antibody molecule. It is important to realize that this division is artificial and in reality the antibody molecule is four polypeptide chains: A researcher can generate several primary antibodies that recognize various antigens have different variable regions , but all share the same constant region. All these antibodies may therefore be recognized by a single secondary antibody. This saves the cost of modifying the primary antibodies to directly carry a fluorophore. Different primary antibodies with different constant regions are typically generated by raising the antibody in different species. For example, a researcher might create primary antibodies in a goat that recognize several antigens, and then employ dye-coupled rabbit secondary antibodies that recognize the goat antibody constant region "rabbit anti-goat" antibodies. The researcher may then create a second set of primary antibodies in a mouse that could be recognized by a separate "donkey anti-mouse" secondary antibody. This allows re-use of the difficult-to-make dye-coupled antibodies in multiple experiments. Limitations As with most fluorescence techniques, a significant problem with immunofluorescence is photobleaching. Loss of activity caused by photobleaching can be controlled by reducing the intensity or time-span of light exposure, by increasing the concentration of fluorophores, or by employing more robust fluorophores that are less prone to bleaching e. Immunofluorescence is only limited to fixed i. Proteins in the supernatant or on the outside of the cell

membrane can be bound by the antibodies; this allows for living cells to be stained. Depending on the fixative that is being used, proteins of interest might become cross-linked and this could result in either false positive or false negative signals due to non-specific binding. An alternative approach is using recombinant proteins containing fluorescent protein domains, e. Use of such "tagged" proteins allows determination of their localization in live cells. Even though this seems to be an elegant alternative to immunofluorescence, the cells have to be transfected or transduced with the GFP-tag, and as a consequence they become at least S1 or above organisms that require stricter security standards in a laboratory.

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Chapter 2 : Antibody Validation for Immunohistochemistry | CST

IMMUNOHISTOCHEMISTRY FOR CARCINOMA OF UNKNOWN â€¢Primary site determination for metastatic IHC for lineage/site specification.

This article has been cited by other articles in PMC. Abstract Immunohistochemistry IHC is an important application of monoclonal as well as polyclonal antibodies to determine the tissue distribution of an antigen of interest in health and disease. IHC is widely used for diagnosis of cancers; specific tumor antigens are expressed de novo or up-regulated in certain cancers. This article deals with the various applications of IHC in diagnosis of diseases, with IHC playing an important role in diagnostic and research laboratories. Antibody, antigen, disease, immunohistochemistry Immunohistochemistry IHC , the utilization of monoclonal and polyclonal antibodies for the detection of specific antigens in tissue sections, is an extraordinarily powerful tool in the armamentarium of the diagnostic surgical pathologist. IHC is an important application of monoclonal as well as polyclonal antibodies to determine the tissue distribution of an antigen of interest in health and disease. It is widely used for diagnosis of cancers because specific tumor antigens are expressed de novo or up-regulated in certain cancers. IHC plays an important role in pathology, particularly in the subspecialties of oncologic pathology, neuropathology, and hematopathology. Utilization studies are rare,[1] but several authors have reviewed the diagnostic utility of IHC in surgical pathology. The site of antibody binding is visualized under an ordinary or fluorescent microscope by a marker such as fluorescent dye, enzyme, radioactive element, or colloidal gold, which is directly linked to the primary antibody or to an appropriate secondary antibody. With the expansion and development of IHC technique, enzyme labels have been introduced, such as peroxidase[8 , 9] and alkaline phosphatase. Other labels include radioactive elements, and the immunoreaction can be visualized by autoradiography. The aim of IHC is to perform most IHC staining by causing least damage on the cell or tissue, and by using least amount of antibody, it finds a way in the tumor typing and tumor markers. Applications Since IHC involves specific antigenâ€”antibody reactions, it has apparent advantage over traditionally used special enzyme staining techniques that identify only a limited number of proteins, enzymes, and tissue structures. Therefore, IHC has become a crucial technique and is widely used in many medical research laboratories as well as clinical diagnostics. Analysis of tumors by these methods is a significant improvement over the conventional prognostic considerations by clinical staging and histologic grading. IHC is used for disease diagnosis, drug development, and biological research. Using specific tumor markers, physicians use IHC to diagnose a cancer as benign or malignant, determine the stage and grade of a tumor, and identify the cell type and origin of a metastasis to find the site of the primary tumor. IHC is also used in drug development to test drug efficacy by detecting either the activity or the up- or down-regulation of disease targets. A panel of antibodies is chosen to resolve such diagnostic problem cases. The selection of antibodies being made is based on clinical history, morphological features, and results of other relevant investigations. Immunohistochemical stains for intermediate filaments are expressed by tumor cells keratin, desmin, vimentin, neurofilaments, and glial fibrillary acidic proteins. Both these tumors are under the growth regulation of the hormones estrogen and androgen, respectively. The specific receptors for these growth regulating hormones are located on respective tumor cells. Tumors expressing high level of receptor positivity would respond favorably to removal of the endogenous source of such hormones or hormonal therapy is administered to lower their levels â€” estrogen therapy in prostate cancer and androgen therapy in breast cancer. The application is used routinely in validation of disease targets as it allows visualizing expression of the target in the affected tissue during the disease process. The concept was introduced as early as the s when fluorescein dye visible under ultraviolet light was tagged to antibodies directed against pneumococci for identification of this organism with specific anti-serum. Another important advantage of IHC is that it can also be used to detect organisms in cytological preparations such as fluids, sputum samples, and material obtained from fine needle aspiration procedures. This can be very helpful in

