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Endogenous oxidants: biosensor monitoring of metabolic conditions in living organisms

The oxidation state of the cells in our body is very important for us: if the normal balance of the distribution of endogenous oxidants is disturbed or if they attack cellular structures, cells are either unable or only partially able to fulfil their functions, and diseases develop. Knowing the oxidation state and one day also having the capacity to modify it with pharmaceuticals, is therefore of crucial importance when disease-related changes occur. However, a suitable method for studying oxidative processes in the whole organism has not been available up until now. Dr. Tobias Dick and his team of researchers at the German Cancer Research Center (DKFZ) in Heidelberg have now developed a biosensor that facilitates real-time measurements of subtle oxidative changes in metabolism and has led to completely new insights into the body.

The oxidation state of molecules in our body depends on many factors. For example, diet, physical activity, diseases and possibly ageing are all factors that impact the spatial and temporal distribution of endogenous oxidants in cells and tissues. For a long time, the opinion prevailed that oxidants – often wrongly referred to as "free radicals" – were harmful as they cause cell damage and promote ageing processes. Antioxidants were presented as health-promoting due to their ability to capture free radicals and thus protect cells against oxidative stress. However, scientists have known for quite some time that these are false myths: endogenous oxidants are chemical messengers that are essential for keeping organism functions going. Hydrogen peroxide plays a key role in these processes.

Endogenous oxidants are a by-product of normal metabolic processes such as cellular respiration during which molecular oxygen is reduced. Under physiological conditions, cellular enzymes control the production, amount as well as spatial and temporal distribution of oxidants in the cells and in fact prevent the production of reactive radicals. Without this control, our body is unable to break down reactive oxygen species into harmless oxygen and water, resulting in the radicals attacking cellular structures.

Oxidants – important messenger substances in the body

Dr. Tobias Dick's research group at the German Cancer Research Center in Heidelberg investigates the function and distribution of endogenous oxidants. "Our research group is specifically focused on the analysis of hydrogen peroxide, which is a specific and controlled signal produced during normal cell metabolism," says Dick. "Hydrogen peroxide was previously regarded as a metabolic waste product. And to this day, many members of the public still believe that oxidants are harmful and

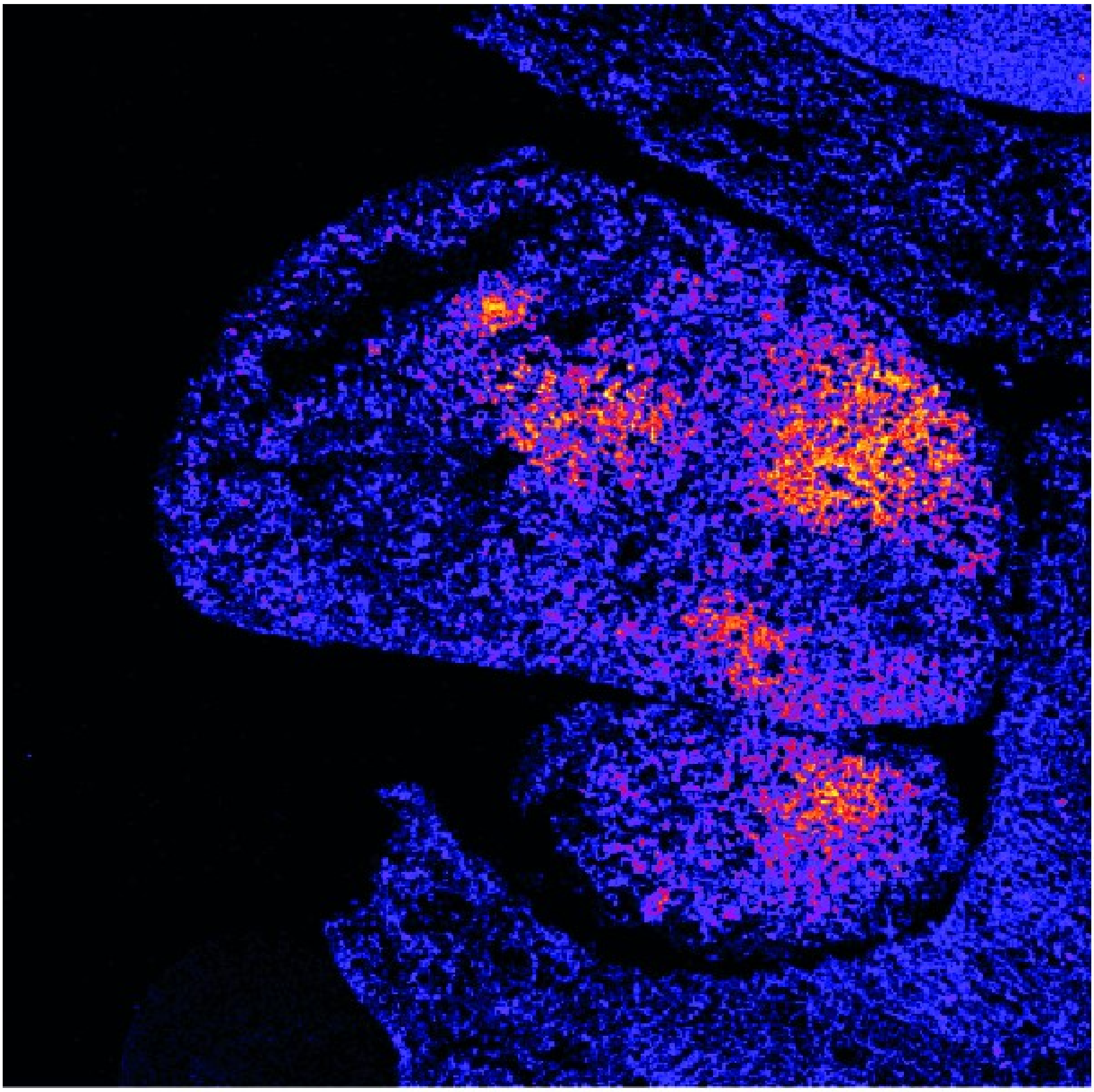
Dr. Tobias Dick's (3rd from the left) research group at the German Cancer Research Center in Heidelberg investigates the function and distribution of endogenous oxidants.

