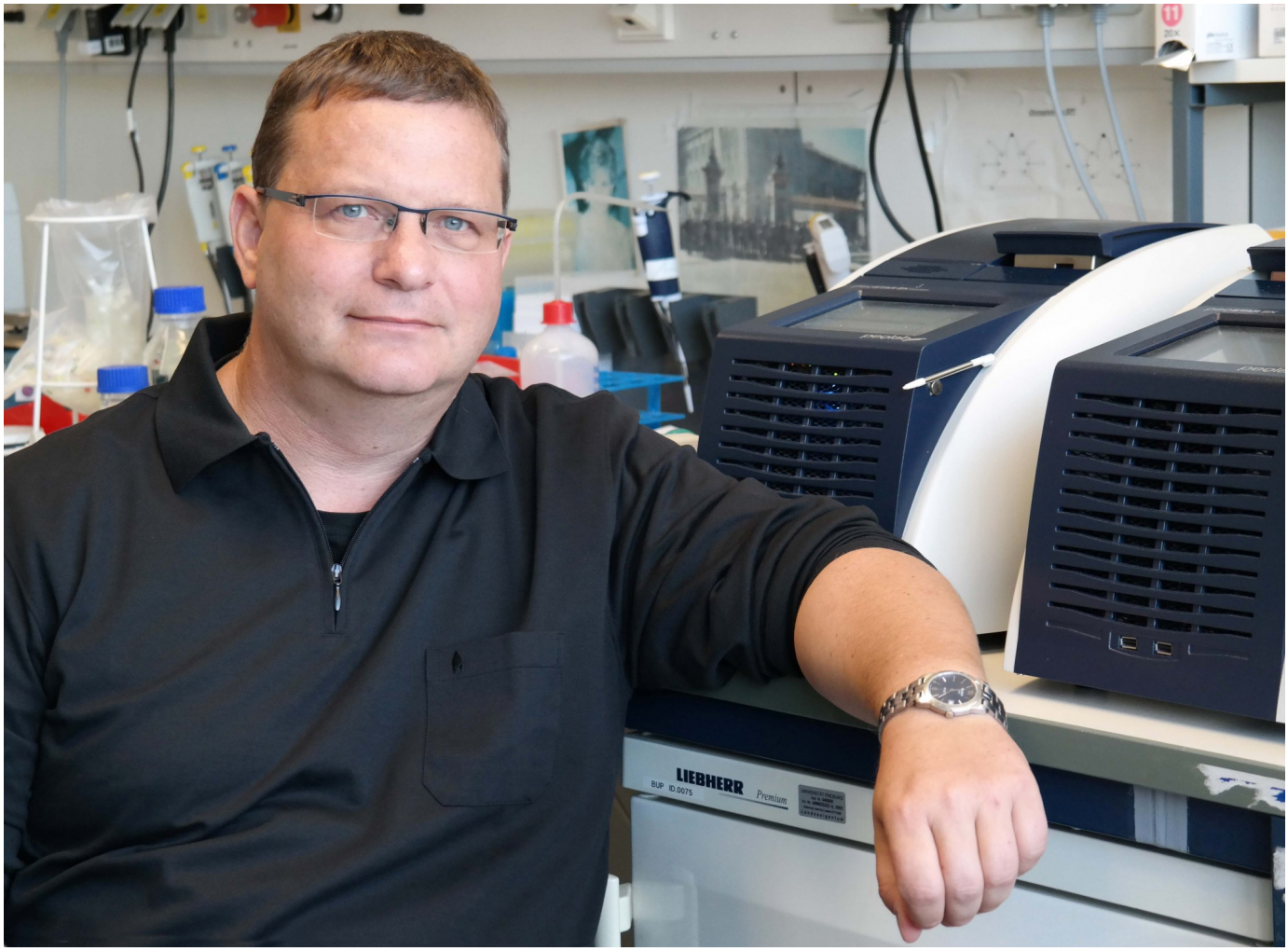
Cathepsin L: overcoming stress in tumours