certain situations such as detection of pneumocystis from the sputum of an immunocompromised patient who needs rapid and precise confirmation of infection in order to begin immediate and appropriate therapy. In Genetics IHC can also be used to determine the function of specific gene products in fundamental biological processes such as development and apoptosis. Using a custom made monoclonal antibody against p53 homologue of the pro-apoptotic pathways of p53 was identified. Neurodegenerative Disorders Degenerative disorders of the nervous system include a wide range of diseases characterized by the dysfunction and death of specific, selectively vulnerable populations of nerve cells. It has played an increasingly important role in the subclassification of neurodegenerative disorders and the development of consensus criteria for their diagnosis. Brain Trauma In the last few years, immunohistochemical staining for beta amyloid precursor protein has been validated as a method to detect axonal injury within as little as 2â€”3 h of head injury. IHC in Muscle Diseases Specific diagnosis of muscular dystrophy is important because of the genetic counseling implications of inherited disease and accurate prognostication. In recent years, abnormalities in several muscle proteins have been identified in muscular dystrophies. Such abnormalities involve proteins located in the sarcolemma, extracellular matrix, cytosol, nucleus, and other sites within muscle fibers. Research Application Much of the current research into the causes of neurodegenerative diseases is directed at identifying the factors that result in the formation of paired helical filaments, the deposition of beta amyloid, cytoplasmic accumulations of alpha synuclein, etc. Consequently, studies to localize and quantify the abnormal proteins that constitute reasons of neurodegenerative diseases are of central importance. IHC using antibodies to beta amyloid, alpha synuclein, ubiquitin, huntingtin, polyglutamine, and others has become a routine tool for a sensitive detection and quantification of these abnormal proteins in both human tissues and in experimental animals that are used to model some of the features of these diseases. IHC is an important tool in diagnostic and research laboratories. Footnotes Conflict of Interest: The use of immunohistochemistry in an oralpathology laboratory, Malays. The differential diagnosis of central nervous system tumors: A critical examination of some recent immunohistochemical applications. Arch Pathol Lab Med. Best practices in contemporary diagnostic immunohistochemistry: Panel approach to hematolymphoid proliferations. Leong AS, Wright J. The contribution of immunohistochemical staining in tumor diagnosis. Histological examination has a majorimpact on macroscopic necropsy diagnoses. Discrepancies between clinical andautopsy diagnosis and the value of post mortem histology; A meta-analysisand review. Immunological properties of an antibody containing a fluorescent group. Proc Soc Exp Biol. Localization antigens in tissue cells. Improvements in a method for the detection of antigen by means of fluorescent antibody. Preparation and application for the localization of antigens. Mason DY, Sammons R. An immunocolloid method for the electron microscope. Essential pathology for dental students. Early detection of axonal injury after human head trauma using immunohistochemistry for beta-amyloid precursor protein. Vainzof M, Zata M. Protein defects in neuromuscular diseases. Braz J Med Biol Res.

