



Getting the Immune System Going Therapeutic Vaccination against Kidney Cancer

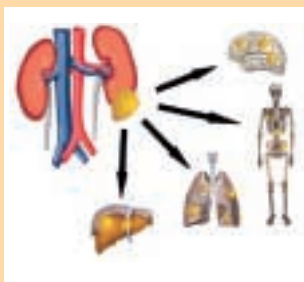
Renal cell carcinoma is the most frequent malignant kidney tumor. There are approx. 14,000 new cases per year in Germany, most of which are discovered by coincidence. There has been hardly any therapy for patients with metastatic renal cell carcinoma, since the tumor cells respond poorly to radiation or chemotherapy. Scientists at the GSF Institute of Molecular Immunology are developing new therapies to activate the patient's immune system to effectively fight tumor cells.

Renal cell carcinoma, or RCC, is an extremely aggressive type of cancer. "At the time of the first diagnosis of a renal cell carcinoma there are often metastases already, e.g. in bones, lungs or brain," says Dr. Bernhard Frankenberger from the GSF Institute of Molecular Immunology. The prognosis is unfavorable – more than three

quarters of the patients with metastases die within two years. Together with the Director of the Institute, Professor Dolores Schendel, he pins great hopes on immunotherapy, because there are indications that the tumor basically can trigger an immune response: it is often infiltrated by defense cells of the immune system, and in about two per cent of



Computed tomography section through a kidney with the finding of RCC renal cell carcinoma.



In cancer the tumor is often not the only evil. Individual tumor cells spread in the body and cause metastases in other organs. Thus, at the time of the first diagnosis of a renal cell carcinoma there are often metastases, e.g., in the bones, lungs or brain.

the patients the tumor recedes spontaneously. The activation of the immune system with cytokines, such as interferon-alpha and/or interleukin-2 as a classical immunotherapy can sometimes result in partial or even complete remission, but is often associated with severe side-effects for the patient.

Long-Term Survivors at the Service of Research

Therefore, the scientists are working on a therapeutic vaccine which is supposed to stimulate the immune system with the help of genetically modified tumor cells. Tumor cells of the cell line RCC26 are used for the vaccination, which comes from a patient whose immune system could apparently fight the tumor particularly well, since – being a long-term survivor – he has survived the outbreak for more than ten years. “These tumor cells may present immunodominant tumor antigens on their surface, which are particularly good at boosting the immune system,” Frankenberger explains. Tumor antigens are specific proteins which distinguish cancer cells from healthy cells. The immune system should really be able to recognize the cancer cells as degenerate cells by these antigens and destroy them. The problem: the tumors

have developed various mechanisms helping them escape the immune cells.

Thus, the RCC26 cells do not have certain costimulatory molecules required to trigger an immune response. In the absence of these molecules defense cells – mainly cytotoxic CD8-positive T-cells – migrate to the tumor cells, where they are deactivated, however, instead of being activated for the fight against the tumor cells.

Therefore, in order to improve the immune reaction against the tumor antigens Prof. Schendel and her colleagues together with Prof. Blankenstein and his group at the Max-Delbrück-Center of Molecular Medicine in Berlin introduced not only the gene for the costimulatory molecule B7.1, but also genes for certain cytokines (such as interleukin-2 and –7) into RCC26 tumor cells to try and ensure that tumor-specific T-cells can proliferate better and maintain a long-lasting immune reaction.

Tumor Antigens on Display

The actual tumor antigens of the cell line RCC26, which can “train” the T-cells for the tumor, remain unknown with this type of therapeutic vaccination, because they are presented by the tumor cell itself. Fortunately the tumor antigens presented by the cell line RCC26 also seem to be present on renal cell tumors of other patients, because RCC26 cells were also recognized by T-cells of other patients. However, T-cells only recognize tumor-specific antigens, if the tumor cell offers them on certain MHC molecules. RCC26 cells present several antigens on an MHC molecule type HLA-A2. For the communication between the antigen-presenting tumor cell and the T-cell to work, the T-cells of the patients must be so-called HLA-A2-restricted cells which recognize this molecule together with a fragment of a tumor antigen.

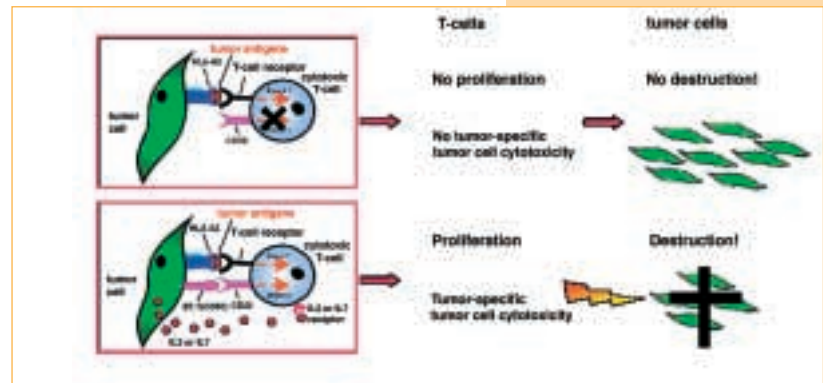
The scientists benefit from a coincidence: “Fortunately the HLA-A2 molecule is expressed in about 50 per cent of the Caucasian population, so that our genetically modified RCC26 vaccine can be used in about half of

all patients,” Frankenberger explains. Apart from the cytotoxic T-cells of the adaptive immune defense, RCC26 cells can also activate natural killer cells (NKs) and non-MHC-restricted (NK-like) T-cells of the innate immune system. NK cells can even recognize tumor cells, when they down-regulate their MHC molecules. Therefore, they are also important for the elimination of degenerate tumor cells. The RCC26 cells apparently have a natural immunogenicity, so that they can activate various effector cells of the immune system.

