

Healthcare industry BW

The toxin of natural killer cells

Natural killer cells kill tumour cells by injecting the protein HMGB1, which blocks the production of cellular energy by aerobic respiration. Researchers from Heidelberg have elucidated this previously unknown cancer defence mechanism and are now in the process of developing a new immunotherapy for treating cancer patients.



Dr. Georg Gdynia, Department of General Pathology at the Institute of Pathology at Heidelberg University Hospital. Gdynia is also the head of the EnFin laboratory and acting director of the "Molecular Tumour Pathology" clinical cooperation unit jointly run by the Institute of Pathology and the DKFZ.

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In May 2016 at its 100th Annual Meeting in Berlin, the German Society of Pathology awarded the Novartis Prize to the Heidelberg researcher Dr. Georg Gdynia for the discovery of a new mechanism that enables cancer cells to be destroyed by the host immune system. In the study published in the journal Nature Communications, Gdynia reports on "the innate immune system's new metabolic weapons that can prevent the formation of tumour metastases and that offer new starting points for developing new treatment concepts and diagnostic test methods" for which he received the award. In addition to Gdynia's team at the Institute of Pathology at Heidelberg University Hospital and the German Cancer Research Center, the study also involved Prof. Dr. Wilfried Roth (now at the University Hospital in Mainz), and scientists from the Heidelberg Institute for Theoretical Studies and the Inorganic-Chemical Institute at the University of Heidelberg.

Exploitation of the innate immune system for immunotherapies

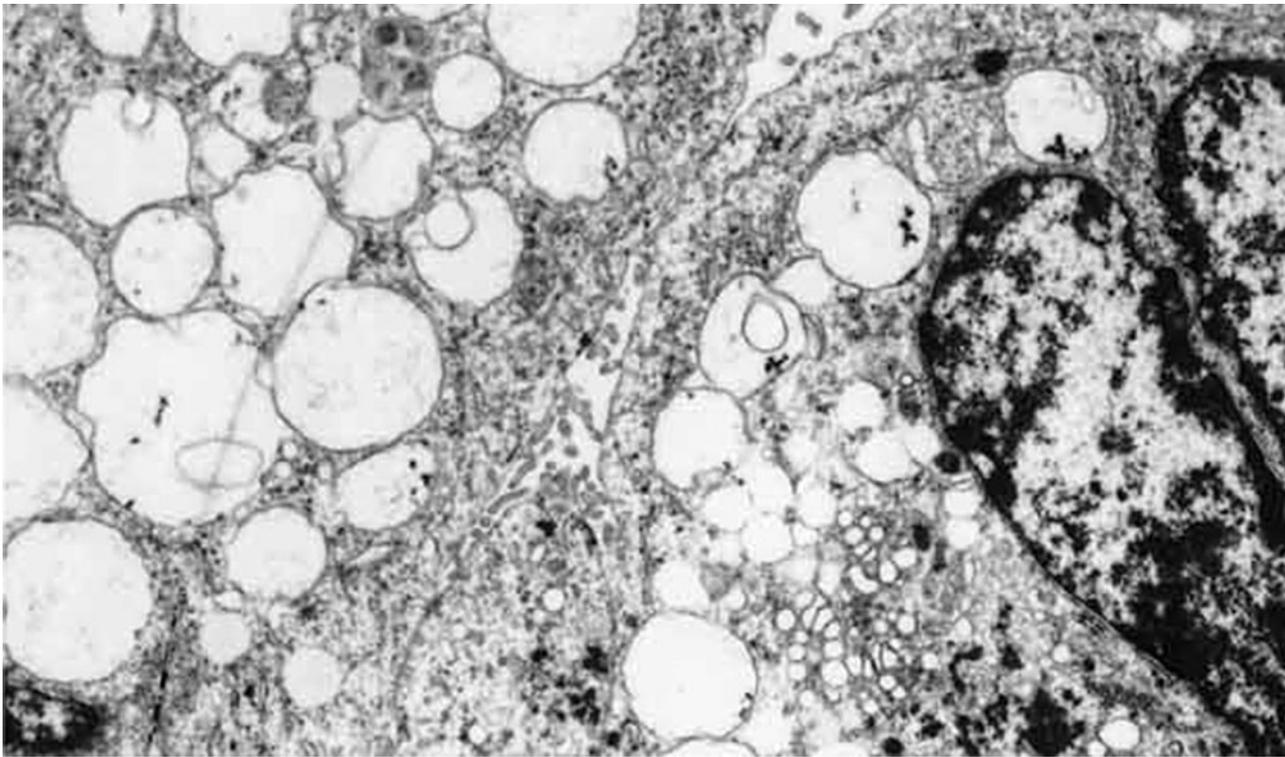
Most immunotherapy approaches for treating cancers target the adaptive immune system, i.e. T lymphocytes that are activated by tumour antigens or camouflage mechanisms used by tumour cells to evade the attack of T lymphocytes. However, Gdynia and his colleagues have discovered a completely different mechanism, which targets cells that are part of the innate immune system – so-called natural killer cells (NK cells). In colon cancer patients, NK cells that patrol the body release a lethal protein cocktail from their cytoplasmic granules when they encounter and recognise tumour cells. The cancer cells often die within minutes. The researchers have now identified the protein "high mobility group box 1" (HMGB1) as the lethal component and highly effective natural agent against cancer. The protein is a cytokine, which also has other functions such as contributing to the maturation and recruitment of other immune cells. It has now turned out to be a potent cytotoxin," says Gdynia.