Chapter 3 : Immunohistochemistry - Wikipedia

In patients with adenocarcinomas of unknown primary site, the focus is on identifying specific subsets in which disease-oriented therapy may be more effective than empiric therapy; this is based upon a combination of clinical features, IHC, and gene expression profiling.

If adenocarcinoma, then predict possible primary site s e. Based on [6â€”9]. The markers in bold are especially useful. CUP is a diagnosis of exclusion, since many studies exclude other tumour types including lymphoma, melanoma and sarcoma, as well as unusual primary rather than metastatic tumours [1 , 2]. Because these other cancer types nevertheless often enter the clinical and pathological differential diagnosis, they must still be considered. Neuroendocrine carcinoma comprises both poorly differentiated tumours, including small cell carcinoma, and well differentiated neuroendocrine tumours, including the old category of carcinoid tumour. Other carcinoma subtypes include carcinomas of solid organs, including hepatocellular, renal, adrenal and thyroid; and transitional cell carcinoma which is often grouped broadly with squamous carcinoma. Related tumours, which may appear similar to carcinoma, include germ cell tumours and mesothelioma [6]. Certain metastatic sites are more likely to harbour metastases from particular primary sites, which can aid diagnosis; and this enables CUPs to be divided into good and poor prognosis categories: Cancer classification is based on the differences in the appearance of different cancers and on their resemblance to the corresponding normal tissues. Tissues are aggregates of cells of similar type and function. Differences between tissues, normal or malignant, are based on the differences in their gene expression. Gene expression depends on the underlying DNa sequence and is regulated at multiple levels including epigenetic and via microRNas miRNas. Such tissue-specific or tissue-restricted genes are often regulatory genes or protein products [10]. Regulatory genes include transcription factors, especially homeobox genes controlling tissue development and maintenance, e. Protein products may be secreted or expressed in or on the cell and include cytokeratins, e. Just as tumours resemble morphologically the tissue from which they were derived, so tumours generally still express some tissue-specific genes, not only in primary cancers but also in metastases [11]. This is demonstrated using the bioinformatics technique of unsupervised clustering applied to cancer mRNa or miRNa gene expression profiles. Samples group cluster together by similarity: In such experiments, cancers of one histological type or subtype cluster together and with the corresponding normal tissue; likewise paired primary and metastatic tumours usually cluster together [11]. Clustering is due at least partly to tissue-specific genes, which explains their utility as diagnostic cancer biomarkers. This raises two general issues for diagnostic work-up in CUP. First, tissue-specific gene expression is better retained in well-differentiated than in poorly differentiated cancers [11]: Second, metastatic tumours are usually harder to diagnose than the corresponding primaries [12 , 13]; metastases might have lower expression of tissue-specific genes than the primary tumour. Most IHC biomarkers have been identified on a candidate basis, as single genes involved in a particular process. For most diagnostic purposes, however, IHC antibodies are used in a panel, including markers expected to be positive and negative in different tumours. This should mean that no single aberrant IHC stain causes incorrect diagnosis. IHC results depend on both the staining technique and microscopic interpretation: Most markers are familiar [6]; newer ones include OCT4, a transcription factor expressed in germ cell tumours, and D, found in mesothelioma and other tumours [6]. These include the classic CK7 and CK20, and newcomers such as Napsin a, a lung aspartic protease [9]; paired box gene 8 PaX8 , a Paired-boX transcription factor regulating gynaecological tissues, kidney and thyroid [8]; and NKX3. Prediction of the primary site of adenocarcinoma using selected immunohistochemistry IHC.

