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New Method for Detecting Antibiotic Resistance: Mutations Emit Light Signals

The exchange of a single gene building block in the genetic material of the tuberculosis bacterium leads to resistance to the antibiotic rifampicin. Scientists of the German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ) and the Universities of Heidelberg and Bielefeld, Germany, have developed a highly sensitive test for detecting this genetic alteration at the level of a single molecule, thus providing information about the resistance status of an infected person.

Many resistances to antibiotics are based on specific mutations in the genetic material of the infectious agents. In the case of life-threatening infections it is vital to determine rapidly which medication will work for the patient. However, commonly used methods of resistance detection are too time-consuming, particularly with microorganisms such as tuberculosis bacteria, which grow very slowly in the culture dish.

Scientists headed by Dr. Jens-Peter Knemeyer of the Division of Functional Genome Analysis at the DKFZ have combined a hybridization method, where small DNA probes bind highly specifically and exclusively to the mutated gene sequence, with confocal microscopy technology. The DNA probes are coupled to a fluorescent dye that flashes under laser light. However, this light signal is emitted only if the probe attaches to the target sequence in the bacterial genetic material. 'Unbound' probe molecules do not emit a signal. Each of these tiny light flashes that occur when the probe and the target molecule bind to each other, detects a single mutated DNA molecule.

By measuring the duration and decay times of the light flashes, the researchers distinguish between real measurement results and the ubiquitous background fluorescence: Due to chemical properties of the molecules involved, spontaneous fluorescence decays much more quickly than the signal emitted by the dye-labeled probe.

Detection of resistance causing point mutations in the genetic material of the tuberculosis bacterium is just one of numerous possible applications of the new method called single-molecule fluorescence spectroscopy. The method has a big advantage: Instead of recording light flashes in a sample solution, as is done in antibiotic resistance detection, the investigation method can also be used in living cells. Dr. Jörg Hoheisel, head of the Division of Functional Genome Analysis at the DKFZ, explains: "Just as we can detect DNA mutations, we can also use suitable probes to detect all molecules in a cell that are characteristic of a specific disease. Since the test identifies single molecules, it is highly sensitive – but reliable at the same time, because we have an internal control using the decay times."

Identification of single-point mutations in mycobacterial 16S rRNA sequences by confocal single-molecule fluorescence spectroscopy. Nicole Marmé, Achim Friedrich, Matthias Müller, Oliver Nolte, Jürgen Wolfrum, Jörg D. Hoheisel, Markus Sauer and Jens-Peter Knemeyer, Nucleic Acids Research Band 34 2006. DOI: 10.1093/nar/gkl495

The task of the Deutsches Krebsforschungszentrum in Heidelberg (German Cancer Research Center, DKFZ) is to systematically investigate the mechanisms of cancer development and to identify cancer risk factors. The results of this basic research are expected to lead to new approaches in the prevention, diagnosis and treatment of cancer. The Center is financed to 90 percent by the Federal Ministry of Education and Research and to 10 percent by the State of Baden-Wuerttemberg. It is a member of the Helmholtz Association of National Research Centers (Helmholtz-Gemeinschaft Deutscher Forschungszentren e.V.).

This press release is available at www.dkfz.de/pressemitteilungen

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