

DIVISION OF GENE REGULATION

MICRORNAS AND RNA BINDING PROTEINS IN CANCER

Our main research objective is to understand the cancerous process in humans and identify essential cancerous genes. The knowledge we gather will allow us to design novel therapeutic approaches. Most human tumors harbor multiple genetic alterations that activate oncogenes, inhibit tumor suppressors and induce genomic instability. As each tumor contains many genetic alterations, the study of the contribution of each alteration to the cancerous phenotype was obscured. In the past, we developed and successfully used an RNA interference (RNAi) approach to inactivate genes in mammalian cells. We used this RNAi system to characterize tumor suppressors, such as the bromodomain containing protein 7 (BRD7) described below.

In the past we initiated studies to identify cancerous microRNAs (miRNAs), a newly emerging gene family encoding for endogenous small RNAs. We developed novel and unique genetic approaches to screen for cancer-causing and cancer-preventing miRNAs. With these tools we discovered and characterized the role of the miR-372 family in tumor growth and metastasis as well as the oncogenic role of miR-221 in glioblastoma.

Interestingly, we noticed that the regions surrounding many functional miRNA targets (identified by our genetic screens, for example) are highly conserved throughout evolution. We postulated that these regions recruit RNA-binding proteins (RBPs) that regulate miRNA function. We performed genetic screens and identified RBPs that can inhibit or potentiate the activity of miRNAs to their target mRNAs. We propose that the genetic interaction between miRNAs and RBPs determines developmental processes and cellular proliferation. How this affects cancer initiation and development is under investigation now.

Functional genetic approaches identify novel tumor suppressor genes

Oncogene-induced senescence is a p53-dependent defense mechanism against uncontrolled proliferation. Consequently, many human tumors harbor p53 mutations and others show a dysfunctional p53 pathway, frequently by unknown mechanisms. Using functional genetic screens with RNAi libraries we identified BRD7 (bromodomain-containing 7) as a protein whose inhibition allows full neoplastic transformation in the presence of wild-type p53 (figure 1). In human breast tumors harboring wild-type, but not mutant, p53 the BRD7 gene locus was frequently deleted and low BRD7 expression was found in a subgroup of tumors (1A&B). Functionally, BRD7 is required for efficient p53-mediated transcription of a subset of target genes (1C). BRD7 interacts with p53 and p300 and is recruited to target gene promoters, affecting histone acetylation, p53 acetylation and promoter activity. Thus, BRD7 suppresses tumorigenicity by serving as a p53 cofactor required for the efficient induction of p53-dependent oncogene-induced senescence.

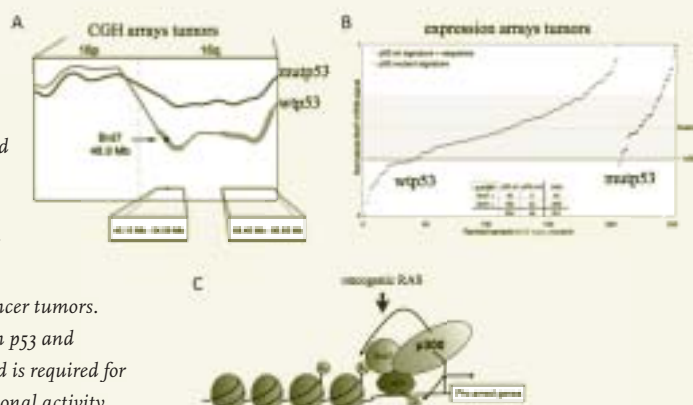


Figure 1:

A. BRD7 gene is deleted in many wild type p53 breast cancer tumors.

B. Expression of BRD7 is significantly low in wild type p53 breast cancer tumors.

C. BRD7 interacts with p53 and acetylated histones, and is required for efficient p53 transcriptional activity.



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Publications

Agami R. *MicroRNAs, RNA binding proteins and cancer.* *Eur J Clin Invest* 2010;40: 370-374

Drost J, Mantovani F, Tocco F, Elkon R, Cornel A, Holstege H, Kerkhoven R, Jonkers J, Voorhoeve PM, Agami R, et al. *BRD7 is a candidate tumour suppressor gene required for p53 function.* 2010. *Nat Cell Biol* 2010;12:380-389

Elkon R, Zlotorynski E, Zeller KI and Agami R. *Major role for mRNA stability in shaping the kinetics of gene induction.* 2010. *BMC Genomics* 2010;11:259

Kedde M, van Kouwenhove M, Zwart W, Oude Vrielink JA, Elkon R, and Agami R. *A Pumilio-induced RNA structure switch in p27-3' UTR controls miR-221 and miR-222 accessibility.* *Nat Cell Biol* 2010;12:1014-1020

Mann M, Barad O, Agami R, Geiger B, and Hornstein E. *miRNA-based mechanism for the commitment of multipotent progenitors to a single cellular fate.* *Proc Natl Acad Sci U S A.* 2010;107:15804-15809

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Mantovani F, Drost J, Voorhoeve PM, Del Sal G, and Agami R. *Gene regulation and tumor suppression by the bromodomain-containing protein BRD7. Cell Cycle* 2010;9:2777-2781

