Liver Cirrhosis - Is that copper or iron in the liver?
Electron-microscope element analysis for fast, differential diagnosis
Liver cirrhosis: Is that copper or iron in the liver?

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.

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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.

This image shows a coarsely tuberous liver cirrhosis. The 30-yr.-old male patient this was removed from received a healthy liver transplant and has been living for more than 10 years without any negative side effects. He had Wilson’s Disease, one of the most frequent hereditary metabolic diseases. His liver was insufficiently capable of disposing of copper and accumulated it instead.

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 H+ Cu2+ 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 EsiVision 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

How are elemental maps and EELS spectra generated?

EFTEM stands for Energy Filtering Transmission Electron Microscope. The electron beam of the EFTEM is directed through the sample (the ultrathin section of liver tissue, for example) and creates a magnified image in the image plane. Thanks to the built-in energy filter, electron-spectroscopic images (ESI) can be generated. For example, if the energy filter is set to 725 eV, then only those electrons that have lost 725 eV will make it to the image plane and contribute to the image. All other electrons are removed by the energy filter.

725 eV is the characteristic energy loss of an electron from an inner-shell ionization of an iron atom. This kind of electron is allowed to pass through by the energy filter and contributes to the image generated on the image plane. Unfortunately, there are other electrons that make it to the image plane which, due to various kinds of multiple energy transfers while passing through the sample, coincidentally also have a total energy loss of 725 eV. Without these other spurious electrons, the ESI image at 725 eV would constitute a direct elemental map of iron, displaying the distribution of iron in the sample.

An elemental map can be calculated however, based on multiple ESI images. At the Electron Microscopy Center of the Faculty of Medicine of the University of Rostock in the Institute of Pathology the iTEM software solution called EsiVision is used. The software remote controls the electron microscope and acquires a series of ESI images at various energy losses via a digital camera. Because ESI images have a very small signal-to-noise ratio, the software optimizes image quality directly during acquisition.

Using what is known as the “two-window method”, two ESI images are acquired: the first one at 725 eV and the second one at a slightly lower energy loss. The second image is then subtracted as a background image from the first image. This eliminates any disturbing background effects – including most contrast segments that are due to the above-mentioned spurious electrons. The result is the desired elemental map showing how much iron has accumulated in the tissue sample. The elemental map is also suitable for quantitative measurement.

Via the EFTEM, thanks to the energy filter and the software, Electron Energy Loss Spectra (EELS) can be recorded. For exact investigation of metal accumulation in tissue, the electron beam is directed at the corresponding position on the sample. The digital camera serves as a detector and records how many electrons lose energy when passing through the sample. Each element has a characteristic EELS spectrum curve. Comparing the EELS with standard spectra determines whether accumulations are of iron or copper.

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Early-stage hemochromatosis was suspected in a 12 year-old female patient. Via electron microscopy and digital image analysis it was possible to confirm this suspicion. The therapy that was initiated immediately prevented her from developing liver cirrhosis. Left: The comparison of the measured energy loss spectrum with the iron standard spectrum proves that the accumulations in the tissue are indeed iron. Iron is characteristic for hemochromatosis. Right: The elemental map shows where and how much iron has accumulated. The red parts represent where the iron is located.

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.

Wilson’s Disease led to acute liver failure in a 30 year-old male patient. He was saved by a liver transplant. The liver removed is shown in fig. 1. Just as with fig. 2, the energy loss spectrum (left) and the elemental map (right) show that the accumulations in the tissue are indeed copper, which is characteristic for Wilson’s Disease. The image analysis software highlights the copper (right) green.

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.

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