Cathepsins are proteases, i.e. enzymes that break down proteins into smaller fragments. They are also involved in the formation of new blood vessels and wound healing. Another thing that cathepsins do is help tumours spread and form metastases in the body. Prof. Dr. Thomas Reinheckel and his team from the Institute of Molecular Medicine and Cell Research at the University of Freiburg are studying how this happens. Insights into the role of cathepsin L in tumour processes could potentially be exploited for developing cancer therapies that target the progression and metastasis of solid tumours.

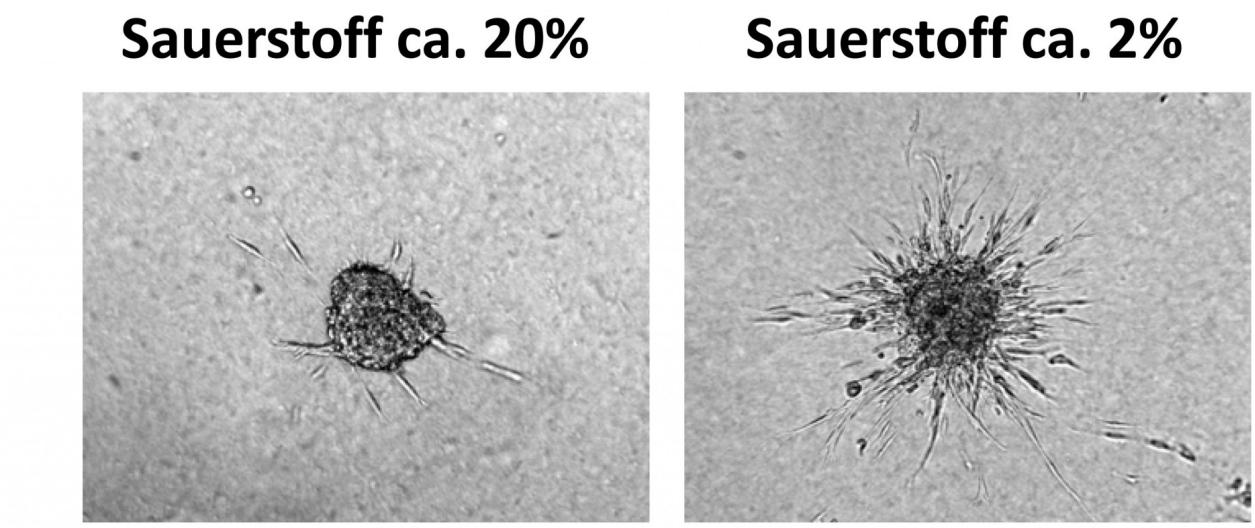


Researcher and physician Prof. Dr. Thomas Reinheckel analyses the mechanism of action of cathepsin L in breast cancer in particular.

Cathepsins play a key role in cellular turnover, they eliminate old cell organelles and degrade proteins. Cathepsins are endoproteases, and are generally found in cellular lysosomes. Humans have around 17 different proteases. These enzymes hydrolyse proteins that enter lysosomes and render the resulting amino acids available as building blocks for further syntheses. Papain, a cathepsin present in papaya fruits, was previously used as a meat tenderizer because of its ability to break down collagen and elastin. Six human genetic diseases that mainly affect the nervous system and bones have been found to be associated with cathepsins: for example, Goldberg syndrome (cathepsin A deficiency), Papillon-Lefèvre syndrome (cathepsin C deficiency), pycnodysostosis (cathepsin K deficiency) and neuronal ceroid lipofuscinosis (cathepsin D deficiency).

