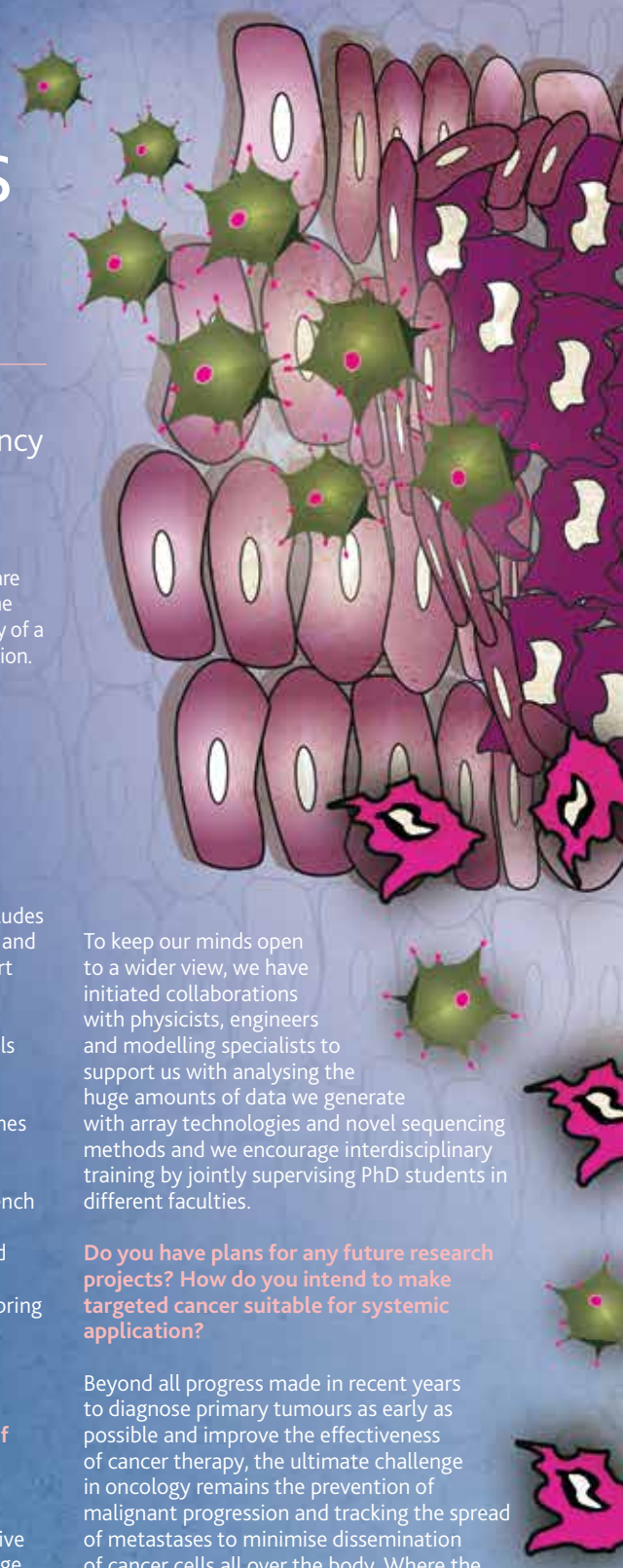
Targeting the dynamics of cancer progression

For over 20 years, **Professor Brigitte M Pützer** has devoted her career towards uncovering the mechanisms of apoptosis deficiency in cancer and their modulation for cancer treatment, while also developing smart tools to specifically target mis-routed cells



Could you begin by outlining your primary research objectives?

The overall goal of our research at the Institute of Experimental Gene Therapy and Cancer Research (IEGT) in Rostock, Germany is to uncover molecular principles of tumour development, cancer spread and the acquisition of chemoresistance. To be more precise, our scientific efforts are devoted to identifying molecular and genetic signatures defining apoptosis deficiency that eventually allow cancer progression, dissemination and metastasis. Ultimately, we intend to make use of these target structures to specifically treat cancer at advanced stages. Our second mainstay is the development of targeted viral vector systems. Such vehicles are meant to enable the selective and exclusive delivery of therapeutic genes by systemic application to highly neoplastic disseminated cancer cells. This is necessary to reach micro-metastases, including those that are as yet eluded from imaging procedures.

Why are the gene signatures that underlie the apoptosis deficiency and metastasis of solid tumours of particular interest?

