



DNA Packaging – A New Approach to Cancer Therapy

Treating cancer is one of the big challenges for modern science. The causes of tumor formation must be understood and methods must be developed allowing degenerate cells to reform to their healthy predecessors or to kill them selectively. In the last few years it has turned out that the packaging of the genetic material DNA in chromatin is of central significance to processes involved in cancer. Scientists at the GSF Institute of Toxicology study a class of enzymes – histone deacetylases – which considerably influence the packaging of DNA and are, thus, a potential point of attack for a cancer therapy.

For more than 30 years a drug has been on the market which can be used for the successful treatment of patients suffering from epilepsy. This is valproic acid, a small organic molecule, which results in the stable absence of seizures in more than 60 per cent of the patients. Valproic acid is well tolerated and has few side-effects, apart from one prominent exception: the sub-

stance is teratogenic, which means that its application during pregnancy may seriously damage the embryo. The embryo's spinal canal is not closed properly and the skull's development is out of proportion.

It was long unclear why valproic acid has such a fatal effect on the embryonic development, but does not cause any serious dam-

age in the adult organism. Together with his colleagues at the time at the Karlsruhe Research Center and at Georg-Speyer-Haus in Frankfurt/Main the toxicologist Prof. Dr. Martin Göttlicher studied the molecular biology processes underlying the teratogenic effect of valproic acid. The results of this work were quite sensational. The scientists did not only find the explanation as to why the substance interferes with the embryonic development, at the same time they showed possibilities of how this active substance could be used to treat certain forms of cancer.

It's the Packaging That Counts

“Our studies at the time showed that valproic acid changes the packaging density of the genetic material DNA, which allows increased activity of many genes,” Göttlicher explains, who has headed the GSF Institute of Toxicology since 2003 and has the Chair of Toxicology at the Technical University of Munich. “In embryonic development this elevated gene expression causes malformations, but with certain cancers the activation of genes seems to have an advantageous effect: the growth of tumor cells is inhibited or they die due to the induction of programmed cell death.”

DNA molecules are in the nucleus of the cell – together with a number of proteins – in a highly organized structure called chromatin. At the first level of packaging the DNA double helix is packaged into so-called nucleosomes: sets of about 200 base pairs of the DNA are wound round a nucleus of eight proteins, the histones. Due to their amino acid composition the histone proteins are positively charged, but they can be modified by enzymes, so that their total charge changes. Thus, a certain class of enzymes – histone acetyl transferases – cause acetyl moieties to be attached, thereby neutralizing the intrinsic charge of the histones. Another class of enzymes – histone deacetylases – can remove these acetyl moieties again and thereby restore the positive intrinsic charge of the histones. Since the components of the DNA – the nucleotides – are negatively charged, the



charge state of the histones assumed to have considerable influence on the packaging density of the DNA in the chromatin and thus also on the accessibility of genes for transcription. In other words: genes in a loosely packaged DNA on acetylated histones are more active than genes in a densely packaged DNA on non-acetylated histones.

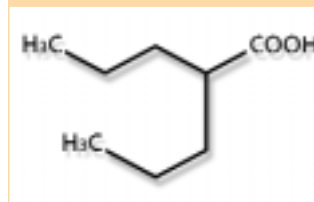
In their earlier studies Göttlicher and his colleagues could show that the antiepileptic valproic acid inhibits the activity of the histone deacetylase enzymes. Therefore, the acetyl moieties can no longer be removed from the histones, the histones are uncharged, the chromatin is packaged less densely, and many genes whose control depends on histone acetylation are, therefore, more active. In embryonic development, which is characterized by a sensitive balance of active and passive genes, this ‘unplanned’ gene activation causes malformations of the medullary tube and the skull. The adult organism is apparently more capable of compensating for the inhibition of the histone deacetylases within certain limits compared to the embryo.

