Treatment of lytic MDA-MB-231 breast cancer bone metastasis in rats with antisense oligonucleotides directed against the SIBLING proteins osteopontin and bone sialoprotein.

Martin R. Berger, Hassan Adwan, Tobias Bäuerle, Youssef Najajreh, Victoria Elazer Gershon Golomb

Breast cancer is often associated with lytic skeletal metastasis. The SIBLING proteins osteopontin (OPN) and bone sialoprotein II (BSPII) are secreted by breast cancer cells and this presumably is related to their specific homing into osseous tissue. To antagonize their effects, specific antisense oligonucleotides (ASOs) were designed against OPN and BSPII and found active in decreasing the expression of the two proteins by Western blot. For testing their potential activity in vivo, lytic metastases were induced in nude rats by injecting 100,000 MDA-MB231 human breast cancer cells into the secondary muscular branch of a femoral artery. Control rats developed lytic metastases after 4 weeks in the respective femur and tibia. Their size was monitored by X-rays. Treatment of these lesions started on the day of tumor cell transplantation by either implanting subcutaneously osmotic mini-pumps, or injecting locoregionally nanoparticles, loaded with ASO, respectively. The pumps were filled with 10 mg of a nonsense oligonucleotide (NSO), or a specific ASO (ASO-OPN-4 or ASO-BSPII-6). The daily dosage delivered by these pumps (3 mg/kg) was extended to 4 weeks by replacing a pump once after two weeks in accordance with their exhaustion rate. The nanoparticles consisting of biologically degradable poly(DL-lactide-co-glycolide) polymer exhibited sustained release over a month; 100 mg were administered once, the corresponding ASO dose was 600 µg.

Treatment results were observed in groups of 4-5 rats, respectively, for up to 12 weeks. Administration of the NSO by osmotic mini-pumps did neither inhibit the occurrence of lytic metastases nor impede their increase in size as the mean lesion size increased 13fold from 33 relative units (R.U., week 4) to 434 R.U. (week 12). Treatment with ASO-OPN-4 reduced the initial lesion size (week 4: 19 R.U; 58 T/C %) as well as the subsequent increase in lesion size. The final size (week 12: 112 R.U) corresponded to an inhibition of 74% compared to NSO-treated rats. Treatment with ASO-BSPII-6 was even more effective as shown by the initial (week 4: 15 R.U; 45 T/C %), and final mean lesion sizes (week 12: 9 R.U; 2 T/C %). Administration of ASO-OPN-4 inhibited the appearance of lytic lesions in 1/5 rats (20%). ASO-BSPII-6 had the same initial effect but caused disappearance of lesions in two further rats, thus effecting complete remissions in 3 of 5 rats (60%).

The nanoparticles (NP) used were characterized in morphology and loading efficiency. Their size varied between 193±81 nm (blank NP) and 233±70 (NPs loaded with NSO, ASO-BSPII-6 and ASO-OPN-4) and their loading efficiency was 49±13% for NSO, 51± 9 for OPN-4 and 55±14% for BSPII.

Administration of nanoparticles loaded with NSO was related to a 5fold increase in mean lesion size from 16 R.U. (week 4) to 85 R.U. (week 12). When this vehicle was loaded with ASO-BSPII-6, the initial (week 4: 9 R.U.) and final mean lesion sizes (week 12: 12 R.U., 14 T/C%) were reduced. This outcome was surpassed by ASO-OPN-4 as shown by initial (week 4: 7 R.U.) and final mean lesion sizes (week 12: 5 R.U., 6 T/C %).

In conclusion, both ASOs are potent means for inhibiting lytic skeletal metastases in a rat model, with ASO-BSPII-6 being more effective following systemic administration and ASO-OPN-4 following locoregional administration.

Constitutive SOCS3 expression confers a growth advantage to a human melanoma cell line

Waraporn Komyod[1], Peter C. Heinrich[1], and Iris Behrmann[2]
[1]Institut für Biochemie, Universitätsklinikum der Rheinisch-Westfälischen Technischen Hochschule Aachen, Pauwelsstr. 30, 52074 Aachen, Germany
[2] Laboratoire de Biologie et Physiologie Intégrée, Université du Luxembourg, 162A, av. de la Faïencerie, 1511 Luxembourg, Luxemburg

