Specialty area: Translational Pathways & Validation course

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Highlights:
- The most used molecular biology techniques are explained
- Molecular biology validation may turn your MRI interpretation up side down
- Pitfalls to avoid when starting out as a novice

There’s more than meets the eye: interpreting you MR images using molecular biology

Target audience: Non-biologists wishing to learn more about validating their MRI findings with basic molecular biology and histology techniques

Purpose: This educational lecture provides a basic explanation of standard molecular biology techniques and explains the use of these techniques in the context of MRI research. When developing MRI techniques, or when using MR methods in new applications, the question arises what the exact physiological or pathological substrate is of the obtained MRI contrast. In those cases, researchers may often use animal models or post-mortem brain specimens to validate the MRI findings using gold standard histological and molecular biology techniques.

Methods: This presentation will focus on the most commonly used techniques, such as immunohistochemistry, Western blots and Elisa essays.

(Immunohistochemistry) refers to the detection of molecular compounds in sections of biological tissue. These compounds may be detected by specific stainings, or by the use of antibodies targeted against the antigen one wishes to visualize, hence the term immunohistochemistry. Antibody stainings may be visualized with a direct method, in which the antibody is labeled with a fluorophore. Alternatively, an indirect method may be used in which a secondary antibody reacts with the primary antibody. This secondary antibody in turn can be labeled with a fluorophore, or may be conjugated to a peroxidase enzyme which will oxidize diaminobenzidine (DAB) to produce a dark brown color. Before immunohistochemistry can be done, the tissue needs to be preserved by chemical fixation or freezing. Depending on the preservation method, the tissue can then be sectioned into very thin slices for staining. Immunohistochemistry gives excellent spatial information about the distribution of proteins in tissue. However, quantification of the amount of protein is difficult. In such cases Western blots can be used as an alternative technique. In this technique, a tissue homogenate or extract is used. The proteins in this sample are separated by gel electrophoresis based on their 3-D structure or size. After separation, the proteins are then stained with antibodies and may be quantified based on the intensity of staining. ELISA (enzyme-linked immunosorbent assay) provides the most quantitative detection method. Similar to Western blots, homogenized tissue is used. The samples are loaded on 96-well plates and immobilized on a solid support. The antigen of interest is then detected by a specific antibody and visualized using the same direct or indirect methods as for immunohistochemistry. Usually, a known concentration range is added for the antigen to create a standard curve, from which the antigen concentration in the sample can be determined.
Results and discussion
Apart from these basic principles, examples will be shown of cases where molecular biology played an essential role in interpreting the MRI findings. Attention will be given to special methodological considerations necessary to avoid interference between the techniques.

Literature:
An excellent introduction to immunohistochemistry can be downloaded from the Dako knowledge center (www.dako.com)