Apoptosis deficiency is the main reason for therapy failure upon cancer treatment with conventional DNA-damaging drugs. Gene expression signatures underlying these defects in death pathways are often associated with tumour recurrence and dissemination, and are therefore useful for allowing us to predict the aggressive potential and metastatic capacity of a certain cancer prior to therapeutic intervention.

To what extent do you rely on novel techniques and technologies in your investigations? How important is it to keep up-to-date with cutting-edge developments?

We continuously extend our repertoire of cutting-edge technologies for drug/gene delivery and in targeting tumours. This includes the use of stem cells as delivery platforms and targeted viruses to deliver therapeutic short interfering RNAs, as well as other genome editing methods like TALENs and CRISPRs technologies. Finally, various animal models are made available to test novel therapies.

We focus on some of the unique approaches that can be used to meet the present challenges in the field and translate the potential of cancer gene therapy from 'bench to bedside' in the near future. We believe that personalised therapy, using advanced molecular profiling technology to match patients with novel targeted agents, will bring cancer care to a new level.

To what extent does IEGT foster a multidisciplinary approach? How important is this to the advancement of your research?

I believe multidisciplinary-orientated approaches in biomedical research can drive innovation. The accumulation of knowledge rises exponentially and paves the way towards increasing specialisation in all disciplines of science. By fragmenting our experimental systems, such as a single pathway within a cancer cell, we shall not lose the view on the larger entities, such as the tissues, organs and organism as a whole. By establishing interdisciplinary collaborations, scientists from other disciplines can help us to integrate technologies and data, and open perspectives for wider concepts to understand nature a little better and, ultimately, solve medical problems.

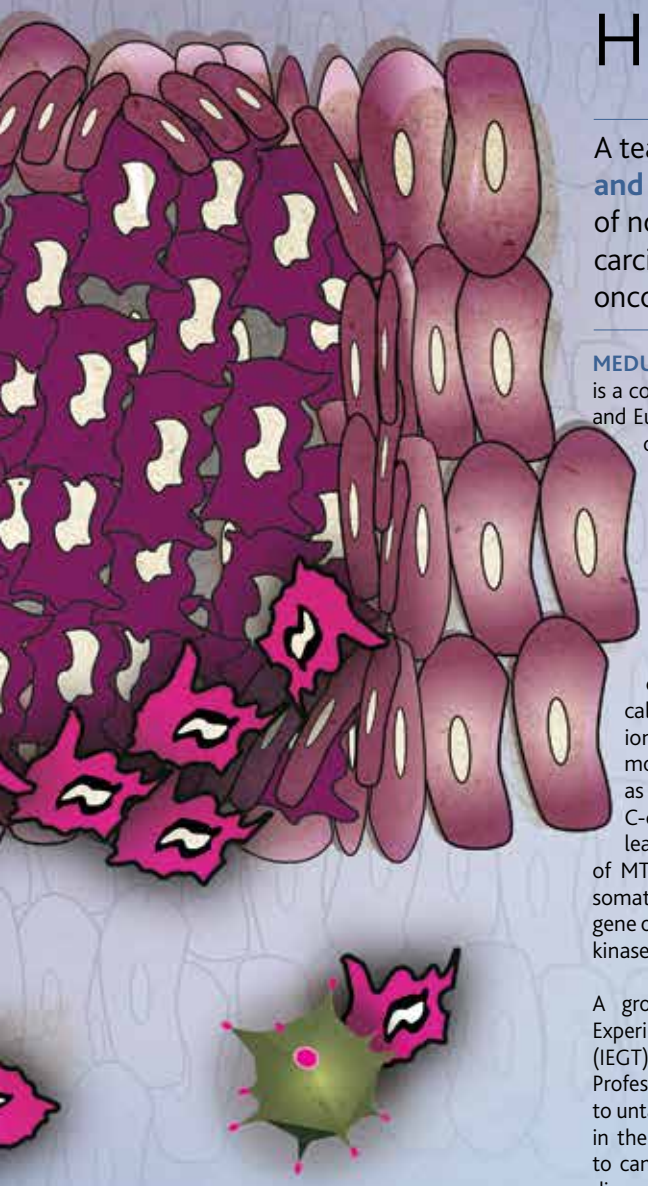
To keep our minds open to a wider view, we have initiated collaborations with physicists, engineers and modelling specialists to support us with analysing the huge amounts of data we generate with array technologies and novel sequencing methods and we encourage interdisciplinary training by jointly supervising PhD students in different faculties.

Do you have plans for any future research projects? How do you intend to make targeted cancer suitable for systemic application?