Chapter 4 : Immunohistochemistry | Cell Signaling Technology

Carcinoma of an unknown primary site is an uncommon clinical syndrome, accounting for approximately 3% of all oncologic diagnoses. Patients in this group are heterogeneous; they have a wide variety of clinical presentations and pathologic findings. A patient should be considered to have carcinoma of.

Sample preparation[edit] Preparation of the sample is critical to maintain cell morphology, tissue architecture and the antigenicity of target epitopes. This requires proper tissue collection, fixation and sectioning. A solution of paraformaldehyde is often used to fix tissue, but other methods may be used. Preparing tissue slices[edit] The tissue may then be sliced or used whole, dependent upon the purpose of the experiment or the tissue itself. Before sectioning, the tissue sample may be embedded in a medium, like paraffin wax or cryomedia. Sections can be sliced on a variety of instruments, most commonly a microtome , cryostat , or Compresstome tissue slicer. Depending on the method of fixation and tissue preservation, the sample may require additional steps to make the epitopes available for antibody binding, including deparaffinization and antigen retrieval. For formalin-fixed paraffin-embedded tissues, antigen-retrieval is often necessary, and involves pre-treating the sections with heat or protease. Reducing non-specific immuno-staining[edit] Depending on the tissue type and the method of antigen detection, endogenous biotin or enzymes may need to be blocked or quenched, respectively, prior to antibody staining. Although antibodies show preferential avidity for specific epitopes, they may partially or weakly bind to sites on nonspecific proteins also called reactive sites that are similar to the cognate binding sites on the target antigen. A great amount of non-specific binding causes high background staining which will mask the detection of the target antigen. To reduce background staining in IHC, ICC and other immunostaining methods, samples are incubated with a buffer that blocks the reactive sites to which the primary or secondary antibodies may otherwise bind. Common blocking buffers include normal serum, non-fat dry milk, BSA , or gelatin. Commercial blocking buffers with proprietary formulations are available for greater efficiency. Methods to eliminate background staining include dilution of the primary or secondary antibodies, changing the time or temperature of incubation, and using a different detection system or different primary antibody. Quality control should as a minimum include a tissue known to express the antigen as a positive control and negative controls of tissue known not to express the antigen, as well as the test tissue probed in the same way with omission of the primary antibody or better, absorption of the primary antibody. Polyclonal antibodies are made by injecting animals with the protein of interest, or a peptide fragment and, after a secondary immune response is stimulated, isolating antibodies from whole serum. Thus, polyclonal antibodies are a heterogeneous mix of antibodies that recognize several epitopes. Monoclonal antibodies are made by injecting the animal and then taking a specific sample of immune tissue, isolating a parent cell, and using the resulting immortalized line to create antibodies. This causes the antibodies to show specificity for a single epitope. Primary antibodies are raised against an antigen of interest and are typically unconjugated unlabeled , while secondary antibodies are raised against immunoglobulins of the primary antibody species. The secondary antibody is usually conjugated to a linker molecule, such as biotin , that then recruits reporter molecules, or the secondary antibody itself is directly bound to the reporter molecule. With chromogenic reporters, an enzyme label reacts with a substrate to yield an intensely colored product that can be analyzed with an ordinary light microscope. While the list of enzyme substrates is extensive, alkaline phosphatase AP and horseradish peroxidase HRP are the two enzymes used most extensively as labels for protein detection. For chromogenic and fluorescent detection methods, densitometric analysis of the signal can provide semi- and fully quantitative data, respectively, to correlate the level of reporter signal to the level of protein expression or localization. The direct method of immunohistochemical staining uses one labelled antibody, which binds directly to the antigen being stained for. The indirect method of immunohistochemical staining uses one antibody against the antigen being probed for, and a second, labelled, antibody against the first. Target antigen detection methods[edit] The direct method is a one-step