The deficiency of certain cathepsins can trigger lysosomal storage diseases, which are inherited metabolic disorders that are caused by defects in lysosomal function and that provoke neurodegeneration. People with such disorders often die at a young age. In contrast, overexpression of cathepsins by immune cells causes bones and cartilage to be destroyed, resulting in arthritic pain. However, these potent enzymes do not just destroy things, they also create new ones, albeit indirectly. "Cathepsins can be secreted by macrophages and immune cells and degrade the extracellular matrix, creating room for new blood vessels," says Prof. Dr. Thomas Reinheckel. Reinheckel and his team at the Institute of Molecular Medicine and Cell Research at the University of Freiburg are mainly focused on investigating the role of cathepsins in cancer, especially in the pathogenesis of breast cancer. As cathepsins promote tumour growth, patients with high cathepsin levels tend to have an unfavourable prognosis.

Stress in the tumour



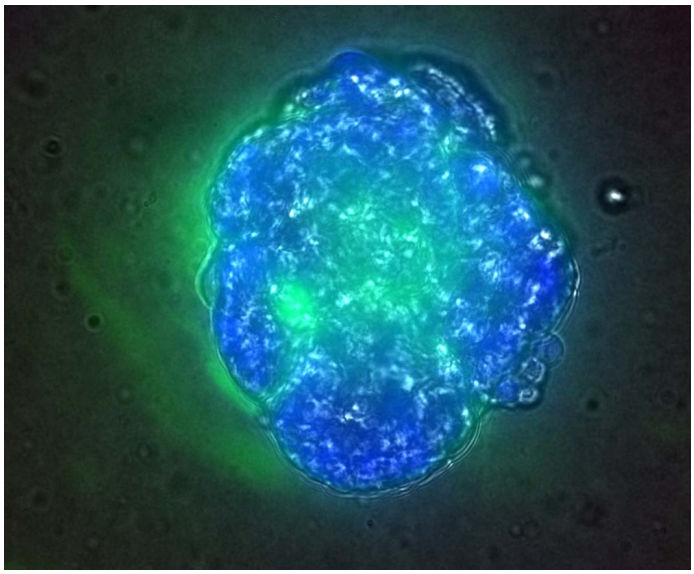
At high oxygen concentrations, breast cancer cell spheroids form fewer spindle-like protrusions (left). In the absence of oxygen, protrusions of the spheroids penetrate the surrounding extracellular matrix (right). Proteases such as cathepsin L promote this aggressive invasion process.

The process of tumour growth and of developing metastases leads to a kind of cellular stress in some cancer cells. Cells inside the tumour are cut off from oxygen and nutrients due to the lack of

blood vessels. The tumour cannot grow and metastasise. Reinheckel found higher than normal cathepsin L (CTSL) levels in breast cancer cells, even though they were in an obviously stressed state. He therefore wondered how cells managed to produce such high cathepsin L levels despite high cancer stress conditions. In the absence of oxygen or nutrients, tumour cells are prompted to initiate a programme that shuts down new protein synthesis in order to save material and energy. The cells achieve this by inhibiting the translation of mRNA into protein. Under stress conditions, newly formed ribosomes fail to associate with cytoskeletal mRNA, preventing the formation of new actin molecules. Moreover, cells that are under stress form granular aggregations in which the mRNA can be temporarily wrapped to protect it from harmful conditions. These stress granules are composed of proteins, translation factors and mRNAs, and free up capacity for the formation of urgently needed proteins. They dissolve again once conditions improve. Reinheckel observed that high levels of cathepsin L are maintained in cancer tissue and was able to show that the translation of CTSL continues as the tumour grows even when cells are exposed to stress.

High CTSL levels in breast cancers enable cells to metastasise, which is associated with a poor survival rate. The usual explanation is that a larger number of cathepsins can degrade more of the extracellular matrix and thus make room for more tumour cells, which are then able to spread and migrate. “This helps the cell to overcome the stress situation,” explains Reinheckel, adding, “higher cathepsin L levels give cells a survival advantage.” He also pointed out that it is an indirect way to induce the migration of cancer cells. Protease enzymes are used by cells as an accessory factor in order to survive in unfavourable environments. “Cathepsin L is not your standard oncogene or tumour promotor,” Reinheckel says.