Growth of melanocytes and many early stage melanoma cells can be inhibited by cytokines whereas late stage melanoma cells have been reported often to be "multi-cytokine resistant". We have shown previously that the transcription factor STAT3 plays a crucial role for the growth arrest of melanoma cells mediated by interleukin (IL)-6-type cytokines. We analysed a melanoma cell line resistant towards the growth-inhibitory effects of IL-6 and oncostatin M (OSM) to better understand the mechanisms leading to cytokine resistance. In spite of expressing receptors (gp130, OSMR) at the cell surface, cytokine stimulation led to little activation of Janus kinase 1, STAT3 and STAT1. We noticed a high-level constitutive expression of SOCS3 (a STAT-inducible feedback inhibitor of Jak/STAT-signalling) that did not further increase after stimulation. Constitutive SOCS3 expression was neither affected by inhibitors of the p38 and Erk MAP kinase pathways nor by presence of dominant negative STAT3. Importantly, upon suppression of SOCS3 by RNAi, cells became more susceptible towards OSM and IL-6: they showed an increased STAT3 phosphorylation and a dramatically increased STAT1 phosphorylation, reminiscent of the Interferon-gamma-like response elicited by IL-6 in $SOCS3^{-/-}$-cells. Moreover, suppression of SOCS3 made the cells sensitive to the anti-proliferative action of IL-6 and OSM, and even control cells not stimulated with cytokine grew slower. Thus, SOCS3 expression apparently confers a growth advantage to this cell line. However, constitutive expression of SOCS3 protein does not seem to be a general phenomenon for melanoma although SOCS3 mRNA, albeit at lower levels than in the cell line mentioned above, could be detected in all 15 cell lines examined. There was no global difference between melanoma cells and normal melanocytes. Thus, the melanoma situation seems to differ from other reported malignancies (lung cancer, breast cancer, mesothelioma, hepatocellular carcinoma, squamous cell carcinoma of the head and neck) characterized by methylation silencing of the SOCS3 gene.

Breast Cancer Risk is Associated with Polymorphisms of *ERCC2, CYP1B1, and HSD17B1* as well as Longterm Hormone Replacement Use

C Justenhoven, U Hamann, B Pesch, S Rabstein, V Harth, C Baisch, T Brüning, C Vollmert, T Illig, Y Ko, H Brauch

According to a current concept the majority of breast cancers develop as a consequence of multiple heritable, polygenic factors as well as endogenous and exogenous exposures. In support of the complex disease hypothesis numerous breast cancer risk associations have been suggested which on the level of environmental exposures include diet as well as use of hormone replacement therapy (HRT), and on the constitutional level genetic polymorphisms of hormone/drug/xenobiotic metabolizing and DNA repair enzymes. So far, the contribution of individual breast cancer risks to the overall risk remains equivocal. A major challenge in current and future breast cancer risk association studies will be to assess the value of such findings within the scope of known and/or predicted concerted biological action. Supportive evidence may come from sufficiently powered studies for the assessment of environmental and genetic risks to be identified in candidate genes of potentially disease relevant pathways.
We investigated whether genetic polymorphisms of enzymes involved in DNA repair and the endocrine pathway are associated with the risk to develop breast cancer. Study participants of the German GENICA population-based breast cancer case-control collection (688 patients, 724 controls) were genotyped at 30 loci of 20 genes to investigate a possible association with breast cancer risk. On the level of single polymorphisms we observed significant risk associations for *ERCC2*_6540_GG (OR 2.06, 95% CI: 1.39-3.07), *CYP1B1*_1358_GG (OR 2.43, 95% CI: 1.27-4.63), and *HSD17B1*_937_GG (OR 0.72, 95% CI: 0.52-0.99). The combination of two polymorphisms increased the risk to 3.7-fold in carriers of the combined genotype *ERCC2*_6540_GG / *ERCC2*_18880_CC. The latter pointed to the *ERCC2*_6540_G / *ERCC2*_18880_C as the at risk haplotype (OR 3.49, 95% CI: 2.30-5.28). Furthermore we observed an increased risk to develop breast cancer in women using HRT for ten or more years (OR 1.79, 95% CI: 1.12-2.87).
Our data showed that various factors contribute to breast cancer risk in the German population. The strongest risk was associated with ERCC2, a DNA repair enzyme involved in nucleotide excision repair and apoptosis. Moderate risks were associated with enzymes of the estrogen metabolism, i.e. *HSD17B1* involved in the conversion of estrone to estradiol, and CYP1B1 involved in the hydroxylation of estradiol, predominantly to 4-hydroxyestrogen. These effects point to a role of elevated estrogen levels in the risk for breast cancer. These findings on the molecular level are in line with our observation of longterm HRT being a breast cancer risk in postmenopausal women. Altogether, our findings contribute to the understanding of the polygenic concept of the development of breast cancer. Whether there are additional risk factors and how genetic as well as epidemiological factors may interact within the context of breast cancer susceptibility is subject of ongoing investigations.

Molecular pathogenesis of human hepatocellular carcinoma: mechanistic and prognostic significance of aberrant methylation

Diego F. Calvisi
Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD and Department of Biomedical Sciences, Section of Experimental Pathology and Oncology Section, University of Sassari, Italy

Aberrant methylation is implicated in the development of various tumors, including hepatocellular carcinoma (HCC). We show here that genome-wide hypomethylation and CpG hypermethylation correlate with biological features and clinical outcome of human HCC. Both parameters reached the highest levels in HCCs from patients with shorter survival length, paralleled by increasing genomic instability and upregulation of DNA Methyltransferases (DNMTs) 1,3A and 3b. A progressive rise in Interleukin-6 levels was responsible for upregulation of DNMT1, but not DNMT3s, in surrounding non-neoplastic livers and HCCs. Analysis of 106 putative tumor suppressor gene promoters identified the genes both universally and specifically inactivated in distinct subgroups of HCCs based on the length of patient's survival. Activation of Ras, Jak/Stat, and Wnt pathways due to epigenetic silencing of Ras, Jak/Stat, and Wnt inhibitors was detected in the majority of HCCs, regardless of clinicopathological features of HCC patients. Furthermore, selective inactivation of genes controlling EGFR signaling and angiogenesis (SOCS4, 5, EGLN2, BNIP3) was associated with an unfavorable outcome. Taken together, our results assign a therapeutic significance to methylation patterns in human HCC. Therapeutic approaches aimed at modifying the methylation status and utilizing novel molecular targets identified in this study may effectively inhibit HCC development and progression.

