Epigenetic modifications for the treatment of oesophageal cancer

Oesophageal cancer is a rare but highly aggressive type of cancer with a rather poor prognosis. The exact causes of the disease are not yet known. However, tobacco, excessive alcohol consumption and heartburn are regarded as major risk factors. The main treatment is surgical removal of the tumour in combination with radio- and chemotherapy, which can potentially improve prognosis. Dr. Theresa Ahrens, a researcher in a group led by Prof. Dr. Silke Laßmann and Prof. Dr. Martin Werner at the Institute of Clinical Pathology at the Freiburg University Medical Centre, has tested a variety of epigenetic drugs that can interfere with the development of oesophageal cancer.

Dr. Theresa Ahrens wants to reduce the migratory capacity of oesophageal cancer cells.

The two main subtypes of oesophageal cancer, oesophageal squamous cell carcinoma and oesophageal adenocarcinoma, differ in pathogenesis and histology. Squamous cell carcinoma can occur anywhere in the oesophagus and mainly affects men over 55. Squamous cell carcinoma is specifically linked to high alcohol and nicotine consumption. While the number of squamous cell carcinoma cases is on the decline, oesophageal adenocarcinoma is becoming increasingly important due to the growing number of hospitalisations. The tumour is usually located on the lower side of the oesophagus, which is why gastro-oesophageal reflux disease is regarded as the major risk factor. "The reflux of gastric acid can damage the oesophageal epithelium; a new epithelium, which forms a protection against gastric acid, then develops," says Therese Ahrens, who also believes that epigenetic factors play a role in this process. Oesophageal cancer is usually diagnosed when prominent symptoms appear and the cancer is already in an advanced stage. The prognosis of oesophageal cancer is therefore quite poor. The most common

symptom is usually difficulty in swallowing, often associated with pain and weight loss. The latter is, however, a symptom of all cancers.

Cancer epigenetics

What causes cancer? Scientists around the world are still working to find out what causes a healthy cell to become a cancer cell. They have been able to show that epigenetic regulation systems can become out of control in various types of cancer, including oesophageal cancer. These systems, which specifically modify the DNA bases and determine which genes are needed or not, are mediated by enzymes. For example, histone deacetylases (HDACs) remove acetyl groups from histones and render the gene non-transcribable and inactive. DNA methyl transferases (DNMTs) prevent gene transcription by attaching methyl groups to specific DNA bases. Therefore, epigenetic modifications that lead to a change in gene expression also control aspects of cell division. "Epigenetic mechanisms have been shown to inactivate tumour suppressor genes. However, epigenetic mechanisms might also activate oncogenes," says Ahrens. For example, hTERT (human telomerase reverse transcriptase) has been shown to be selectively expressed in tumour cells and thus contribute to the immortalisation of cancer cells. microRNAs have also been shown to be involved in the epigenetic regulation of gene expression; however, they exert their effect on the post-transcriptional level.

Inhibition of HDACs and DNMTs

The team is part of the DFG-funded Collaborative Research Centre of Medical Epigenetics (CRC 929) and has investigated the effects of HDAC and DNMT inhibitors on two types of oesophageal carcinoma lines (a non-neoplastic oesophageal cell line as well as an oesophageal cancer cell line). The researchers are hoping that their work might lead to the use of epigenetic drugs to either activate genes that help fight oesophageal cancer or inhibit genes that promote its development. Ahrens investigated three different HDAC and two different DNMT inhibitors individually and in various combinations for their ability to interfere with oesophageal cancer development.

Single inhibitors did not damage the cancer cells, but the combination of 5-azacytidine (DNMT inhibitor) and MS-275 (HDAC inhibitor) had a more pronounced effect. Although the two epigenetic regulation systems have different effects, they nevertheless work along the same lines: they epigenetically enhance or diminish inhibitory signals. "Generally, the different levels of epigenetic control are closely interconnected. Synergistic effects can be achieved and the specificity of cancer cell inhibition increased by targeting different epigenetic levels, DNA methylation on the one hand and histone acetylation on the other. The removal of acetyl groups from histones compacts chromatin and renders genes inactive while the attachment of methyl groups to DNA suppresses gene activity," says Ahrens. In cancer cells, DNA methylation inactivates tumour suppressor genes. HDAC inhibitors such as MS-275 enhance the acetylation of histones, resulting in the transcription of tumour suppressor genes such as p21. In other words, HDAC-mediated histone acetylation leads to the transcription of inactive (tumour suppressor) genes, thus restoring the gene's original function.