Gene Activation to Control Cancer Cells

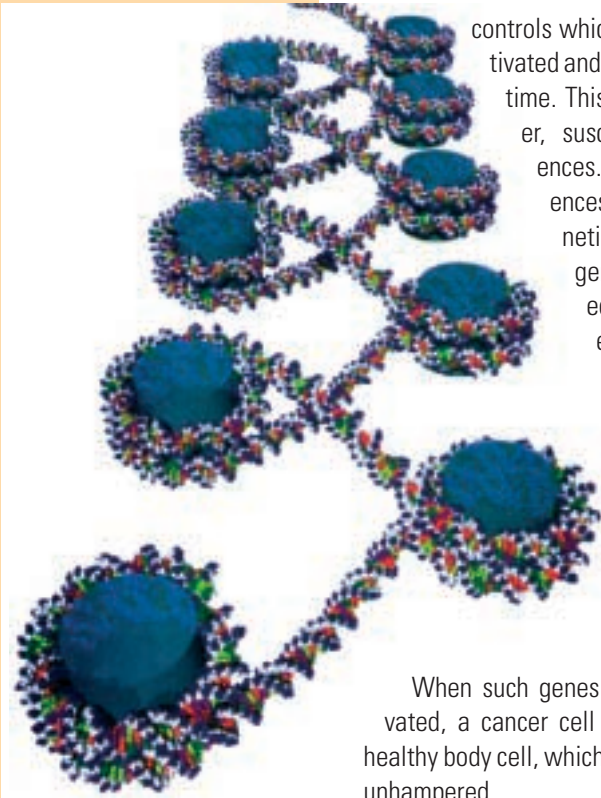
The gene activation caused by the inhibition of histone deacetylases could – the scientists speculated – however, also be a promising concept for the therapy of certain tumor diseases. In all body cells a highly complex program

Valproic acid, a drug successfully applied for epilepsy shows a teratogenic effect: administration during pregnancy can cause severe damage to the embryo: the spinal canal is not closed properly. The two sides of the active substance were the starting point for the groundbreaking work by Prof. Göttlicher and his colleagues.

The picture shows mouse skeletons (left: healthy, right: damage to the fetus following treatment with valproic acid). Photographs: Prof. Dr. H. Nau, Dr. K. Hoffmann, Tierärztliche Hochschule Hannover (University of Veterinary Medicine Hanover)



Small molecule with big effect: valproic acid



The packaging of DNA in chromatin. About 200 nucleotides per nucleosome are wound round a core (greenish blue) of eight histone proteins. The histones can be enzymatically modified by ubiquitin, phosphate, methyl and acetyl moieties. This changes the packaging density of the DNA in the chromatin.



Valproic acid has been used successfully for the treatment of epilepsy for more than 30 years. Prof. Dr. Martin Göttlicher, now Director of the GSF Institute of Toxicology, discovered together with colleagues that valproic acid also has an effect against tumor cells. Meanwhile encouraging phase I, II and III studies on patients with acute myeloid leukemia are under way.

controls which genes must be activated and deactivated at which time. This program is, however, susceptible to interferences. Environmental influences or defects in the genetic material may cause genes to be deactivated, although their expression is really important, e.g., for protecting the cell from uncontrolled growth, for its differentiation in accordance with its purpose or for inducing programmed cell death.

When such genes are wrongly deactivated, a cancer cell can develop from a healthy body cell, which may then proliferate unhampered.

It was known from tumor research that the modification of chromatin is disturbed in many types of cancer cells and that, therefore, the expression of numerous genes is dysregulated. For example, in certain leukemia cells the mechanism for the acetylation and deacetylation of histones is faulty – too much histone deacetylase activity is apparently bound to certain genes. This changes the chromatin structure, so that important regulatory genes remain ‘silent’.

For the treatment of such tumors it would be ideal, if changes in the chromatin, which cause irregular silencing of genes due to histone deacetylases could be reversed. There are a number of natural and synthetic substances which can inhibit these enzymes, including valproic acid. Its special advantage as a therapeutic drug would be that it has long been approved as a pharmaceutical drug and there is extensive experience with effects and side-effects – albeit for a different application.

The scientists first studied the antitumor effect of valproic acid on animal models for breast and colon cancer, on carcinoma cell

cultures and on human leukemia cells. The results were extremely promising. Many tumor cells reformed, redifferentiated to more normal cells or were eliminated by programmed cell death. There were, however, also cancer cells which did not react to the inhibition of histone deacetylases.

Promising Building Block for Therapy

Meanwhile clinical partners have also started studies with valproic acid as an antitumor drug. At Krankenhaus Nordwest in Frankfurt/Main a phase I/II study on dosaging and tolerance is being conducted, and at the University of Ulm the AML study group (AMLSG) is conducting a phase II and phase III study on patients with acute myeloid leukemia to find out what the effect of valproic acid is in combination with conventional and other innovative antitumor drugs.

