DIVISION OF MOLECULAR GENETICS

ROLE OF POLYCOMB-GROUP GENES IN TRANSCRIPTIONAL REPRESSION, STEM CELL FATE AND TUMORIGENESIS

Our lab has a long-standing interest in epigenetic gene regulation dictated by chromatin modifications. We study the mechanism of transcriptional repression by Polycomb-group (Pc-G) protein complexes, and the effects of deregulation of Pc-G genes on development, Cell cycle control, cancer formation and stem cell maintenance. In addition, we are performing large-scale genetic screens in primary cells and in cancer-predisposed mice to identify cancer-relevant networks of oncogenes and tumor-suppressor genes. Model organisms comprise Mouse and Drosophila.

Functional characterization of Pc-G protein complexes Repressive Pc-G proteins and counteracting Trithorax-group (Trx-G) of nucleosome remodeling factors are involved in maintenance of proper gene expression patterns during development at the level of chromatin structure. Pc-G protein complexes control large sets of genes including Hox gene clusters and the INK4a/ARF tumor suppressor locus. At least two biochemical distinct evolutionary highly conserved Pc-G protein complexes can be distinguished. The first (PRC2) contains EzH1/EzH2 (SET domain proteins acting as Histone H3 methylases), Su(z)12, Eed and histone deacetylases. The second large complex (PRC1) encompasses Bmi1/Mel18, M33/MPc2, Mph1/Mph2 and Ring1b/Ring1a together with other more loosely associated proteins is required throughout development. To study Pc-G, function we focus on representative members of PRC1 and PRC2 in gain- and loss-of-function studies in mice and Drosophila. In genetic and biochemical experiments we identified the Bmi1/Ring1b heterodimer as an E3 ubiquitin ligase for monoubiquitination of histone H2A (collaboration with G. Buchwald and T. Sixma, Division II). Conditional Ring1b loss-of-function experiments indicate an essential role for maintenance of Pc-G repression in development and stem cell maintenance. An outstanding question is how the activity of PcG enzymes is regulated; we recently obtained evidence that phosphorylation of Bmi1 is required for E3 ligase activity of PRC1 and by mutating the essential phosphorylation sites demonstrated that these are important for Bmi1's oncogenic capacity. In addition we are studying the function of the deubequitinating enzyme Usp3 that binds to mono-ubiquinated H2A and can remove this mark. A major unresolved question is where and how Pc-G complexes bind to chromatin. We have performed genome-wide surveys of where PRC1 and PRC2 complexes bind to the Drosophila and mammalian genomes using DAMid profiling on tilling arrays (collaboration with B. van Steensel, Department of Gene Regulation). This highlighted binding of both PRC1 and PRC2 to distinct domains of 10-140 Kb containing ± 400 target genes in Drosophila and identified ± 800 mammalian targets. These comprise conserved developmental regulators that control differentiation. In recent in vivo 4C (chromatin conformation capture on Chip) experiments we demonstrated that these domains interact in vivo in 3D nuclear space in Drosophila neural tissues.

Connections between Pc-G gene repression, control of stem cell fate and cancer formation We originally identified Bmi1 as an oncogene that cooperates with cMyc in the induction of B and T-cell lymphomas in mice, underscoring the connection between deregulation of Pc-G repression and cancer. In contrast, Bmi1 knockout mice show severe progressive proliferation defects and increased apoptosis of lymphoid and myeloid cells, resulting in severe lymphopenia. In addition, also Bmi1-deficient primary embryo fibroblasts (MEFs), neural precursors and many other primary cell types show proliferation defects. We demonstrated that these defects are in part due to increased levels of the tumor suppressors p16INK4a and p19ARF, that are critical regulators of the RB and the p53 tumor suppressor pathways. As such, the INK4a/ARF locus acts as an important tumor-prevention mechanism in normal cells and stem/precursor cells. Using the mammary fat pad

