

## DIVISION OF MOLECULAR CARCINOGENESIS

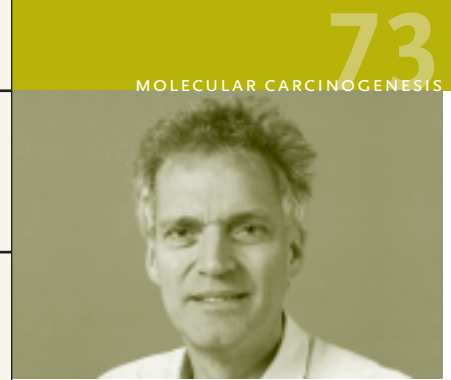
### FUNCTIONAL GENOMICS

My group uses functional genomics technologies to identify mechanisms of resistance to cancer drugs and to find novel cancer-relevant genes. We use various types of genetic screens to achieve these goals.

**Identification of mechanisms of drug resistance** Unresponsiveness to therapy is a recurring problem in the treatment of cancer. It is therefore important to identify the molecular pathways that contribute to unresponsiveness to cancer therapeutics. We use loss-of-function genetic screens with large sets of shRNA vectors to identify genes that contribute to drug resistance, in particular to the new classes of targeted therapeutics. In the past year, we have focused on the identification of genes whose suppression contributes to resistance to receptor tyrosine kinase (RTK) inhibitory drugs and their downstream signaling pathways. To do this, we used the H3122 non-small cell lung cancer cell line, which harbors the *EML4-ALK* translocation. This translocation renders these cells highly sensitive to the small molecule ALK kinase inhibitors TAE684 and PF02341066 (also known as Crizotinib). In a genome-wide shRNA screen, we identified both components of the SWI/SNF chromatin remodeling complex as well as components of the MEDIATOR complex as genes whose inhibition confers resistance to ALK inhibition. Interestingly, knockdown of these genes also conferred resistance to crizotinib in *MET*-amplified lung cancer cell lines and to the EGFR inhibitory drug gefitinib in lung cancers having an activated version of the *EGFR*. These data suggest that the MEDIATOR and SWI/SNF complexes play critical roles downstream of RTK signaling. Consistent with this, knockdown of either of these genes caused a significant increase in phospho-ERK and phospho-AKT, even in the presence of RTK-inhibitory drugs. We also found that knockdown of the MEDIATOR or SWI/SNF complex components caused a marked increase in phospho-EGFR, even in the presence of RTK inhibitory drugs like gefitinib. We are currently investigating how knockdown of key components of the MEDIATOR and SWI/SNF complexes can cause activation of the ERK and AKT signaling pathways and affect EGFR activity. Importantly, high throughput sequencing of these MEDIATOR and SWI/SNF components suggests that mutations in these genes may occur in lung cancer. Whether these mutations are associated with resistance to RTK inhibitory drugs remains to be investigated.

**Functional studies on de-ubiquitinating enzymes** The Androgen receptor (AR) belongs to the nuclear receptor super-family and is essential for male sexual development and maturation, as well as prostate cancer development. Regulation of AR signalling activity depends on several post-translational modifications, one of these being ubiquitination. We screened a short hairpin library targeting members of the de-ubiquitination enzyme (DUB) family and identified the X-linked DUB USP26 as a novel regulator of AR signalling. USP26 is a nuclear protein that binds to AR via three important nuclear receptor interaction motifs, and modulates AR ubiquitination, consequently influencing AR activity and stability. Our data suggest that USP26 assembles with AR and other co-factors in sub-nuclear foci, and serves to counter-act hormone-induced AR ubiquitination, thereby contributing to regulation of AR transcriptional activity.

Histone ubiquitination has emerged as an important epigenetic mark in regulating gene expression and thereby plays an essential role in the development of higher organisms. Moreover, aberrant histone ubiquitination is frequently seen in cancer. However, most of these studies have been carried out in yeast and have focused on mRNA coding genes. The *Drosophila* ubiquitin protease USP36 is a H2B specific DUB. Using a human USP36 specific antibody we found that USP36 is strictly nucleolar in localization. We then looked at the pre-rRNA levels by quantitative RT-PCR using primers specific for 5'ETS (rRNA External Transcribed Spacer) upon USP36 knock down. A dramatic reduction in the pre-rRNA levels in the absence of



Division head, group leader René Bernards

**René Bernards PhD** Group leader  
**Katrien Berns PhD** Academic staff  
**Annette Dirac PhD** Senior Post-doc  
**Michiel van der Heijden MD PhD** Senior Post-doc  
**Michael Hölzel MD PhD** Senior Post-doc  
**Sidong Huang PhD** Senior Post-doc  
**Prasanth Kumar PhD** Post-doc  
**Ian Majewski PhD** Post-doc  
**Rianne Oosterkamp MD** Clinical fellow  
**Ernst-Jan Geutjes MSc** PhD student  
**Floris Groenendijk MD** PhD student  
**Guus Heynen MSc** PhD student  
**Jasper Mullenders MSc** PhD student  
**Anirudh Prahallad MSc** PhD student  
**Chong Sun MSc** PhD student  
**Astrid Bosma** Technical staff  
**Annemiek Gennissen** Technical staff  
**Marielle Hijmans MSc** Technical staff  
**Wipawadee Grertrum** Technical staff

### Publications

Ashworth A, Bernards R. *Using functional genetics to understand breast cancer biology. Cold Spring Harb Perspect Biol.* (Polyak, Rosen and Bissell, eds). 2010 (in press)

