Website address:
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Targeting epigenetic DNA modifications for the diagnosis of cancer

Epigenetic DNA modifications have been shown to play a role in carcinogenesis and are therefore a promising target for the early detection of cancer. Dr. Daniel Summerer and his research group at the University of Konstanz have developed a method that enables the direct and site-specific identification of epigenetic changes. The method represents a new approach in the field of epigenetic analysis as well as a major step towards simpler cancer diagnosis.

Since all human cells contain virtually the same genetic information, differentiation into cells with different physical characteristics (nerve cells, skin cells and immune cells) must occur at the gene expression level. Epigenetic processes play a crucial role in determining the fate of cells, and epigenetic modifications of the DNA are passed on to daughter cells. The most important epigenetic mechanism that controls gene expression in humans is the methylation of cytosine, resulting in 5-methylcytosine (mC). “Methylated cytosines play a key role in the regulation of chromatin structure and hence the accessibility of DNA for the transcription machinery. Transcription is the first step in gene expression,” says Dr. Daniel Summerer, head of the research group “Chemical Biology of the Genetic Code” at the University of Konstanz.

DNA methylation can repress or prevent the transcription of genes. This also applies to the large number of genes involved in the development and growth of tumours. Repression of tumour suppressor gene expression can for example lead to genetic alterations in the cells, and as a consequence to cancer. “Cancer cells usually have characteristic mC patterns, which can be used as biomarkers for the diagnosis of cancer,” explains Summerer. Current methods used for the detection of methylation patterns in cancer-relevant genes are however not sufficiently sequence-selective, and the application of several methods is required in order to detect this modification at specific genomic loci.

No analysis without the right tools

Nucleic acids and their analogues have long been the only molecules that can be programmed to recognize any DNA sequence, including longer ones such as genes. This is thanks to the Watson-Crick base pairing (G-C, A-T) rules, according to which specific hydrogen bonds form between the four canonical nucleotides adenine (A), guanine (G), thymine (T) and cytosine (C). “Epigenetic DNA alterations such as the addition of a 5-methyl group to cytosines do not however prevent complementary bases from hybridizing; unmethylated and methylated cytosines cannot therefore

Dr. Daniel Summerer and his research group at the University of Konstanz have developed a new approach that enables the site-specific detection of epigenetic modifications in genomes.
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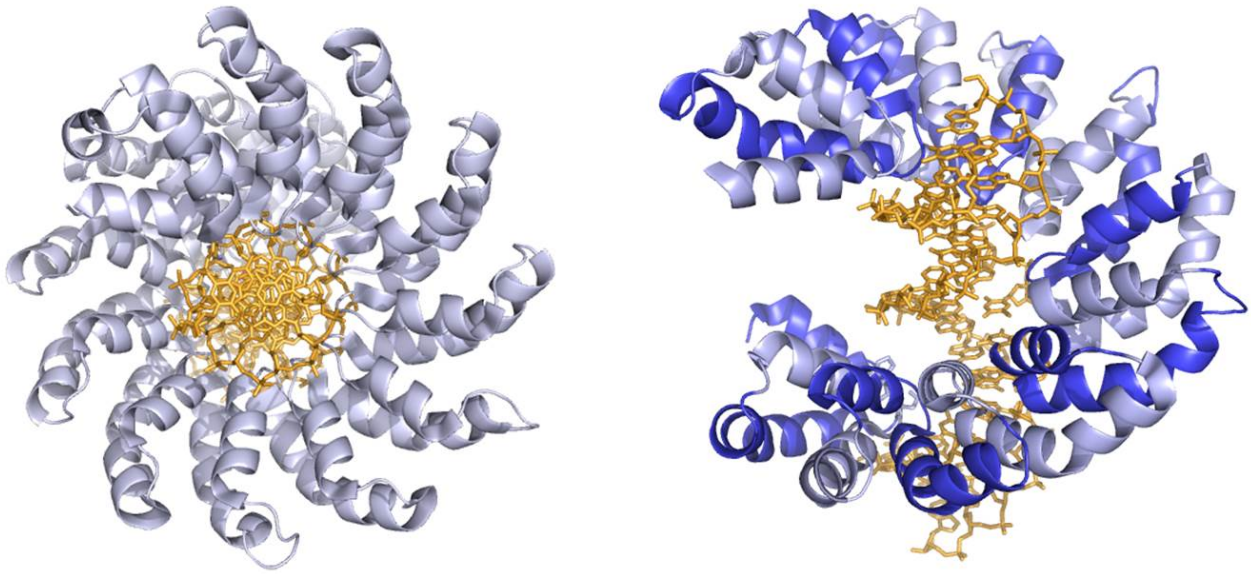
be discriminated on the basis of base pairing," explains Daniel Summerer. Current methods for the detection of methylated cytosines therefore have to take several detours and involve several steps. The first step is a non-specific differentiation of non-methylated and methylated cytosines, for example by selective binding or chemical transformation. This then allows the sequence to be determined using methods based on the rules of base pairing. "The ability to detect the level of mC modification directly and at specific genomic loci depends on the availability of molecules that can be programmed to recognize any desired DNA sequence," explains Summerer.