Division head, group leader
Maarten Van Lohuizen

Maarten Van Lohuizen PhD Group leader
Elisabetta Citterio PhD Senior Post-doc
Jean Bernard Beaudry PhD Post-doc
Jaap Kool PhD Post-doc
Karim Nacerdine PhD Post-doc
Inka Pawlitzky PhD Post-doc
Gaetano Gargiulo PhD Post-doc
Anke Sparmann PhD Post-doc
Bas Tolhuis PhD Post-doc
Nienke de Vries PhD Post-doc
Bart Westerman PhD Post-doc
Cesare Lancini PhD Post-doc
Waseem Akthar PhD Post-doc
Michela Serresi PhD Post-doc
Joep Vissers MSc PhD student
Martijn Koppens PhD student
Marleen Blom Technical staff
Paulien Cornelissen Technical staff
Danielle Hulsman Technical staff
Ellen Tanger Technical staff
Els Verhoeven Technical staff
Huub van Vugt Technical staff

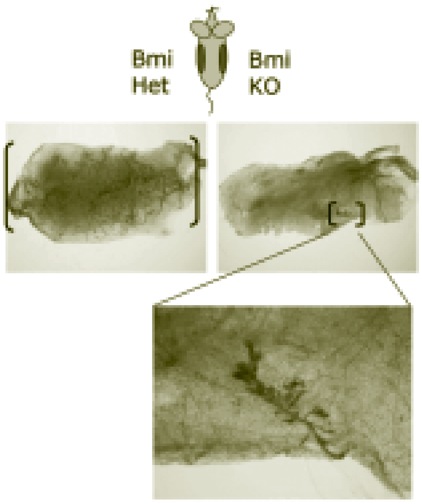

Figure 1: Mammary fat pad transplantation assay. Ductal outgrowth is severely impaired upon transplantation of Bmi1-/- (KO) when compared to Bmi1+/- (Het). This suggests a severe mammary epithelial precursor (stem) cell defect in Bmi1 deficient mice.

Publications

Kool J, Uren AG, Martins CP, Sie D, de Ridder J, Turner G, van Uitert M, Matentzoglu K, Lagcher W, Krimpenfort P, Gadiot J, Pritchard C, Lenz J, Lund AH, Jonkers J, Jane Rogers J, Adams DJ, Wessels L, Berns A, van Lohuizen M. *Insertional mutagenesis in mice deficient for CDK inhibitors p15Ink4b, p16Ink4a, p21Cip1, p27Kip1 reveals cancer gene interactions and correlations with tumor phenotypes. Cancer Res. 2010;70:520-31*

Mattison J, Kool J, Uren AG, de Ridder J, Wessels L, Jonkers J, Bignell GR, Butler A, Rust AG, Brosch M, Wilson CH, van der Weyden L, Largaespada DA, Stratton MR, Futreal PA, van Lohuizen M, Berns A, Collier LS, Hubbard T, Adams DJ. *Novel candidate cancer genes identified by a large-scale cross-species comparative oncogenomics approach. Cancer Res. 2010;70:883-95*

De Vries NA, Bruggeman SW, Hulsman D, de Vries HI, Zevenhoven J, Buckle T, Hamans BC, Leenders WP, Beijnen JH, van Lohuizen M, Berns A, van Tellingen O. *Rapid and robust transgenic high-grade glioma mouse models for therapy-intervention studies. Clin Cancer Re*s. *2010;16:3431-41*

