

*Molecular Oncology in Martinsried*

# Up and down p53

Cancer research's most-loved tumour suppressor is also Heiko Hermeking's favourite. Taking p53 as a starting point his group browses the regulation network, looking for its molecular players, functions and levels of regulation.

It is the gene most often mutated in human tumours: p53. It encodes a transcription factor that is activated by DNA damage, leading to G1 and G2 arrest and – under some circumstances – to senescence and apoptosis. When p53 is corrupted, the cell lacks its most important brake for cell cycle control, paving the way for malignancies. To understand tumorigenesis, cancer researchers are digging deep into its networks of regulation.

“We are interested in the signalling upstream and downstream of p53,” says Heiko Hermeking, head of an independent research group at the Max-Planck-Institute (MPI) of Biochemistry at Martinsried near Munich. In 2000 he returned to Germany's southern metropolis, after some postdoc years in the US. He definitely found a good position, as the MPIs of the Max-Planck society are famous for their uniquely good working conditions for young scientists. Independent research groups, especially, are designed as stepping stones for young group leaders, giving them seven years to establish their research programme.



Heiko Hermeking (below, left) and his p53 networkers

The Munich region – for many, *THE* place to be in German life sciences – wasn't new to Hermeking though. He received his biology degree from the Ludwig-Maximilians-Universität in Munich and entered the p53 field not very far away from his cur-

rent lab. While working as a PhD student in Dirk Eick's group at the GSF Research Center he studied the c-MYC oncogene and, surprisingly, found that activation of this transcription factor in turn induces p53 (*Science* 1994, 265, p.2091). At that time DNA damage was the only factor known upstream of p53. “Also it was the first time that the field of cellular oncogenes was connected to the p53 tumor suppressor.”

## Arrest. But how?

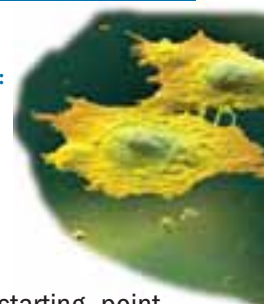
His fruitful work on the interaction between c-MYC and p53 signalling eventually brought Hermeking to the lab of a leading p53 expert: Bert Vogelstein at Johns Hopkins University in Baltimore. During his postdoc there, he concentrated on events downstream of p53. He searched especially for the unknown molecular mediators of the G2 arrest induced by p53.

What he found was 14-3-3sigma, a gene whose expression is induced by p53 upon gamma irradiation or other DNA damaging agents. Introduced into cycling cells it results in a G2 arrest (*Mol Cell* 1997, 1, p.3). 14-3-3sigma's role in the p53 pathway was a fertile discovery. His MPI group, among other groups, is still working on it.

To find 14-3-3sigma, Hermeking used serial analysis of gene expression (SAGE) to quantify mRNAs of the two states – with or without irradiation. SAGE

had been developed in Vogelstein's lab two years earlier. Unlike microarrays, which are used for similar purposes, the method is based on sequencing, so it allows quantifying gene expression without prior information about genes that are thought to be ex-

Possible result of p53 mutation: primary colon cancer cells



pressed. “Compared to microarrays, SAGE is an unbiased approach that does not involve hybridizations which may be misleading,” Hermeking says.

## Becoming an European expert

With SAGE Hermeking also continued his work on c-MYC, identifying CDK4 as one of its transcriptional targets. Before, it was unclear by which mechanisms c-MYC promotes cell-cycle re-entry, driving proliferation. The finding helped to link c-MYC's oncogenic nature with its cell cycle effects (*PNAS*, 2000, 97, p.2229).

When he returned to Germany in 2000, he started his own lab as one of Europe's first SAGE experts. His group further explored the potentials of its gene expression analysis, amongst others delivering more contributions to c-MYC's transcriptome (*PNAS* 2002, 99, p.6274) and identifying genes involved in the senescence of the prostate epithelial cells, which is presumably a tumor suppressive process (*Cancer Res* 2002, 62, p.6255).

Furthermore, the group elucidated the role of epigenetic silencing of 14-3-3sigma for hyperproliferative skin diseases (*Oncogene* 2003, 22, p.5519) and prostate cancer (*Oncogene* 2004, 23, p.9034; *Cancer Res* 2005, 65, p.4218). The fact that 14-3-3sigma is inactivated in many tumours (*Semin Cancer Biol* 2006, 16, p.214) shows that it has good tumour suppressive qualities itself.