DNA binding proteins according to the construction kit principle

Two years ago, Summerer took notice of so-called TALEs (transcription activator-like effectors). "TALEs are modular proteins with a central repeat domain that consists of a variable number of amino acid repeats. These repeat domains specifically bind to a single DNA base in the target sequence and can be joined together in any order," says Summerer. In the laboratory, these repeats can be joined to create extended DNA stretches, which can subsequently be expressed in cells of interest. They are therefore fully programmable and are widely used, for example for the specific modification of the genome of living organisms (genome engineering). They have not previously been used for the detection of epigenetically modified DNA bases.

The idea of using TALE proteins for this particular purpose came after Summerer read two papers on the crystal structure of TALE-DNA complexes in the journal Science. "The crystal structure of the complexes suggested that the TALEs interacted with the DNA in the major groove that contained epigenetic modifications such as the 5-methyl group of mCs," says Summerer who concluded that

such modifications would also affect the binding of TALEs. In order to verify his assumption, Summerer and his research group went on to investigate the DNA binding properties of TALEs.



Model of a TALE-DNA complex seen from above (left) and from the side (right); green/blue: TALE proteins, yellow: DNA (images generated with The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC)
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New impulses for epigenetic analyses

In fact, TALEs turned out to be ideal tools for discriminating between methylated and non-methylated cytosines. "We found that the binding of TALEs to DNA, even to long sequences, is strongly inhibited by the presence of single mCs. For example, the binding of TALEs to a sequence 17 nucleotides long is inhibited 75-fold," says Grzegorz Kubik, first author of the study and student representative of the Konstanz Research School Chemical Biology, where the research project took place.

This difference in binding capacity is the basic characteristic that enables the detection of the mC content of a DNA sequence and has the potential to be used in conjunction with a wide variety of DNA detection methods, including quantitative PCR, microarrays and next-generation sequencing. "Direct methods of this kind have the potential to facilitate the detection of methylated cytosines considerably and could also be used for the identification of epigenetically modified DNA bases at specific gene loci in living cells," says Kubik. Evidence for this arose from studies carried out by research groups that used TALEs in in vivo genome engineering projects and found that they exhibited reduced activity in methylated regions of the genome.

Summerer's initial studies were carried out with zebra fish genomes, but have since been expanded to human cancer genes. The detection of single mCs at specific gene loci (i.e. typing) and the detection of mC patterns in larger genome regions (i.e. profiling) are obvious applications of TALEs in epigenetic research and diagnostics. "The use of TALE proteins is thus a major step towards the development of simpler methods for the diagnosis of cancer," says Summerer. A patent application has been filed and the method will be used in the future for research into biological functions of disease-related epigenetic DNA modifications. "We would be interested to meet any industrial partners who could support us in the further development of the method. Another option is of course to set up our own company," concludes Summerer.

Further information:
Dr. Daniel Summerer
Research group "Chemical Biology of the Genetic Code"
University of Konstanz
Tel.: +49 (0)7531/ 88-5669
E-mail: daniel.summerer(at)uni-konstanz.de

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Bettina Baumann
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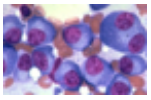

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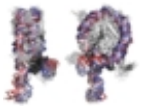

Epigenetics – heritable traits without changing the DNA sequence