First Steps to the Clinic

In a clinical phase I/II study started last year twelve patients are treated with the RCC26 vaccine at the Urological Clinic of Ludwig-Maximilians-University in cooperation with Dr. Heike Pohla’s Clinical Cooperation Group. “In these patients the vaccine has not shown any toxic side-effects yet. But a final conclusion as to the effect of the vaccination is not possible yet, since the immune monitoring accompanying the study is still continuing,” says Frankenberger.

Despite the comparatively good immunogenic properties of the RCC26 vaccine, vaccination with tumor cells may also have disadvantages, because tumor cells generally tend to be weak antigen-presenting cells. Since in many cases there are no MHC class II molecules on tumor cells, they often cannot activate any CD4-positive helper T-cells. Therefore, the scientists follow another approach in parallel with the vaccination with genetically modified RCC26 cells: “professional antigen-presenting cells, such as dendritic cells (DCs) can stimulate the immune system much better: they induce both CD8 and CD4 T-cells, they carry costimulatory molecules on their surface, and they produce substances which tend to be beneficial to the immune system,” Frankenberger explains. Therefore, DCs should also be applied in the fight against renal cell carcinoma. As opposed to the vaccination with RCC26, however, it is advantageous for the development of a DC-based vaccine, if the RCC-associated tumor antigens are known. Then the RNA

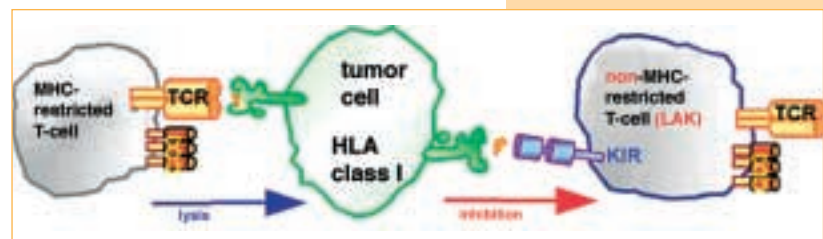


coding for these tumor antigens could specifically be introduced into the DCs. “The DCs produce the antigens coded by the RNA and present them on their surface, where they are ideally recognized by tumor-specific T-cells. This can stimulate the immune system and allow a specific reaction to the tumor,” says Frankenberger.

New Sleuths at Work

This therapeutic approach is substantially driven by great progress in the methods by which potential tumor-associated antigens (TAAs) can be identified. Prof. Schendel’s group investigates which antigens are presented by the tumor cells in cooperation with laboratories in Tübingen and in the US, where the peptides presented by the tumor cells on HLA-A2 are isolated and sequenced. Differential transcriptome analyses, in which the transcript quantities of the potential

To improve the immune reaction against the tumor antigen, Prof. Schendel and her colleagues introduced the gene for the costimulatory molecule B7.1 into RCC26 tumor cells as well as genes for certain cytokines (such as interleukin-2 and -7). They are supposed to stimulate the proliferation of tumor-specific T-cells and thereby maintain a long-lasting immune reaction.



Schematic diagram of the regulation of MHC-restricted and non-MHC-restricted T-lymphocytes. With their specific T-cell receptor (TCR) classical cytotoxic T-cells recognize the HLA/peptide complex expressed on tumor cells. This interaction is a prerequisite for the activation of these killer cells. Non-MHC-restricted T-cells, in turn, are regulated by MHC/peptide complexes in a mirror image: the binding to inhibitory receptors (KIRs) induces negative signals which deactivate non-MHC-restricted T-cells.

Good Craftsmanship – The Future GMP Laboratory of the GSF

The production of dendritic cells (DCs) loaded with RCC antigens is pursued within a Collaborative Research Center. "We are currently testing the production and loading of the DCs in accordance with "Good Manufacturing Practice" guidelines, with the aim of using their application in a clinical study early in 2007," says Iris Bigalke, head of the new GMP laboratory of the GSF, which is being set up. Her task will be to generate the DCs for application in patients under the conditions of the GMP with her working group: all pharmaceutical drugs to be used in patients are subject to strict legal regulations. Since cell therapy products are sterile, they must be manufactured in cleanroom conditions, i.e. in an environment free from bacterial contamination and particles. A pressure cascade ensures that no external contaminated air can enter the cleanrooms. Preparations for commissioning such a cleanroom system for the production of cell preparations have been under way at the GSF for two years.