HMGB1 is a toxin for the energy metabolism of tumour cells

The Heidelberg researchers isolated human HMGB1 protein by stimulating NK cells obtained from the blood of healthy blood donors. Treatment with pure HMGB1 led to the destruction of cancer cells in mice with human colon tumours; the tumours shrank and sometimes even disappeared completely. The cytotoxic effect of the protein on cancer cells was suppressed by the HMGB1-specific inhibitor glycyrrhizin.

A variety of experiments involving cancer cell lines and animal experiments as well as computer models led to the elucidation of the mechanism of HMGB1-induced cell death. This mechanism fundamentally differs from the events that lead to the death of cells in classical forms of necrosis. HMGB1 blocks a glycolytic enzyme (i.e. the tetrameric isoform of pyruvate kinase M2) that is necessary for cellular respiration. The aerobic (oxygen-dependent) generation of energy from glucose in the mitochondria then comes to a halt, so that energy can only be generated by anaerobic glycolysis, which is a relatively ineffective way of producing it. Cancer cells that depend on oxygen for energy production will then die.

As the researchers showed many years ago, this type of cell death leads to the formation of giant mitochondria that form hollow spheres. In another paper recently published in the journal "Molecular & Cellular Oncology", the researchers report on an HMGB1-resistant cancer cell population whose energy metabolism is adapted to anaerobic glycolysis. These particularly aggressive tumour cells, which are also largely resistant to radio- and chemotherapy, are the



Appearance of giant mitochondria following the HMGB1-induced death of a tumour cell – electron microscope image.
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major source of tumour recurrences and metastases. They therefore play a critical role in the fate of cancer patients. The researchers found that, under certain conditions, HMGB1 can also kill some of these so-called anoxic cancer cells.

A spin-off company focused on the diagnosis of anoxic tumour cells

These findings illustrate how important it is to know the quantity of anoxic cells in the tumour. This information allows physicians to anticipate more accurately how likely the cancer is to grow back, or how well it will respond to standard medication, and then choose the adequate therapy accordingly and as early as possible after diagnosis. At present, Gdynia's group of researchers are developing the world's first test to allow oncologists to determine the proportion of highly aggressive cells in their patients' tumours. The researchers plan to use this test called EnFin (short for "energetic fingerprinting") to establish a company. Company foundation is funded under the "EXIST research transfer" programme run by the German Federal Ministry for Economic Affairs and Energy.

HMGB1's ability to act as a toxin for the tumour-specific breakdown of glucose makes it an interesting object for cancer therapy. Gdynia comments: "Immunotherapies normally aim to enhance the immune system so that it can better recognise and combat cancer cells. Therapy with HMGB1 would have the advantage that it uses the weapons of the natural immune system without depending on its functionality, and still manages to target cancer cells in a very selective manner." Obtaining the protein is very difficult, as absolutely the right one has to be caught. There are countless HMGB1 variants that only marginally differ from each other, but each has a different function. Only the HMGB1 variant from the granules of killer cells is able to kill cancer cells, nuclear HMGB1 cannot. Gdynia and his colleagues have filed two patents: one for the scientific technique that they used to obtain the protein from killer cells and another for the new therapy concept.

Original papers:

Gdynia G, Sauer SW, Kopitz J, Fuchs D, Duglova K, Ruppert T, Miller M, Pahl J, Cerwenka A, Enders M, Mairbörl H, Kamiński MM, Penzel R, Zhang C, Fuller JC, Wade RC, Benner A, Chang-Claude J, Brenner H, Hoffmeister M, Zentgraf H, Schirmacher P, Roth W. The HMGB1 protein induces a metabolic type of tumour cell death by blocking aerobic respiration. *Nat Commun.* 2016 Mar 7;7:10764. doi:10.1038/ncomms10764. PubMed PMID: 26948869; PubMed Central PMCID: PMC4786644.

Adelheid Cerwenka, Jürgen Kopitz, Peter Schirmacher, Wilfried Roth, Georg Gdynia. HMGB1: the metabolic weapon in the arsenal of NK cells. *Molecular & Cellular Oncology.* <http://dx.doi.org/10.1080/23723556.2016.1175538>



The Institute of Pathology at Heidelberg University Hospital (director: Prof. Dr. Peter Schirmacher) is the largest institute of its kind in Germany.
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