staining method and involves a labeled antibody e. FITC -conjugated antiserum reacting directly with the antigen in tissue sections. While this technique utilizes only one antibody and therefore is simple and rapid, the sensitivity is lower due to little signal amplification, in contrast to indirect approaches. The indirect method involves an unlabeled primary antibody first layer that binds to the target antigen in the tissue and a labeled secondary antibody second layer that reacts with the primary antibody. As mentioned above, the secondary antibody must be raised against the IgG of the animal species in which the primary antibody has been raised. This method is more sensitive than direct detection strategies because of signal amplification due to the binding of several secondary antibodies to each primary antibody if the secondary antibody is conjugated to the fluorescent or enzyme reporter. The indirect method, aside from its greater sensitivity, also has the advantage that only a relatively small number of standard conjugated labeled secondary antibodies needs to be generated. For example, a labeled secondary antibody raised against rabbit IgG, which can be purchased "off the shelf", is useful with any primary antibody raised in rabbit. With the direct method, it would be necessary to label each primary antibody for every antigen of interest. Counterstains[edit] After immunohistochemical staining of the target antigen, a second stain is often applied to provide contrast that helps the primary stain stand out. Many of these stains show specificity for specific classes of biomolecules, while others will stain the whole cell. Troubleshooting[edit] In immunohistochemical techniques, there are several steps prior to the final staining of the tissue antigen, which can cause a variety of problems including strong background staining, weak target antigen staining, and autofluorescence. Furthermore, autofluorescence may be due to the nature of the tissue or the fixation method. These aspects of IHC tissue prep and antibody staining must be systematically addressed to identify and overcome staining issues. IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. It is also an effective way to examine the tissues. This has made it a widely used technique in the neurosciences , enabling researchers to examine protein expression within specific brain structures. Its major disadvantage is that, unlike immunoblotting techniques where staining is checked against a molecular weight ladder, it is impossible to show in IHC that the staining corresponds with the protein of interest. For this reason, primary antibodies must be well-validated in a Western Blot or similar procedure. The technique is even more widely used in diagnostic surgical pathology for immunophenotyping tumors e. More recently, Immunohistochemical techniques have been useful in differential diagnoses of multiple forms of salivary gland, head, and neck carcinomas. Many clinical laboratories in tertiary hospitals will have menus of over antibodies used as diagnostic, prognostic and predictive biomarkers. Examples of some commonly used markers include: Used to identify tumors as well as in neuroscience research.

Chapter 5 : Immunocytochemistry - Wikipedia

According to immunohistochemistry (IHC) visualized tumor markers such as enzymes, oncogenes, tumor-specific antigens, tumor suppressor genes and tumor proliferation markers, doctors can efficiently predict oncogenesis and diagnose a cancer as benign or malignant, determine the stage and the grade of a cancer.

For further assistance, please contact our technical service department. Lack of Staining Test or Action Lack of antigen. Check protein expression by in situ hybridization in some rare cases translation may be blocked even though mRNA is detected. Antibodies do not work due to improper storage. Follow storage instructions on the datasheet. In general, aliquot antibodies into smaller volumes sufficient to make a working solution for a single experiment. Inactive primary or secondary antibodies. Test reporter system independently to assess reagent viability. Try increasing the fixation time or try a different fixative. Reduce the duration of the immersion or post-fixation steps. If immersion fixation cannot be avoided for example, collection of postmortem tissues or biopsies in pathology lab , antigens may be unmasked by treatment with antigen retrieval reagents. Incompatible secondary and primary antibodies. Use a secondary antibody that will interact with the primary antibody. For example, if the primary antibody was raised in rabbits, use an anti-rabbit secondary antibody. Antigen was destroyed before incubation with the primary antibody. If quenching of endogenous peroxidase was done prior to the addition of primary antibodies, block peroxidase after incubation with the primary antibody. Epitope altered during fixation or embedding procedure. Try restoring immunoreactivity through various antigen retrieval techniques. Antigen retrieval was ineffective. Increase the time of treatment or change the treatment solution. Reagents omitted or used in wrong order. Repeat staining and confirm that correct reagents are used and are added in the correct order. High Background Test or Action High concentration of primary and or secondary antibodies. Titer antibody to determine optimal concentration needed to promote a specific reaction of the primary and the secondary antibodies. Hydrophobic interactions of the antibody and proteins in the tissue. Lower the ionic strength of the antibody diluent particularly monoclonal antibodies respond well to reducing the salt concentration. Non-fat dry milk is another option. Non-specific binding of secondary antibody. Use an antibody that has cross-reactive IgG species removed absorbed against sample species. Avoid letting the tissue dry during the staining procedure. Reagents sticking to old or poorly prepared slides. Start over with freshly prepared or purchased slides. Background due to ionic interactions. Increase the ionic strength of the diluent buffer. Empirically determine the conditions that preserve tissue morphology while restoring the immunoreactivity of the antigen. Tissue sections falling off slide. Empirically determine an additional or alternative fixative. Use freshly prepared, adequately charged slides. Tissue section appears torn or folded. Air bubbles under section. Re-cut sections using a sharp blade, or ignore damaged areas when analyzing the results. Poor resolution of tissue morphology Cut thinner tissue sections. Ice crystals may have destroyed morphology of frozen sections. Cut smaller pieces of tissue for more thorough immersion fixation. Autolysis of tissue leading to staining of necrotic debris. Increase the fixation time, ratio. Consider using cross-linking fixative.