Bernards R, Filipowicz W, Livingston DM, Mihich E. *Twenty-second Annual Pezcoller Symposium: RNA Biology and Cancer. Cancer Res.* 2010 (in press)

Bernards R. *It's diagnostics, stupid. Cell* 2010;141:13-7

Dirac AMG, Bernards R. *The de-ubiquitinating enzyme USP26 is a regulator of androgen receptor signaling. Mol. Can. Res.* 2010;8:844-54

Epping MT, Meijer LAT, Krijgsman O, Bos JL, Pandolfi PP, Bernards R. *TSPYL5 suppresses p53 levels and function by physical interaction with USP7. Nature Cell Biol.* 2010 (in press)

## Publications (continued)

Hölzel M, Huang S, Koster J, Øra I, Lakeman A, Caron H, Nijkamp W, Xie J, Callens T, Asgharzadeh S, Seeger RC, Messiaen L, Versteeg R, Bernards R. *NF1 is a tumor suppressor in neuroblastoma that determines retinoic acid response and disease outcome.* *Cell* 2010;142:218-29

Mullenders J, Fabius AWM, van Dongen MMW, Kuiken HJ, Beijersbergen RL, Bernards R. *Interleukin-1R-Associated Kinase2 Is a Novel Modulator of the Transforming Growth Factor Signaling Cascade.* *Mol. Can. Res.* 2010;8:592-603

Van der Heijden MS, Bernards R. *Inhibition of the PI3K pathway: hope we can believe in?* *Clinical Cancer Res.* 2010;16:3094-9

endogenous USP36 was observed, indicating that USP36 has a role in the regulation of mammalian ribosomal RNA (rRNA) gene transcription. We are currently asking how USP36 control rRNA gene transcription.

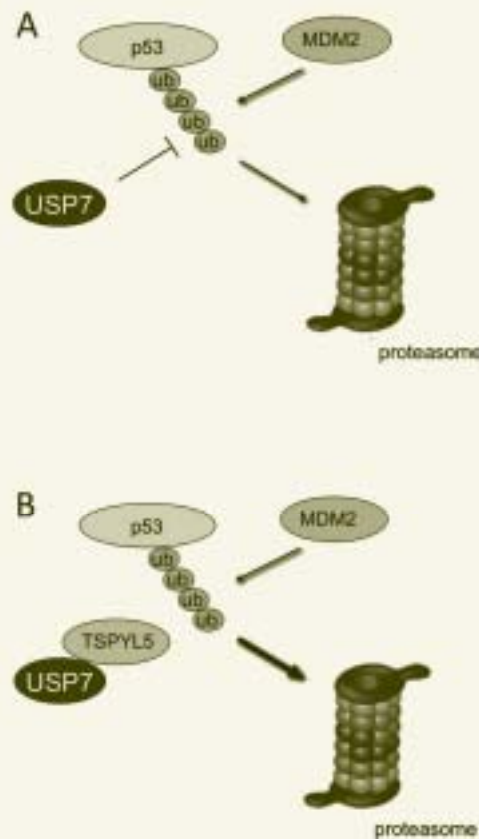


Figure 1: TSPYL5 regulates p53 turnover. The degradation of the p53 tumor suppressor protein is regulated by both ubiquitination by the ubiquitin ligase MDM2 and deubiquitination by the DUB USP7. When TSPYL5 is over-expressed in breast cancer as a result of gene amplification, TSPYL5 binds USP7, thereby preventing it from interacting with p53. Consequently, the balance between ubiquitination and deubiquitination is shifted towards increased polyubiquitination of p53, causing an increase in p53 destruction by the proteasome. As a result, TSPYL5 over-expressing breast cancer cells are functionally deficient in p53.

TSPYL5 is a gene of unknown function located at a region on chromosome 8q22, which is frequently amplified in breast cancer. Using mass spectrometry, we found that TSPYL5 physically interacts with ubiquitin-specific protease 7 (USP7)/herpesvirus-associated ubiquitin-specific protease (HAUSP). USP7 is the DUB for the p53 tumor suppressor and we find that TSPYL5 opposes the activity of USP7 towards p53, resulting in increased p53 ubiquitination (figure 1). We found that TSPYL5 reduces p53 protein levels and inhibits activation of p53 target genes. Moreover, expression of TSPYL5 overrides p53-dependent proliferation arrest and oncogene-induced senescence in multiple assays. These data identify TSPYL5 as a novel breast cancer oncogene that critically modulates the p53-USP7 network.

**High throughput kinome sequencing** Cancer cells often contain multiple genomic alterations that together are responsible for the deregulated growth. Cancer cells often depend on the continued presence of these genomic alterations and sudden inhibition of the signals that emanate from these genomic alterations frequently results in death of the cancer cells, a phenomenon coined “oncogene addiction”. Conversely, the presence of downstream activating mutations (e.g. in KRAS) confers potent resistance to EGFR inhibitory drugs in colon cancer. The presence of specific changes in the genomes of cancer cells can therefore have strong predictive value for responsiveness to therapies that target these mutations. Since most of the mutations identified to date that predict responses to targeted therapies are in kinase genes or their downstream signaling pathways, we set out to develop a dedicated high-throughput sequencing platform to identify kinase mutations in large numbers of tumors. In collaboration with Agilent Technologies (Santa Clara, CA USA), we developed and validated a “kinome capture” platform that allows us to rapidly sequence 586 kinase and kinase-related genes through the enrichment of their coding sequences by hybridization selection. We are currently using this platform to find novel mutations in subgroups of breast cancer that are currently difficult to treat with conventional therapies. We aim to identify novel kinase targets for therapy that can be exploited to improve the therapy of these types of breast cancer.