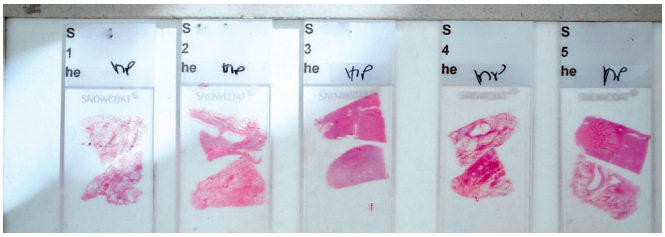
Pathology

Departing from the script

by Hanno Carisius



What factors enable tumors to grow? Heiko Hermeking and his team use tumor samples from patients to verify the results of their experiments. *Source: Jan Greune*

Countdown to catastrophe: Tumor biologist Heiko Hermeking studies the factors that cause cells to disregard natural constraints and opt out of their assigned roles in differentiated tissues.

All cancers originate in a loss of control, when a single cell suddenly begins to divide more often than it should. Under the microscope, the deregulated cell cannot be distinguished from its neighbors in the same tissue or organ, and its progeny also look normal.

It takes time for such misfits in a homogeneous cell mass to become recognizable as being different from the cells around them. And by this time, the tumor has taken on a life of its own. Molecular biologist Heiko Hermeking at LMU's Institute of Pathology, wants to understand why.

Hermeking compares the tumor cell with a vehicle in which the accelerator pedal is stuck in position. Whether a car is travelling at 100 km/h on the autobahn or racing at top speed when this happens can make a big difference to the outcome, but slowly growing tumors can actually be more dangerous than the rapidly growing ones, he says. Fast-growing tumors tend to provoke the body's immune system into mounting a counterattack that eliminates the rogue cells. Slowly growing ones are more likely to evade detection. And tumors that can continue to grow for many years are bad news, he explains. "Genetic changes which do not immediately cause chaos and force cells to commit suicide are often the more dangerous ones," says Hermeking.

So keeping the accelerator pressed to the floor is usually not a good strategy for a growing tumor – although Hermeking prefers not to use the term "strategy" when it comes to tumors. "A tumor does not behave as it does because it is following a well thought out plan," he points out. "On the contrary, a tumor results from a local breakdown in the system. Tumor cells are subject to the rules of evolution, he says. They simply make use of the biological possibilities provided by their genetic apparatus, which determines the metabolic capacities they possess and the complement of molecules they produce. And for the past 25 years, Hermeking has been probing how this process of adaptation works.

Hermeking has been based in the Institute of Pathology at LMU for the past 6 years. Visitors who enter the building from the Winckelstrasse embark on a journey through time. Columned halls, broad staircases, busts of former directors arrayed in niches along the walls. The succession stretches back for nearly a century. Max Borst, for instance, was Director when the building was opened in 1928 (its construction took only 2 years) as a replacement for the old Institute on Nussbaumstrasse, which was no longer large enough to serve its original purpose. Borst was responsible for developing a method for the "histogenetic definition of tumors". This provided a systematic method for the differentiation of tumor types classifying tumors, and still serves as the basis for the classification scheme used by the World Health Organization. The aura of a bygone age persists when one enters the wing in which the laboratories are located – but not for long! It soon becomes clear that time has not come to a standstill here.

Modern laboratory equipment is everywhere. "Pathologists here examine some 50,000 tissue samples every year," says Hermeking – mostly in order to determine whether a patient's tumor is benign or malignant. He himself is only indirectly concerned with such histopathological investigations however.

When Ludwig von Buhl took over the direction of Munich University's first Institute of Pathology on Nussbaumstrasse in 1875, he seems to have divined how the subject would develop. When the Institute opened, he declared that it would "permit a division of labor, focusing not only on pathological anatomy and histology, but also on chemical, physical and experimental studies, with a view to understanding the nature of

pathological phenomena and defining the conditions that give rise to them. In other words, from the beginning, observational diagnostics and microscopy have co-existed alongside chemical, physical and biological – in short, experimental – methods, in the quest for a better understanding of cancer and more effective ways of fighting it. The complementarity between basic, experimental research – such as that in which Hermeking is engaged – and clinical diagnostics remains constitutive.

As Head of the Laboratory for Experimental and Molecular Pathology, Hermeking is a dedicated basic researcher, but he collaborates closely with his clinical colleagues. However, his own "patients" are four-legged creatures. Whenever the problem in hand cannot be answered in cell culture systems, his group turns to the mouse as an experimental model organism.

Hermeking also has plans to work with so-called spheroids in future. Spheroids are three-dimensional cell cultures consisting of various cell types, which are organized in ways that reproduce important aspects of the structure of the kind of tumor one wants to study. They can be derived from mice but Hermeking also collaborates with specialists who generate them from patients' tissues. "This allows us to significantly reduce the number of animal experiments we do," he says, "and in many cases we get the results quicker."

Amplification of DNA: With a PCR machine, virtually any stretch of genomic DNA can be synthesized in large amounts. *Source: Jan Greune*

Tumor cells in disguise

In addition, Hermeking and his coworkers make use of all the standard molecular biological methods to reconstruct what goes on in tumor cells. State-of-the-art sequencing machines that perform Next Generation Sequencing now enable researchers to identify the genes that drive the growth of an individual patient's tumor, while a variety of molecular histological methods and imaging techniques are used to characterize tumor tissue. Many patients consent to the use of their tissue samples for research purposes. This allows Hermeking and his colleagues to check whether their laboratory findings in mouse are also hold for tumor growth in humans. He has no favorite method, he says. "All are necessary to give us the complete picture." But from the aesthetic point of view, he adds, confocal laser microscopy is in a class of its own. And this tribute to the continuing relevance of light microscopy places him firmly in the long tradition of his predecessors in the Institute.