Solid Tumors

In cooperation with the Karlsruhe Research Center Göttlicher and his colleagues from the GSF Institute of Toxicology recently also found a connection between a malfunction of histone deacetylases and the development of solid colon tumors. Such tumors often develop on the basis of a defect in the so-called APC (adenomatosis polyposis coli) tumor suppressor gene. Among other things failure of this gene results in the increased production of a certain histone deacetylase, HDAC-2. Apparently elevated HDAC-2 amounts have great significance to the further destiny of the cancer cells. Isolated colon tumor cells have been observed to only be able to survive, if there are increased amounts of HDAC-2. Recent research investigates the question why an inhibition of histone deacetylases results in the death of these tumor cells. The genome-wide search for genes which are activated by the inhibition of histone deacetylases in colon tumor cells shows that the balance between inductors and inhibitors of programmed cell death is apparently decisively controlled by the histone deacetylase activity. On top of that components involved in the recognition of tumor

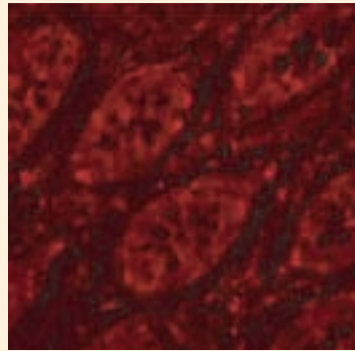
cells by the immune system also depend on the activity of histone deacetylases.

The greater occurrence of histone deacetylase HDAC-2 in colon tumors raises the question whether this enzyme makes a vital contribution to tumor development and whether any tumors can develop in the absence of HDAC-2. The availability of genetically modified mice now allows us to investigate this question with the help of mouse tumor models.

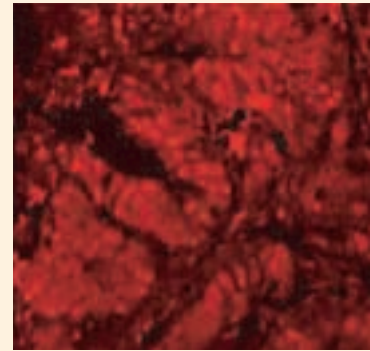
In view of the future possibility of inhibiting HDAC-2 for therapeutic purposes it is important that the organism as a whole can tolerate this inhibition of the enzyme to a certain extent. The genetically modified mice already provide important information here: they are viable despite the absence of HDAC-2. Although they are smaller than their unmodified siblings from birth and do not go through their development without any defects, these analyses are enough reason to hope that the inhibition of HDAC-2 in the adult organism which has developed a tumor does not result in any serious damage. The development of colon tumors is reduced in

Colon

normal



carcinoma



immunofluorescence HDAC2

The expression of histone deacetylase 2 (HDAC2) in normal and tumor tissue in a patient with colon carcinoma. The enzyme was made visible by immunofluorescence with HDAC2-specific antibodies. In tumor tissues much more HDAC2 is expressed than in healthy tissue.

mice without HDAC-2, although it is not completely absent – up to the stage of benign polyps. The question remains unanswered to what extent the further development of such adenomas all the way to the malignant carcinoma is influenced by the absence of HDAC-2, and whether it is a worthwhile perspective to develop selectively effective inhibitors for HDAC-2.

Cancer Award



In March 2006 Prof. Martin Göttlicher and Prof. Thorsten Heinzel, formerly at Georg-Speyer-Haus in Frankfurt/Main, now at the University of Jena, were awarded the experimental Cancer Prize 2006 of the Deutsche Krebsgesellschaft (German Cancer Society). Excerpt from the reasons: "The work done by the award-winners resulted in the better understanding of a fundamental mechanism of the development of cancer and – based on this knowledge – (...) the approval of a new cancer drug in the foreseeable future."

In cooperation with the Technical University of Munich and Klinikum rechts der Isar the concepts developed in fundamental research are reviewed in studies on tumor patients. The scientists use samples from human colon tumors to investigate whether the predictions based on cell culture and mouse experiments can be confirmed and there is actually a connection between increased histone deacetylase amounts and the reduction of certain growth inhibitors and triggers of cell death. They expect these studies to provide indicators for those tumors which respond to treatment with inhibitors for histone deacetylases. These studies are funded by the Deutsche Forschungsgemeinschaft (German Research Foundation) within the framework of the Collaborative Research Center 456 "Target Structures for Selective Tumor Interventions."



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