Chapter 6 : [Full text] Development and validation of an immunohistochemistry assay to assess | CMAR

Agrin immunohistochemistry facilitates the determination of primary versus metastatic origin of liver carcinomas Author links open overlay panel Ã•ron SomorÃ¡cz MD a 1 PÃ©ter TÃ¡trai PhD a 1 GÃ¡bor HorvÃ¡th a AndrÃ¡s Kiss MD, PhD a PÃ©ter Kupcsulik MD, PhD, DSc b Ilona Kovalszky MD, PhD, DSc c Zsuzsa Schaff MD, PhD, DSc a.

In contrast, immunohistochemical samples are sections of biological tissue , where each cell is surrounded by tissue architecture and other cells normally found in the intact tissue. Immunocytochemistry is a technique used to assess the presence of a specific protein or antigen in cells cultured cells, cell suspensions by use of a specific antibody, which binds to it, thereby allowing visualization and examination under a microscope. It is a valuable tool for the determination of cellular contents from individual cells. Samples that can be analyzed include blood smears, aspirates, swabs, cultured cells, and cell suspensions. There are many ways to prepare cell samples for immunocytochemical analysis. Each method has its own strengths and unique characteristics so the right method can be chosen for the desired sample and outcome. Cells to be stained can be attached to a solid support to allow easy handling in subsequent procedures. This can be achieved by several methods: Suspension cells can be centrifuged onto glass slides cytospin , bound to solid support using chemical linkers, or in some cases handled in suspension. Concentrated cellular suspensions that exist in a low-viscosity medium make good candidates for smear preparations. Dilute cell suspensions existing in a dilute medium are best suited for the preparation of cytospins through cytocentrifugation. Cell suspensions that exist in a high-viscosity medium, are best suited to be tested as swab preparations. The constant among these preparations is that the whole cell is present on the slide surface. For any intercellular reaction to take place, immunoglobulin must first traverse the cell membrane that is intact in these preparations. Reactions taking place in the nucleus can be more difficult, and the extracellular fluids can create unique obstacles in the performance of immunocytochemistry. In this situation, permeabilizing cells using detergent Triton X or Tween or choosing organic fixatives acetone, methanol, or ethanol becomes necessary. Antibodies are an important tool for demonstrating both the presence and the subcellular localization of an antigen. Cell staining is a very versatile technique and, if the antigen is highly localized, can detect as few as a thousand antigen molecules in a cell. In some circumstances, cell staining may also be used to determine the approximate concentration of an antigen, especially by an image analyzer. Methods[edit] Further information on immunochemical methods: Immunohistochemistry There are many methods to obtain immunological detection on tissues, including those tied directly to primary antibodies or antisera. A direct method involves the use of a detectable tag e. Alternatively, there are many indirect methods. In one such method, the antigen is bound by a primary antibody which is then amplified by use of a secondary antibody which binds to the primary antibody. Next, a tertiary reagent containing an enzymatic moiety is applied and binds to the secondary antibody. When the quaternary reagent, or substrate, is applied, the enzymatic end of the tertiary reagent converts the substrate into a pigment reaction product, which produces a color many colors are possible; brown, black, red, etc. Use of one of these reagents after exposure to the necessary enzyme e. Alternatively the secondary antibody may be covalently linked to a fluorophore FITC and Rhodamine are the most common which is detected in a fluorescence or confocal microscope. The location of fluorescence will vary according to the target molecule, external for membrane proteins, and internal for cytoplasmic proteins. In this way immunofluorescence is a powerful technique when combined with confocal microscopy for studying the location of proteins and dynamic processes exocytosis , endocytosis , etc.