cause oxidative stress. However, these substances have important signalling functions in the body, they are essential for normal cell processes, be they responses to hormones or immune reactions. Hydrogen peroxide is always involved and instrumental in the regulation of enzymes. "In terms of evolution, this is an old and elementary mechanism, which is found in varying forms in all organisms," says Dick.

Oxidants play a particular role in the adaptation of cells to stress situations. For example, physical strain unleashes mechanisms that protect cells and enable them to adapt to the new situation: "Hydrogen peroxide is thought to be the mediator," said the cell biologist. "Mitochondria produce hydrogen peroxide when they are overstrained, and this is a signal for the rest of the cell to adapt to the altered situation, for example by forming a larger number of mitochondria. A number of transcription factors and enzymes respond to the hydrogen peroxide molecules, which then transfer the signal to the nucleus, where an adaptation to new conditions takes place."

Luminescent biosensor gives insights into the organism

When and where oxidants transfer signals in the context of the whole body is still barely understood. The researchers from Heidelberg have developed a method to investigate organismic processes which allows them to visualise changes in hydrogen peroxide concentrations in tissue sections: the biologists use a genetically encoded biosensor to assess the presence and distribution of oxidants in the tissue sections under the microscope. The biosensor is a protein that emits fluorescence signals upon excitation. The genetic information of the sensor is integrated into the genome of mice. These mice then express the proteins in all cells. Upon removal, the tissue is immediately cryoconserved in liquid nitrogen to maintain the in vivo redox state of the biosensor. However, the researchers found that this natural state changed quickly when the samples were thawed. They therefore developed a chemical preservation method in which alkylating agents are poured over the tissue as it is cut. The biosensor is thus quickly and effectively blocked in its original condition. "No matter what will



Formation of oxidants, visualised on a tissue section using biosensors.

subsequently be done with the tissue section – the original redox state of the bionsenor is maintained," explains the biologist.

Analysing oxidants in the entire body

The DKFZ researchers have used the biosensor to investigate oxidants in a variety of tissues, for example a growing tumour or a muscle under starvation conditions. "We had expected hydrogen peroxide signals, but were actually able to see them with the biosensor," says the scientist. The biologist regards these positive results as a first step on the path to being able to analyse the impact of diseases and drugs on the distribution of oxidants in the entire body. And they hope that they will eventually be able to pharmacologically influence abnormal oxidation conditions. "Pro- or antioxidative agents have been available for quite some time," says Dick. "But it has always been difficult to investigate them in living organisms, clarify where they end up and how they act. With our biosensor we are now able to obtain insights into these processes."

Investigations are currently mainly performed on tissue sections. In collaboration with researchers from the University of Munich, so-called in vivo imaging was performed on living organisms. In order to investigate the situation in multiple sclerosis, the researchers were able to have a real-time look into the spinal cord. This type of application is currently still rather limited because the light signals can barely be perceived in deeper tissue sections. “The techniques are being further developed and will in future increase the possibilities of performing investigations in living organisms,” says Dick. Organs that are removed and subsequently kept in culture might lead to falsified results when they are removed from their natural context. “This is why a method that enables us to preserve biosensor redox states in tissue sections is so important for us,” says Dick. “This enables us to preserve the biosensor’s state at the time of its removal. However, the disadvantage is that this is only a snapshot of the situation at a particular point in time.”

Peroxiredoxins – the most sensitive endogenous biosensors

In a second project, the researchers from Heidelberg are aiming to make the biosensor even more sensitive. “Rather than developing a completely new principle, we came up with the idea of using an endogenous detection system, i.e. peroxiredoxins,” says Dick. Peroxiredoxins have the highest hydrogen peroxide sensitivity of all proteins. The new biosensors couple the reaction of a peroxiredoxin molecule to a fluorescent signal and are so sensitive that they can visualise the smallest metabolic changes in oxidant production. The new sensors were initially tested in yeast cells where the researchers were even able to observe the movement of the oxidants between individual subcellular structures (Morgan et al.). The next step will involve testing the peroxiredoxin-based sensors in the mouse model. However, the researchers' long-term goal is to use the biosensors for discovering drugs that enhance or suppress oxidative signals, i.e. drugs that specifically alter the oxidation state of cells. “We still have to establish the method in the animal model before we can use it for human application,” says the cell biologist.

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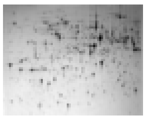

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