All pharmaceutical drugs to be used with patients must be produced in an environment free from bacterial contamination and particles. With her group Iris Bigalke, head of GMP laboratory of the GSF which is being set up, will produce cell drugs in such clean room conditions in the future. An overpressure system ensures that no contaminated air enters the room.

The production protocols and the standard operating procedures (SOPs) for the DCs are developed in parallel at a preparatory laboratory. It often takes one to two years until a laboratory method can be implemented into GMP practice, since not all methods and reagents used in research are also suitable for GMP production. Apart from that, much larger numbers of cells are required for a therapeutic application than are needed for scientific purposes, so that sometimes existing methods must be established almost from scratch with different reagents or equipment. In addition extensive documentation and quality control are required.

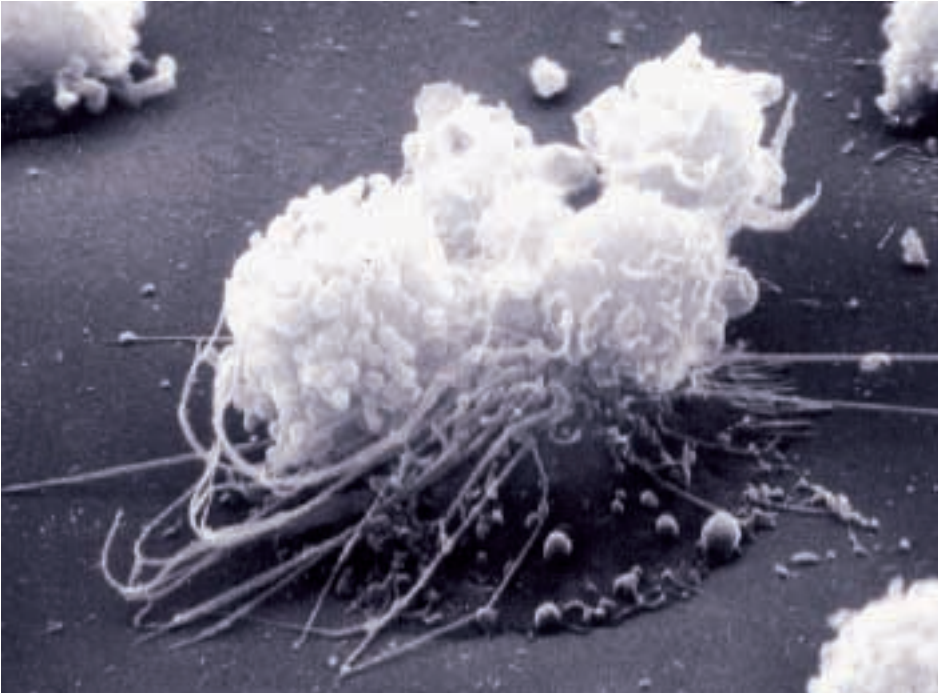


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The planned GMP laboratory (GMP = Good Manufacturing Practice) at the Innovations- und Gründerzentrum Biotechnologie (IZB, Innovation Center for Young Entrepreneurs in Biotechnology) will accommodate an area for production of cell drugs and a separate quality control area.



TAA in tumor cells and in normal renal cells are compared, help to discover overexpressed tumor-specific antigens which are not found in normal cells at all or only in small quantities. The samples for such studies were obtained by the scientists from the GSF Institute of Pathology. "Our aim is to find as many tumor-associated antigens as possible and to introduce them into the DCs in the form of RNA, in order to offer the immune system many different points of attack against the tumor," Frankenberger explains.

The same antigens can also be used to track the success following a vaccination of the patient: if the patient responds to the vaccination, antigen-specific cytotoxic T-cells

must be present in the patient's blood, which are stimulated to proliferate upon contact with the respective antigens and produce immunological messenger molecules.

"As a third pillar in the immunotherapy of renal cell carcinoma we also want to develop a therapy with tumor-specific transgenic T-cells at our institute in the near future," Frankenberger elaborates. The scientists introduce tumor-specific receptors into T-cells by genetic engineering, which then – as is the case with passive immunization with antibodies – specifically trace and fight isolated tumor cells in the patient's blood or micrometastases.

New Collaborative Research Center for Immunity Research

Fundamental work by Prof. Kolb had shown that the infusion of donor lymphocytes following bone marrow transplants can cause the elimination of leukemia cells, that this is due to T-cell-mediated immunity and that, therefore, the immune system is capable of curing cancer. On the basis of this discovery a new transregional Collaborative Research Center of two Helmholtz Centers – the GSF and the Max-Delbrück-Center in Berlin, the two Munich Universities, Humboldt University Berlin as well as Charité in Berlin – was established.

The aim of this work is to understand the foundations of the immunity mediated by specific T-lymphocytes as well as to develop new forms of the therapy of malignant diseases and chronic infections by transferring such T-cells into the patient.

In parallel with the vaccination with genetically modified RCC26 cells, the GSF immunologists pursue another new approach in the fight against renal cell carcinoma: they use dendritic cells from the patient's blood, which have an important function in immune defense due to effective antigen presentation.



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