Is that Copper or Iron in the Liver?

Liver Cirrhosis: Electron-microscope Element Analysis for Fast, Differential Diagnosis

Liver cirrhosis can be due to various causes, including alcohol abuse and viral hepatitis B. Two of the most frequent hereditary metabolic diseases are also indicated: hemochromatosis with pathological iron accumulation and Wilson's Disease with pathological copper accumulation. In order to distinguish them, the Institute of Pathology of the University of Rostock uses energy-filtering transmission electron microscopes for investigation of tissue sections. Thanks to the energy filter and software by Olympus Soft Imaging Solutions for digital image acquisition and image processing, elemental maps and energy loss spectra can be generated. These make it possible to obtain diagnoses quickly, even in early stages of the disease, and begin appropriate treatment early.

Beyond just High Resolution

Electron microscopes perform valuable services in pathology. Structural analyses through electron microscopy play a critical role, for example, when an unknown viral infection is suspected, and with hereditary metabolic diseases. They are also useful when investigating glomerulonephritis, carcinoids and less differentiated melanomas. For purely ultrastructural investigation, electron microscopy has, however become less significant in the past few years for pathomorphological diagnostics. The reasons behind this development have to do with the introduction of immune histochemical detection procedures for antigens, of in-situ hybridization techniques as well as the polymerase chain reaction (PCR). The energy-filtered transmission electron microscopes (EFTEMs) available today can do much more, however, than simply show very fine structures of ultrathin sections at high resolution. It is conveniently easy to conduct elemental analysis with them, similar to Energy Dispersive X-Ray analysis (EDX).

Samples Remain Intact

The EFTEM makes two kinds of elemental analysis possible. Spot analyses can be
done on selected areas of a sample by detecting the electron energy loss spectra of these areas, known as EELS (Electron Energy-Loss Spectroscopy). Electron spectroscopic images (ESI) may also be made of large areas of a sample.

Elemental maps can be generated based on the ESI. In comparison with chemical analyses and most physical methods such as atomic absorption spectrometry (AAS), there is one big advantage. Investigating an ultrathin section in the EFTEM can be repeated as often as desired. Samples are not dissolved in fluid first, nor destroyed during the investigation. Another advantage is that the heterogeneity of the objects being investigated is detectable. This cannot be done with dissolved or homogenized samples.

Two Types of Liver Cirrhosis

Due to such analytical electron-microscopical capabilities, new horizons have opened up in the field of pathology. Two different types of genetic liver cirrhosis serve to demonstrate. Aside from detecting mutation, these forms of cirrhosis can only be distinguished diagnostically through elemental analysis. Both represent metabolic breakdowns due to hereditary genetic defects and are not related to environmental causes or acquired via alcohol abuse or hepatitis infection. The first is hereditary hemochromatosis resulting from a selective mutation of the HFE gene (C282Y) on chromosome 6. This mutation prevents iron from being discharged properly from the body and results in an accumulation of iron in the liver. Over a longer period of time this will result in liver cirrhosis which can lead to liver failure. The second is Wilson's Disease, which also leads to liver cirrhosis and ultimately to liver failure. Here a mutation within the gene responsible for the discharge of copper is at fault - the $\text{H}^+ \text{Cu}^{++}$ ATPase on chromosome 13.

Early Diagnosis
An important question to be answered regards the distinction between these two types of liver cirrhosis - and being able to do so at an early stage. This differential diagnosis needs to be fast, easy and unambiguous and at an early stage so that the later consequences can be avoided. For such an early diagnosis the heavy metal deposits in the liver cells (hepatocytes) have to be investigated. This can be done via analytical electron microscopy in the EFTEM. Determining the nature of any accumulation in tissue samples is done quickly and precisely via the acquisition of EELS spectra using the Olympus Soft Imaging Solutions Esi-Vision software solution for the iTEM platform.

**Characteristic Curves**

The EFTEMs used are a Zeiss 902 A with prism filter, a 912 A and a LIBRA with Omega filter. The electron beam is aimed at the heavy-metal accumulating lysosomes and remaining parts within the hepatocytes. A detector measures the energy loss of electrons during transmission through these areas on the sample. This energy loss depends on the type of atoms the electrons encounter on their passage and thus yields information about the composition of the sample. Displayed graphically, these EELS spectra can be easily compared with the standard spectra of the elements suspected of being present. This makes it possible to quickly and clearly distinguish between hemochromatosis with its characteristic iron accumulation and Wilson's Disease with its characteristic copper accumulation. The elemental maps are also generated via EFTEM and the software and show where and how much of the respective element has accumulated within the tissue samples.

**Conclusion and a Glance at the Horizon**

Analyzing elements via EFTEM is so sensitive and precise that two hereditary metabolic diseases - hemochromatosis and Wilson's Disease - can be recognized at an early stage. Caught early, therapy then has a good chance of preventing the life-threatening effects of advanced liver cirrhosis. The software conveniently manages spectra, images and other related documents with a clearly structured archive database. The software also offers an integrated report generator. This EFTEM and software 'dynamic duo' are not just for quick and reliable diagnosis of metabolic diseases. This method is also suitable for detecting any kind of deposits and accumulations within tissue. It has been used to show that over the long term, body implants made of very durable metal are broken down biologically (biodegradation). For example, inner ear protheses or osteosynthetic plates and screws made of gold, platinum or titanium can exhibit surprising levels of biodegradation. Other applications include analysis of asbestos (silicon, iron and manganese) and
anthracosis (silicon, carbon) and argyria (silver). Quickly differentiating between melanomas and tattoos containing amalgam (mercury, silver, tin, zinc) is another application. An article on this subject is being prepared for publication.

References:

Please ask the authors to obtain further bibliography

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