Beyond all progress made in recent years to diagnose primary tumours as early as possible and improve the effectiveness of cancer therapy, the ultimate challenge in oncology remains the prevention of malignant progression and tracking the spread of metastases to minimise dissemination of cancer cells all over the body. Where the scalpel, X-ray and chemotherapy reach their limits, smart gene delivery vehicles will be the choice to fight neoplasm once it becomes systemic. I strongly believe that the spread of cancer cells can ultimately be tackled only by applying what we call 'smart vehicles' based, for instance, on viral vectors. I am sure that vectors with ablated natural tropism and solely equipped with a distinct affinity for cancer cells are feasible future therapies. What we were able to prove for a prototypic single oncogene driven tumour should hold strong for comparable forms of cancer.

Hunting tumour cells on tour

A team of researchers at the **Institute of Experimental Gene Therapy and Cancer Research** in Rostock, Germany is investigating the use of novel viral vectors to direct genetic therapies to medullary thyroid carcinoma cells, with the aim of inhibiting a crucial dysfunctional oncogene and stopping the spread of this aggressive form of cancer



Making use of adenoviral vectors to deliver targeted genetic therapies to cancer cells has great potential for helping to treat cancer once it has metastasised

MEDULLARY THYROID CARCINOMA (MTC) is a common form of cancer throughout the US and Europe accounting for approximately 5 per cent of all thyroid cancers. It is routinely treated with surgery and radiation therapy but often metastasises before treatment is initiated, limiting the success of the intervention. Consequently, the development of effective methods of treatment is considered an important area of research. MTC generates from a subset of thyroid cells, the parafollicular cells (C-cells), which secrete the hormone calcitonin that regulates the levels of calcium ions in the blood – an important signalling molecule. The cancerous phenotype arises as a result of either a germline-derived C-cell mutation – inherited by the individual, leading to a near 100 per cent probability of MTC development – or after acquisition of somatic, dominant-activating mutations, in the gene of a membrane-spanning receptor tyrosine kinase (RET).

A group of researchers at the Institute of Experimental Gene Therapy and Cancer Research (IEGT) in Rostock, Germany is being led by Professor Brigitte Pützer in a twofold quest: firstly, to untangle the molecular basis of the breakdown in the apoptosis signalling pathways that leads to cancer progression; and secondly, to identify disease-specific biomarkers that can be used in the development of novel viral vector systems able to target malignant cells for gene delivery-based therapies. Pützer is closely supported in this work by an international team of more than 20 researchers. One model system in which Pützer's team is carrying out their investigations is in the role of the activated RET oncogene in the development of MTC, and to specifically target and treat MTC with virus-derived vectors expressing transdominant-negative forms of RET.

THE RET ONCOGENE

RET is a cell membrane-anchored receptor tyrosine kinase. Its physiological form regulates cell proliferation after dimerising when stimulated by its native ligand growth factor, activating the receptor to trigger growth signalling inside the cell. If RET carries one of several known mutations, dimerisation occurs independent of the presence of the growth-factor ligand. In this state RET continuously signals into the cell to stimulate cell growth, even in the absence of growth factors, resulting in uncontrollable proliferation and survival. The continuous firing of intracellular signalling inevitably provokes cancer development.

Early work by the team, published in the *Journal of the National Cancer Institute*, demonstrated how the malignant phenotype of MTC can be reverted by overexpression of dominant-negative RET (dnRET) mutants. By forming inactive heterodimers with the oncogenic protein receptor at the cell membrane, dnRET inhibits the signalling activity and prevents further cell proliferation. Additionally, and perhaps most importantly, the team demonstrated that this interference with the oncogenic receptor restores the apoptotic pathways in MTC cells, reversing the cancer's development. Pützer elaborates: "We showed that orthotopic medullary thyroid carcinoma of RET transgenic mice were considerably smaller than their initial size after a single intratumoral injection of an adenoviral vector delivering dnRET". Having identified a potential gene target, the next challenge faced by the team was to develop a mechanism by which the dnRET could be targeted specifically to MTC cells for therapeutic effect without the need to inject the vector directly into the tumour.

DEVELOPMENT OF TARGETED VIRAL VECTORS

Adenovirus-based vectors are commonly used in gene therapy because of their ability to infect a wide range of cell types, their rapid replication and their capacity to incorporate large transgenes into the viral DNA without integrating into the host genome to prevent insertional mutagenesis. Since the transgene, dnRET, is required only in MTC cells to neutralise dysfunctional RET, and to increase the efficiency of viral infection for tumour cells, a targeting approach had to be developed. The strategy that Pützer and her team have taken to achieve this is to identify cancer cell-specific ligands and attach them on the viral surface after depletion of the native receptor-binding entities. By doing this, the vector would be redirected only to the malignant cell type, leaving non-affected normal host cells free from the potentially cytotoxic effect of this cancer treatment. Conventional cancer treatments, such as chemotherapy and radiation, are not able to make a distinction between healthy and neoplastic cells and thus, can cause widescale damage to non-cancerous tissue.