Chapter 7 : Immunohistochemistry (IHC) in cancer

ProPath's Immunohistochemistry Laboratory is a CAP-accredited laboratory directed by Rodney T. Miller, M.D., an experienced diagnostic pathologist and immunohistochemist with a broad background in methodology and applications of Immunohistochemistry.

Glucocorticoid receptor GR activity has been associated with chemotherapy resistance and poor outcomes in patients with triple negative breast cancer TNBC. The aim of this study was to develop an immunohistochemistry IHC assay to assess GR expression in archival formalin-fixed, paraffin-embedded human invasive breast carcinoma samples. Precision and reproducibility of the GR IHC assay was determined by conducting multiple staining runs of four invasive breast carcinoma samples using replicate serial sections. Analysis of the paired TMA cores was performed by averaging the scores of the two cores for each case. Equivalent cellular patterns of GR reactivity were observed in all replicates from the multiple staining runs; coefficients of variation did not exceed 4. A robust and reproducible GR IHC assay was successfully developed for use in invasive breast carcinoma tissues. Differences in GR expression between larger single tissues and smaller TMA cores illustrate the heterogeneity of the disease, as well as potential intratumoral heterogeneity. Preliminary investigation found that the addition of mifepristone, a GR antagonist, significantly increased the cytotoxic effect of chemotherapy in both preclinical in vitro and in vivo models of GR-positive TNBC. As a result of these findings, additional studies of mifepristone chemotherapy combinations are underway in breast cancer. The use of immunohistochemistry IHC assays to evaluate ER and PR status and the use of IHC and fluorescence in situ hybridization to evaluate HER2 status are well established in the clinical evaluation of newly diagnosed invasive breast carcinomas. Materials and methods Antibody specificity testing Three different GR antibody candidates were chosen for comparative analysis: Upon determination of preliminary assays, staining patterns in test tissues were compared. Ethical approval for the use of human tissue samples in this study was not required, as the samples came from commercial tissue banks and did not contain any personal identifiers. The three antibodies demonstrated mostly equivalent staining patterns in the tissues tested positivity within the same regions of cells. The BuGR2 clone detected cytoplasmic and nuclear isoforms of GR, although with a broader staining pattern that appeared to be less specific compared with the other clones. It also did not appear to recognize stromal GR antigens. Thus, D8H2 was chosen as the optimal clone for further assay development and validation. In general, GR is recognized in invasive breast carcinomas, normal breast tissue, stromal cells, and T-lymphocytes. Both clones produced equivalent results, with only one sample showing tumor positivity, which is consistent with published rates of GR expression. The D8H2 antibody concentration, antibody incubation time, antigen retrieval reagents and methods, and antibody detection system were all tested as part of the optimization process. The D8H2 concentrations tested ranged from 1: Progressive iterative steps were employed based on the results of prior staining runs to identify the conditions that demonstrated accurate cellular localization of GR, a broad dynamic range of GR expression, an appropriate signal-to-noise ratio, and acceptable performance in positive and negative tissue controls. Rabbit IgG Cell Signaling Technology [S] was used at the same concentration as D8H2 to determine any nonspecific ie, antibody constant region staining inherent in the detection reagents or tissues or arising in tissues; rabbit IgG does not control for the unique GR antigen binding region of clone D8H2. This automated platform uses a capillary gap process 29 for all reagent changes, including antibody incubation, detection steps up to and including counterstaining, and intervening washes. D, Los Angeles, CA, USA , which is biotin-independent and reduces the potential for background or nonspecific staining from endogenous biotin, was used for primary antibody detection. Between all incubation steps, slides were extensively washed with tris-buffered saline containing 0. A percent score was used to semiquantitatively assess tumor GR expression in samples with at least viable invasive carcinoma cells. A board-certified pathologist scored nuclear tumor staining in the total area of viable tissue section available; areas of