Hermeking and his team are now particularly interested in the question of how some tumor cells manage to leave their sites of origin, enter the circulation and find their way to another tissue where they seed a new tumor, a metastatic tumor. "To do so, tumor cells make use of a sort of tumor mimicry", Hermeking explains. The tumor cell adopts a disguise, so to speak, by exploiting biological programs that are otherwise activated only during the growth and development of the embryo in the womb. At this point in development, many organs and tissues have not yet fully differentiated. Their cells are in a labile state, and can be induced to take on any one of a variety of forms and functions. This, incidentally, confirms that tumors are opportunists. They make use of the mechanisms open to them – and this is what makes them so difficult to fight.

For example, tumor cells can change their character by undergoing an epithelial-mesenchymal transition (EMT). An epithelial cell in a mucous membrane can "forget" that it is part of a flat sheet of cells and turn into a mobile fibroblast. Hermeking and his team recently identified a regulatory program that triggers this transformation, which can prove lethal unless tightly controlled.

At the heart of this program are two transcription factors – proteins that regulate the activity of specific genes or sets of genes – called Myc and p53. Hermeking refers to them as master switches. Myc promotes cell division, while p53 is a "tumor suppressor" and acts as a brake on cell proliferation. But, being master switches, they actually regulate a wide range of processes within cells, not only those that are directly related to carcinogenesis Take p53, which is named for its molecular weight. Among other things, p53 helps to inhibit cardiac hypertrophy, the pathological replacement of injured muscle cells by scar tissue that often occurs

following a heart attack. "p53 is a central component in the organism's response to stress, and its function is not confined to specific cell types," says Hermeking. However, unlike Myc, it is not essential for survival of the developing organism. Mice that lack p53 due to a mutation develop normally. However, they are highly sensitive to all forms of stress, such as DNA damage or induced ischemia. Mutant mice in which Myc is missing, on the other hand, die before birth.

There's a poster on the wall behind Hermeking's desk that looks about as complicated as a street-map of Tokyo. What it actually shows is a kind of circuit diagram of carcinogenesis. Each node represents a switch in the program that controls how cells behave, and the edges depict how the switches are connected. In this scheme, p53 and Myc stand out as central hubs, and have been extensively investigated. Indeed, according to Hermeking, literature searches now turn up around 30,000 publications that refer to Myc, while p53 is cited in some 80,000 research papers.

We owe the recognition of some of the salient connections and central junctions in this network to Hermeking and his coworkers. But the road map that describes the many routes to malignancy consists of more than links between proteins. So-called micro-RNAs, i.e., short fragments derived from longer ribonucleic acid molecules, which are both structurally related to, and derived from the genomic DNA, also play a role in carcinogenesis. By interacting with the messenger RNAs that direct the assembly of proteins, specific micro-RNAs can inhibit the synthesis of certain proteins. Moreover, some years ago, Hermeking and his team showed that p53 and Myc are involved in regulating the production of specific micro-RNAs. This is another of the complex interaction networks that Hermeking hopes to dissect.

More recently, Hermeking's group uncovered a molecular interaction that links inflammation reactions to the formation of metastases. In this context, the synthesis of a specific micro-RNA, miR-34a, is turned off by signals that originate in the tumor's environment and are responsible for provoking local inflammation. Synthesis of miR-34a is activated by the tumor suppressor p53, which in turn initiates a process that serves to localize the tumor. However, if inflammation processes inhibit the production of miR-34a, this brake on tumor growth is disabled, allowing cells to escape from the primary tumor and form metastases. It is particularly important to understand how such secondary tumors form, because they are what actually cause 90% of all cancer deaths.

Hermeking compares the progress of his field of research with what one sees through the viewfinder of a camera: As one adjusts the focus, the picture gradually becomes sharper, and one can recognize ever finer details. Discoveries that completely alter the landscape and make it necessary to rewrite the textbooks are very rare.

Can carcinogenesis be prevented? Hermeking doesn't yet have a final answer to this question. But experiments in animals suggest that a healthy, balanced diet can suppress the growth of tumors. Conversely, eating too much sugar or fat can provoke inflammation, which in turn promotes tumorigenesis. "A healthy lifestyle probably cannot prevent the appearance of tumorous cells, but it looks as if lifestyle factors can have an impact on their further development."

At least in mice, there is evidence that, in addition to dietary factors, lack of exercise, too much weight and chronic inflammation promote the growth of tumors. Is that also true of people? "That may well be so," Hermeking replies. "But one thing's for sure. In these respects, people exhibit far more diversity than mice do."

Prof. Dr. Heiko Hermeking
Now Professor of Experimental and Molecular Pathology at LMU, Hermeking (b.1966) studied Biology at LMU, where he also obtained his doctorate and completed his *Habilitation* in Cell Biology. After his PhD, he worked as a postdoc in the Oncology Center at Johns Hopkins University Medical School in Baltimore (Maryland), and as a Research Associate at the Howard Hughes Medical Institute. On his return to Germany, Hermeking headed a research group in Molecular Oncology at the Max Planck Institute for Biochemistry in Martinsried, and later served as Professor of Molecular Tumor Pathology at the University of the Ruhr in Bochum, before moving back to LMU in 2009.

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