To identify MTC-specific ligands, Pützer's team carried out biopanning on a human MTC-derived tumour cell line as well as from orthotopic MTC from transgenic mice, using phage-display peptide libraries. They successfully identified a peptide that

TARGETED SYSTEMIC THERAPY OF RET-ONCOGENE INDUCED MEDULLARY THYROID CARCINOMA BY 'SMART' AD-VECTORS

OBJECTIVES

To elicit the potential of specifically target advanced stage metastatic cancer cells with custom-made adenoviral (Ad) vectors. Tumour cell-specific surface moieties will be unveiled to define peptide-based redirection of vectors for systemic application. Specific and selective treatment combines newest oncogene depletion strategies with individualised cancer targeting. The studies include the detection of gene expression signatures and their relevance for apoptosis and chemoresistance, tumour invasiveness and progression. The investigations will provide novel therapeutic approaches to combat advanced cancer disease.

KEY COLLABORATORS

Professor Jens Pahnke, University and DZNE Magdeburg, Germany; **PD Dirk Nettelbeck;** DKFZ and University Hospital, Heidelberg, Germany; **Professor Andreas Wree;** Rostock University Medical Center,Rostock, Germany; **Professor Henning Dralle,** University of Halle, Germany; **Professor Sir Bruce AJ Ponder,** University of Cambridge, UK

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BRIGITTE PÜTZER is Professor of Molecular Biology at the University of Rostock and Director of the Institute of Experimental Gene Therapy and Cancer Research at the University Medical Center. She received her PhD and MD degrees at the University of Essen. Following a postdoctoral fellowship at McMaster University in Canada, she continued her research as group leader at Essen University and gained her habilitation (venia legend). She has worked in the field of molecular cancer biology for over 20 years. Her research interests span from elucidating mechanisms of apoptosis deficiency and cancer progression to vector development for anticancer treatment, particularly targeted viral gene transfer.

Institute for Experimental Gene Therapy and Cancer Research

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not only specifically bound to MTC cells *in vitro* and *in vivo*, but was also efficiently internalised.

POTENTIAL FOR TREATING METASTATIC CANCER

Compared to other types of cancer, MTC is extremely aggressive and characterised by early metastasis. This makes treatment with conventional cancer therapies difficult because metastases are particularly resistant against chemotherapy and radiation, which significantly reduces the prognosis of the disease for patients.

Making use of adenoviral vectors to deliver targeted gene therapies to cancer cells has great potential for helping to treat cancer once it has spread, and for protecting the vast bulk of innocent normal cells from any toxic intervention. The researchers have made this possible by ablating the surface features of the virus that provide it with a native affinity for a large host range, and substituting these for the identified peptide that is mainly present on the cells of interest. The beauty of this system is that the modified viral vectors can be injected intravenously and can spread throughout the body through the circulatory system until they encounter an MTC cell which they are programmed to recognise. Through the interaction between the MTC cell-specific surface properties that complement the targeting peptide, the virus is internalised into the cancer cell, delivering the dnRET gene therapy that stops pathological cell proliferation and revives the intrinsic apoptosis signalling pathway leading to cell killing. This mechanism is as effective for identifying and tackling a primary tumour, secondary tumour, or a single metastatic cell, which makes it a valuable therapy for treating this highly aggressive cancer.

This therapy has been demonstrated *in vivo* using a mouse model with a number of established disseminated human-derived MTC tumours. A systemic injection of therapeutic gene-carrying virus into the tail vein of mice showed that the adenoviral particles rapidly co-localise to multiple sites of cancer. Consequently, the tumours at these sites were shown to reduce in size, demonstrating not only the successful and specific targeting of cancer cells, but additionally the effectiveness of the gene therapy for preventing the unregulated cell growth that is characteristic of the cancer cells in the absence of treatment. During this experiment, the tumours in the control mouse – which was injected with unmodified adenovirus – expanded from their initial size without notable cell death.

Whilst this project focuses on targeting the viral vectors to MTC cells, the same approach can be adopted for other types of tumour or small subpopulations of cancer cells, namely metastasis-inducing cancer stem cells, that are potentially responsible for the formation of distant malignant tumours to combat the specific cause of this life-threatening disease.