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Interplay between RNA-binding proteins and miRNAs regulates gene expression microRNAs (miRNAs) interact with 3'-Untranslated regions (3'UTRs) of messenger RNAs (mRNAs) to control the expression of a large proportion of the protein coding genome during normal development and cancer. RNA-binding proteins (RBPs) potentially control the biogenesis, stability, and activity of miRNAs (figure 2A). In recent years we have demonstrated that miRNA accessibility to target mRNAs can be controlled by RBPs. We provided first proof of principle to this phenomenon with an RNA binding protein called DND1, a gene required for proper differentiation and survival of primordial germ cells. In addition, we generated genome-wide tools for large-scale screen analysis to identify RBPs whose expression controls miRNA accessibility to target mRNAs. This has led us to the identification of RBPs required cell cycle control, as well as for optimal p53 function (figure 2B).

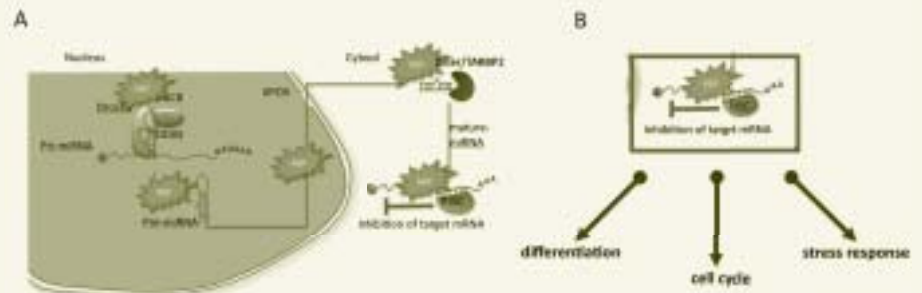


Figure 2: A. Interplay between RNA-binding proteins (RBP) and miRNA biogenesis and activity. B. Binding of RNA-binding proteins (RBP) to target mRNAs influences miRNA activity. This affects cellular processes such as differentiation, cell cycle and stress response.

A Pumilio-induced RNA structure switch in p27-3' UTR controls miR-221 and miR-222 accessibility

Key regulators of 3' untranslated regions (3' UTRs) are microRNAs and RNA-binding proteins (RBPs). The p27 tumor suppressor is highly expressed in quiescent cells, and its downregulation is required for cell cycle entry after growth factor stimulation. Intriguingly, p27 accumulates in quiescent cells despite high levels of its inhibitors miR-221 and miR-222. Here we show that miR-221 and miR-222 are underactive towards p27-3' UTR in quiescent cells, as a result of target site hindrance. Pumilio-1 (PUM1) is a ubiquitously expressed RBP that was shown to interact with p27-3' UTR. In response to growth factor stimulation, PUM1 is upregulated and phosphorylated for optimal induction of its RNA-binding activity towards the p27-3' UTR. PUM1 binding induces a local change in RNA structure that favours association with miR-221 and miR-222, efficient suppression of p27 expression, and rapid entry to the cell cycle. We have therefore uncovered a novel RBP-induced structural switch modulating microRNA-mediated gene expression regulation (figure 2).

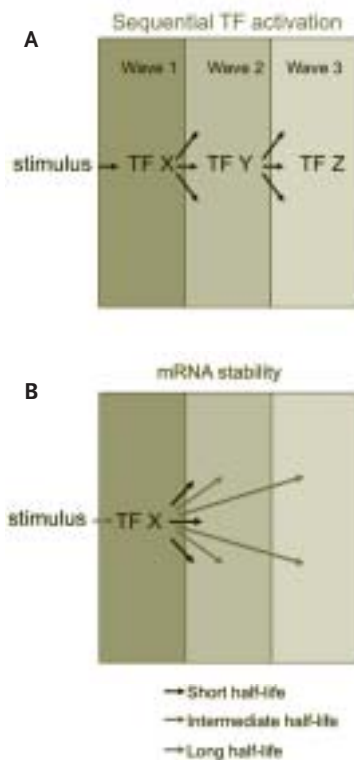


Figure 3: A. Kinetic induction created by sequential transcription activation. B. Kinetic induction created by differential stability of mRNA.

Major role for mRNA stability in shaping the kinetics of gene induction

mRNA levels in cells are determined by the relative rates of RNA production and degradation. Yet, to date, most analyses of gene expression profiles were focused on mechanisms which regulate transcription, while the role of mRNA stability in modulating transcriptional networks was to a large extent overlooked (figure 3A). In particular, kinetic waves in transcriptional responses are usually interpreted as resulting from sequential activation of transcription factors. We therefore examined on a global scale the role of mRNA stability in shaping the kinetics of gene response (figure 3B). Analyzing numerous expression datasets we revealed a striking global anti-correlation between rapidity of induction and mRNA stability, fitting the prediction of a kinetic mathematical model. In contrast, the relationship between kinetics and stability was less significant when gene suppression was analyzed. Frequently, mRNAs that are stable under standard conditions were very rapidly down-regulated following stimulation. Such effect cannot be explained even by a complete shut-off of transcription, and therefore indicates intense modulation of RNA stability. Taken together, our results demonstrate the key role of mRNA stability in determining induction kinetics in mammalian transcriptional networks.