The secret behind cathepsin L’s stress resistance



Breast cancer cells (blue) embedded in the extracellular matrix form three-dimensional tumour cell spheroids. High proteolytic activity (green) is found in and around the centre. © Research group of Prof. Dr. Reinheckel, University of Freiburg

In order to shed light on this observation, Reinheckel’s research team focused on the association of mRNAs with polyribosome complexes and discovered why CTSL is also effectively produced under conditions of stress. Polyribosome complexes are made of an mRNA with several protein-synthesising ribosomes associated with this mRNA, and the more complexes the better. These complexes normally ensure that large quantities of a protein are effectively synthesised from a single mRNA molecule. The researchers found that in breast cancer cells, cathepsin L mRNA was constantly associated with polyribosome complexes, while other mRNAs in the same cell were shut down under stress conditions. Why is CTSL resistant to stress-induced inhibition of translation? And why not other proteins?

According to Reinheckel there is evidence that cathepsin L mRNA has a genetic motif that allows the synthesis of proteins even though the normal initiation machinery is blocked. This motif is called IRES (internal ribosomal entry site) and is probably of viral origin. “The IRES element is present in cathepsin mRNA and thus allows protein expression independently of normal processes,” says Reinheckel. Viruses often have IRES elements because they want to ensure the formation of

their own viral particles when a host cell is infected. “From the cells' perspective, it makes perfect sense to increase cathepsin L production in stress situations,” says Reinheckel. Cells inside tumours only have two possibilities, either they die due to lack of oxygen and nutrients, or they find ways to overcome stress, for example by inducing the formation of new blood vessels and synthesising proteins that promote their further growth.

Inhibition with restriction

Interestingly, Reinheckel and his team have discovered another method that CTSL uses to ensure its own translation. The enzyme also evades the other mechanism that is initiated when cells are under stress. “We do not know how this happens yet, but we have seen that the mRNA of cathepsin is not encapsulated into stress granules, which are, as mentioned before, translationally silent protein/mRNA accumulations formed in stressed cells,” says the molecular biologist who believes that microRNAs play a key role in this process. The researchers used transgenic mice that carry the human cathepsin L gene to find out more. When they crossed the transgenic mice with tumour mice, Reinheckel and his team found that the transgene promoted tumour growth.

Although this has huge potential in tumour treatment, it also has its disadvantages as the exact therapeutic window is not known. The complete inhibition of cathepsins (which is the same as cathepsin deficiency) could have a fatal outcome. Inhibitors that impede cathepsin extracellularly could present an alternative with fewer adverse effects. Such inhibitors could prevent tumour growth without interfering with cellular enzymes. “Inhibition obviously cannot cure cancer, it just creates an unfavourable environment that cells find it harder to adapt to,” says Reinheckel, suggesting that a combined use of chemotherapy and kinase inhibitors would be an effective treatment for tumours.

Article

14-Dec-2015
Stephanie Heyl
BioRegion Freiburg

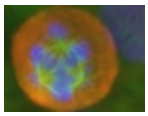
Further information

Prof. Dr. Thomas Reinheckel
Institute of Molecular Medicine and Cell Research
University of Freiburg
Stefan-Meier-Str. 17
79104 Freiburg
Tel.: +49 (0)761 203 - 9606
Fax: +49 (0)761 203 - 9634
E-mail: thomas.reinheckel(at)uniklinik-freiburg.de

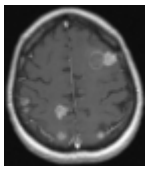
- University of Freiburg: Institute of Molecular Medicine and Cell Research

---

The article is part of the following dossiers



Cancer – basic research, successes and trends



Tumour metastasis

stress | cancer | metastases