## What the crystals tell

“14-3-3sigma is very interesting because it interacts with phosphorylatable binding sites in hundreds of different proteins. In p53's network the molecule acts like an amplifier, enabling p53 to control many processes simultaneously,” Hermeking explains. To understand the network regulated by 14-3-3sigma, the repertoire of the lab was enlarged once more. Analysis of the crystal structure of unbound 14-3-3sigma was used to find out more about the isoforms and dimerization of the protein (*Cell Res* 2005, 15, p. 219; *Cell Cycle* 2006, 5, p. 2920). Also proteomics was introduced to identify pro-



teins interacting with 14-3-3sigma (*Mol Cell Proteomics* 4, p785).

As a whole new layer of regulation, microRNAs have become increasingly interesting in the last years. Therefore, analysing p53's network for microRNA components was the next project for Hermeking and his group. They were able to identify a microRNA gene as a direct target of the transcription factor p53: miR-34a, whose expression is up-regulated 30-fold by p53. It induces apoptosis and G1-arrest when re-introduced into cells (*Cell Cycle* 2007, 6, p.1586).

#### A few weeks too late

Unfortunately, the hype around the small RNAs is so big, that three other groups had the same idea of searching for miRNAs that are induced by p53. Within one month, all four of them published more or less the same results – a group from Israel and a group from Johns Hopkins in the same issue of *Molecular Cell* and another group from Cold Spring Harbor in *Nature*.

Hermeking, being a few weeks later, couldn't place his group's work in a similarly ranked journal. Instead, he chose to submit the manuscript to *Cell Cycle*, a relatively new journal, attracting scientists with its rapid publication process. "It's strange," Hermeking says, "c-MYC induces miRNAs as well, that was published two years ago. Why wasn't that studied earlier for p53? Also then, several groups came to the same conclusion simultaneously!" However, although displeased about the coincidence, he says, "I'm happy that we were able to publish it at all."

As the microRNA work was a return to gene expression analysis, naturally SAGE would have come into play again. The only problem with SAGE was that it takes a lot of time. "For the 14-3-3sigma experiments in Baltimore it took me half a year to get the data. In those days, we sequenced for three months, day and night," Hermeking says.

#### Thanks to a generous budget

So for the recent microRNA study in his German lab he decided to try to use a SAGE-related approach. His group generated libraries of microRNAs which were then analysed by parallel pyro-sequencing. "In fact, we outsourced the sequencing part to 454 life sciences. We sent the library to them and got back the data a few weeks later," Hermeking says.

Nearly unthinkable for an average university research group in Germany, Hermeking, supported by the generous Max-Planck-Society, paid for this service with institutional money. "Although we had a lot of grant funding during the last years, too, these miRNA experiments were paid out of the group's budget," he says. For him this is one of Max-Planck's major benefits, the freedom to initiate projects quickly, without writing proposals first. "It may spoil you though," he admits, thinking of his limited time left at the MPI of Biochemistry.

#### One year for a new system

Little hands-on time, therefore, was required for the screen itself. However, to address the question of what the microRNAs found in the screen do when reintroduced into cells ectopically, the lab had already developed a conditional expression system for microRNAs. "That was the really time-consuming work done in the lab before," Hermeking says. It took a year to establish the new and stringent Tet-based system published last year (*Nucleic Acids Res* 2006, 34, e119). It was worth the trouble, though. In contrast to other systems it has all the necessary components on one vector. "We can now generate a pool of cells within a week to find which inducibly expresses the microRNA of interest," he says.

This rapid conditional expression system proved useful as a knock-down method, too, when testing a candidate found in their recently conducted proteomics screen for p53 interactors – the cullin protein Cul7. Among other tests, Cul7's inhibitory effect on p53 was demonstrated using the new system (*PNAS* 2007, 104, p.11388).

#### Soon somewhere else

At the moment 50 percent of all projects are concerned with the new p53-regulated microRNAs, Hermeking says. The other half are projects continuing the successful work on 14-3-3sigma and c-MYC. However, as generous MPI days are numbered, more interesting than the question of *what* next, is the question of *where* next.

*Lab Times* therefore wanted to know whether he will move to a German university. He could, because he holds a habilitation, a postdoc degree which in Germany is still a prerequisite for a position as a professor in most cases. Or is he looking for other positions in Germany, Europe, the US, internationally? Hermeking won't tell yet. "I'll be somewhere else next year," he says, smiling vaguely.

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