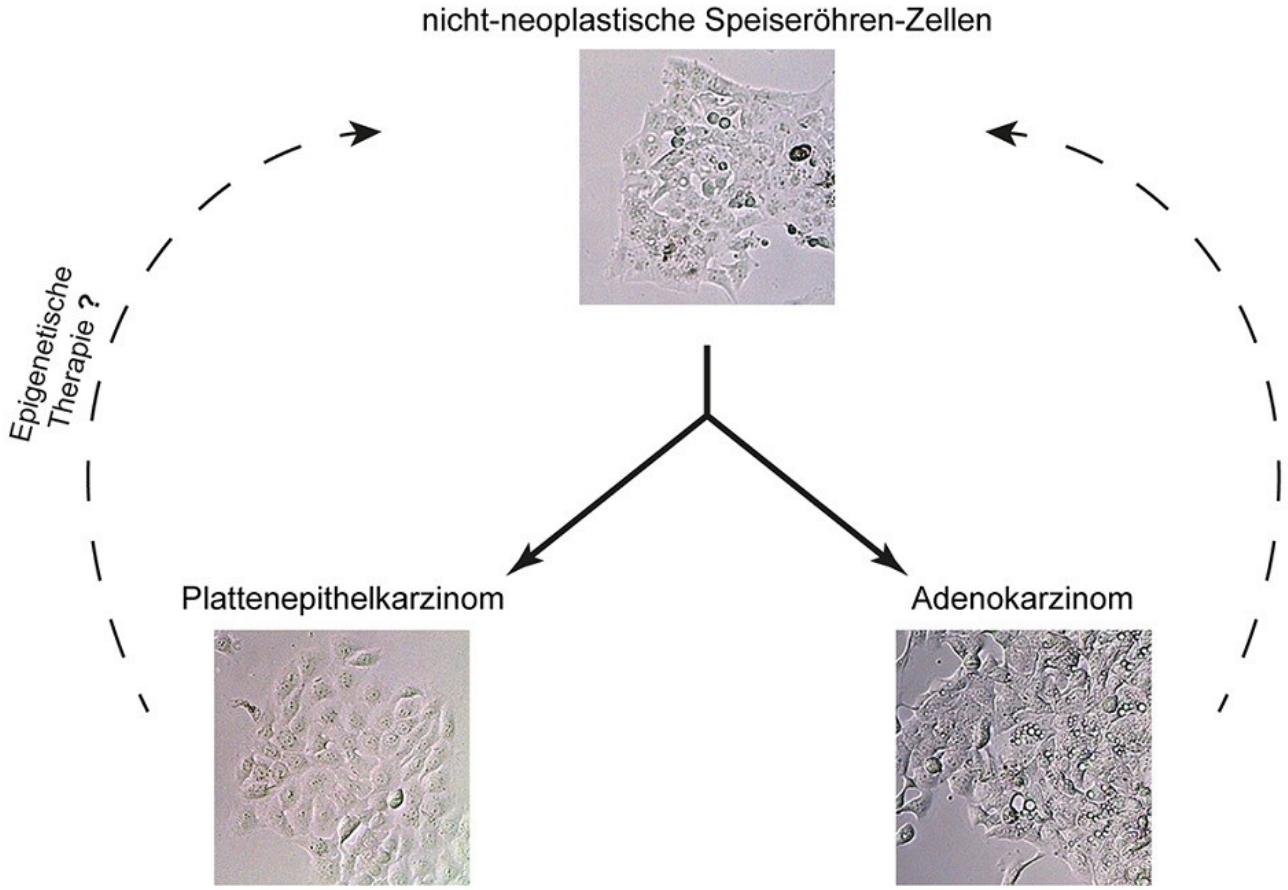
Thargeted therapies

Ahrens was able to show that the epigenetic drugs influenced the regulation of important genes in malignant oesophageal cancer cell cultures, and either attenuated or eliminated cancer cells.

These initial cell culture experiments showed that the epigenetic drugs selectively inhibited the migration of cancer cells, and were even able to trigger the death of some cells. Despite the similar expression of HDACs, normal, non-neoplastic oesophageal cells were not affected. The DNMT inhibitor 5-azacytidine is a synthetic nucleoside and cytosine analogue that prevents the synthesis of proteins due to its incorporation into DNA or RNA. Non-dividing cells are relatively unaffected by treatment with this antimetabolite. However, dividing non-neoplastic, i.e. healthy cells, also seem to be unaffected by the epigenetic drugs.

"One theory is that the healthy cells have simply stopped the cell cycle and used the time to repair the DNA damage," says Ahrens. Existing chemo- and radiotherapies used to treat oesophageal cancer are fairly unspecific and also affect healthy cells. The goal is therefore to develop targeted therapies for all kinds of tumours, i.e. therapies that interfere with specific molecules needed for tumour growth, and thus reduce any potential adverse effects of the drugs. In the future, targeted therapies might also involve epigenetic therapies, i.e. medicines that trigger antitumoral effects in cancer cells by removing cancer-associated methylations or histone modifications.

Hoping to redifferentiate cancer cells



The researchers hope that epigenetic inhibitors will help turn abnormal cancer cells of squamous cell and adenocarcinomas into better-natured cells.

After conception, epigenetic factors contribute to the development of a variety of cell types, specialised tissues and organs. Fully differentiated cells cannot normally go back to an undifferentiated state. A former epithelial cell of the oesophagus that has become an abnormal cancer cell might to a certain degree develop back into an epithelial cell. "Cancer can be thought of as a state of cellular dedifferentiation, cells that take a step back in their development," says

Ahrens. If the researchers could stop the dedifferentiation of cancer cells, they would be able to enhance their specialisation. However, this can also be done in small steps. “I don’t think we will be able to turn a cancer cell into a completely normal epithelial cell again. But I do believe that we will be able to make cancer cells less malignant,” says Ahrens. It can be assumed that cancer cells that are unable to migrate are also less likely to form metastases.

Even though these HDAC and DNMT inhibitors have been used for treating rare leukaemias in Europe since 2008, there is still a long way to go before they can be used to treat solid tumours such as oesophageal cancer.

Article

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genetic diagnostics | cancer | metastases | epigenetics | cell differentiation | basic research | therapy | drug discovery