cytoplasmic or stromal staining, in situ carcinoma, necrosis, or obviously poorly fixed areas of tissue were not evaluated. Results GR IHC assay precision and reproducibility Inter- and intra-assay variation of the GR IHC assay was assessed in a panel of four invasive breast carcinoma samples that comprehensively covered the expected range of GR expression in clinical samples. Within- and between-run precision were determined from multiple staining runs performed on different days by at least two different operators using different automated staining platforms. The tissues used in each run were replicate serial sections, with three sections per sample for GR expression and one section per sample as a negative control. The samples reacted as expected, and equivalent cellular patterns of GR reactivity were observed in all replicates. Minor and graded changes in GR expression were noted in immunostaining abundance scores that could be attributed to increases or decreases in the amounts of tumor in each serial section. Table 3 TMA percent tumor stain comparison Abbreviation: Discussion Previous analysis has shown that GR expression in ER-negative breast cancer is associated with chemotherapy resistance. Six patients had GR-positive disease, and of those, two had a complete clinical response and two had a partial response. Treatment was generally well tolerated with the exception of dose-limiting neutropenia resulting from elevated plasma levels of paclitaxel, which the authors felt was likely due to delayed clearance of nab-paclitaxel when coadministered with mifepristone. The variation in GR expression likely reflects the heterogeneity present in TNBCs as a group, as well as intratumoral heterogeneity. Methodological differences in assay development eg, antibody selection, tissue sampling, etc make it difficult to compare results with previously reported analyses of GR expression in patients with breast cancer. Therefore, larger tissue samples, including core needle biopsy or excision specimens, may be preferable for assessing GR expression and heterogeneity rather than the limited amount of tissue available for evaluation via TMA. The development and use of a validated assay technique to detect GR expression is necessary to help reduce the potential for discordant test results, which could impact therapeutic decisions. Human colon adenocarcinoma tissue was used as an internal negative control in this study for GR specificity rather than null xenograft tumor sections and cell lines because of the similarities in processing the human FFPE colon adenocarcinoma tissues to that of the TNBC FFPE tissues. While xenografts and cell lines used to create xenografts can demonstrate conclusive assay specificity, they can sometimes behave differently with IHC assays compared with human FFEP tumor tissues because of differences in composition, morphology, and format. Also, this assay was not optimized for cell lines, and cell lines were not readily available. Thorough precision testing demonstrated consistent and reproducible staining and established the robustness of the GR IHC assay. However, this cutoff may be modified based on the outcomes of GR antagonist therapy in clinical populations. Application of the GR assay in a clinical setting will help provide additional guidance on the most appropriate cutoff thresholds. This assay is currently being utilized in clinical trials of the GR antagonist, mifepristone, in patients with TNBC, where it will provide additional information on the effects of GR antagonism on cytotoxic chemotherapy. The University of Chicago has received research support, in the form of medicine and placebo tablets, from Corcept Therapeutics. Corcept also pays the University market-based fees to perform limited laboratory services. The authors report no other conflicts of interest in this work.

Chapter 8 : Immunohistochemistry

Immunohistochemistry is an effective, commonly used, adjuvant technique used to diagnose primary and metastatic neoplasms of the lung and pleura. Because of its relative ease of use and specificity, immunohistochemistry has largely replaced mucin histochemistry and electron microscopy in diagnosing pulmonary and pleural neoplasms.

Chapter 9 : Applications of immunohistochemistry

Immunohistochemistry (IHC) involves the process of selectively imaging antigens (proteins) in cells of a tissue section

by exploiting the principle of antibodies binding specifically to antigens in biological tissues.