Peric-Hupkes D, Meuleman W, Pagie L, Bruggeman SW, Solovei I, Brugman W, Gräf S, Flicek P, Kerkhoven RM, van Lohuizen M, Reinders M, Wessels L, van Steensel B. *Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. Mol Cell. 2010;28:603-613*

transplantation model, we revealed the essential role of Bmi1 in mammary epithelial stem cells and precursors and ductal tree development (figure 1). Genetic studies showed that the proliferative defects but not the observed premature differentiation upon loss of Bmi1 in mammary epithelial precursors is in part mediated via INK4a/ARF. Importantly, these studies revealed a dual role for Bmi1/Pc-G: controlling both proliferation and differentiation. A key characteristic of cancer cells is their unlimited self-renewal. In this respect, cancer cells resemble stem cells, and accumulating evidence suggests that many forms of cancer may indeed contain cells carrying stem cell markers. In studying the proliferation defects in Bmi1 deficient mice we discovered that Bmi1 is required for proliferation and self-renewal of neural stem cells. Importantly, loss of the INK4a/ARF locus rescues the proliferation & renewal defects, indicating it also is a critical Pc-G target in neural stem cells. Using a transplantable Glioma model we demonstrated a critical role for Bmi1 in brain tumor maintenance (figure 2). Interestingly, Bmi1 acts in this tumor setting in an Ink4a/ARF-independent manner on cell adhesion and migration. These results, together with the recently established role of Bmi1 in hemapoietic stem cells and leukaemic stem cells, suggest a common conserved role for Bmi1-containing Polycomb complexes in maintenance and expansion of stem cells or committed progenitors and in the pathogenesis of tumors originating from the neoplastic transformation of these cells. The possible broader relevance of these findings for human cancer is further underscored by the amplification of BMI1 in Mantle cell lymphomas and a subset of brain cancers and the overexpression of BMI1 in various tumor types including non-small cell lung cancer, breast cancer, prostate cancer and liver cancer. Conditional transgenic- and knockout models are currently used to investigate the role of Pc-G genes in various tissue stem/progenitors and in solid cancers that develop in these tissues.

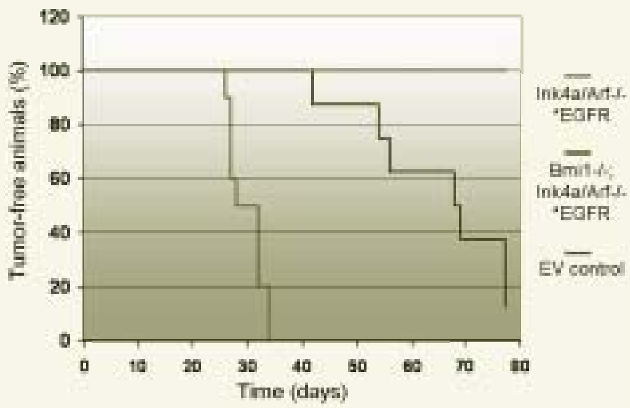

Figure 2: Severely reduced Glioma formation of Bmi1-/- transformed astrocytes. Survival curves indicate that astrocytes oncogenically transformed by loss of INK4a/ARF and activation of EGF-receptor signaling rapidly form agressive gliomas whereas tumor formation is delayed upon transplantation of Bmi1-deficient transformed astrocytes orthotopically transplanted in the forebrain of recipient mice.

In vivo genetic screens to identify new groups of collaborating oncogenes or tumor suppressors In close collaboration with A Berns (this Division), J Jonkers (Division of Molecular Biology) and D Adams and A Bradley/The Sanger Centre, Hinxton, UK, we have developed high-throughput insertional mutagenesis techniques and are now extending and optimizing these types of screens to other cancer relevant models such as breast cancer. The power of this approach as a cancer gene discovery platform is highlighted by the first completed screens in hemapoietic tumors induced in wild type, p53 or p19Arf deficient mice. We recently extended these screens to p15Ink4b, p21 and p27 defcient mice and to Pten-deficient mice prone to MMTV-induced mammary tumorigenesis.These screens yielded over 10.000 insertion sites implicating over 300 loci in tumorigenesis and uncovered new pathway-specific oncogenes and candidate tumor-suppressors. Cross species comparative analysis with a large array-CGH dataset of human cancer cell lines revealed both new and novel candidate oncogenes and tumor-suppressor genes. The role and mechanism of action of several of these new putative oncogenes or tumor suppressors, is under investigation hemapoietic- and mammary fat pad cell-transplantation systems.