Glycogen Synthase Kinase 3beta (GSK3beta) as a key component of estradiol signalling pathway?

Grisouard Jean,
Medunjanin Senad, Strauth Stephanie, Hermani Alexander and Mayer Doris

German Cancer Research Centre (DKFZ), Research Group Hormones and Signal Transduction, Heidelberg, Germany

The involvement of GSK3 in the glycogen metabolism was first discovered 20 years ago. This enzyme was then described as a regulator of a large number of other cell functions. For example, GSK3 plays a role in insulin and growth factors signalling, in cell motility, cell proliferation and cell survival [1]. Interestingly, this kinase was also involved in the control of gene expression via the regulation of transcription factors, including estrogen receptor alpha (ERalpha). Furthermore, GSK3 is involved in estrogen-independent activation of ERalpha [2]. Indeed, the regulation of estrogen signalling is under the influence of its receptor phosphorylation. Therefore, kinases such as GSK3 and more specifically GSK3beta may have a strong impact on the regulation of ERalpha function and activity.

Some results obtained in our lab described the involvement of GSK3beta in estrogen-dependent activation of ERalpha [3]. We suggest a novel mechanism for the regulation of ERalpha-mediated estrogen signalling controlled by a dual action of GSK3beta. More precisely, we demonstrated that GSK3beta phosphorylates ERalpha in the AF-1 domain of the receptor at two serine residues motifs. Under resting conditions, GSK3beta phosphorylated ERalpha at Ser-102, -104, and 106 motif and stabilised the inactive receptor within the cytoplasm. Upon E2 stimulation, GSK3beta was phosphorylated via the PI3K/Akt/PKB pathway resulting in inactivation of the enzyme in the cytoplasm. This allowed ERalpha release and translocation into the nucleus, where ERalpha was phosphorylated at Ser-118 residue motif. The aim of my work was to clarify the role of GSK3beta regarding Ser-118 phosphorylation of the ERalpha within the nucleus. The effects of this phosphorylation on estrogen-dependent modulation of ERalpha transcriptional activity were also studied.

In vitro kinase assays first depicted that GSK3beta phosphorylated ERalpha at Ser-118. Then, the addition of a GSK3 inhibitor (LiCl) on MCF-7 cells in culture stimulated with E2 decreased Ser-118 phosphorylation and inhibited ERalpha-controlled luciferase activity. Further experiments using the siRNA technology and transfection of cells with GSK3beta dominant negative mutant may help to better understand the effects of GSK3beta regarding ERalpha signalling pathway.

[1] Frame S and Cohen P (2001) *Biochem. J.*, **359**: 1-16.
[2] De Servi B., A. Hermani, S. Medunjanin and D. Mayer. (2005) *Oncogene*, **24**: 4946-55.
[3] Medunjanin S., A. Hermani, B. De Servi, J. Grisouard, G. Rincke and D. Mayer. (2005) *J. Biol. Chem.*, **280**: 33006-14.

The Calcium Binding Proteins S100A8 and S100A9 as Novel Markers for Human Prostate Cancer

Alexander Hermani*, Rainer Grobholz#, Lutz Trojan‡, Peter Angel§, Doris Mayer*

German Cancer Research Centre, *Research Group Hormones and Signal Transduction, §Division of Signal Transduction and Growth Control, Heidelberg, Germany, University Hospital Mannheim, #Department of Pathology, ‡Department of Urology, Mannheim, Germany

Abstract

S100 proteins comprise a family of calcium-modulated proteins that have recently been associated with epithelial tumours. We examined the expression of two members of this family, S100A8 and S100A9, together with the S100 receptor RAGE in human prostate adenocarcinomas using histochemical staining procedures. S100A8, S100A9, and RAGE were up-regulated in prostatic intraepithelial neoplasia and preferentially in high-grade adenocarcinomas, whereas benign tissue was negative or showed weak expression of the proteins. The analysis of S100A9 in patient serum additionally revealed significantly elevated S100A9 serum levels in cancer patients compared to BPH (benign prostatic hyperplasia) patients or healthy individuals [1].
In cell culture experiments S100A8 and S100A9 were identified as extracellular factors which are able to induce MAP kinase and NF-κB signalling pathways and to stimulate the migration of prostate cells [2]. Thus, S100A8 and S100A9 are linked to the activation of important features of prostate cancer cells. Furthermore, S100A8 and S100A9 may represent novel players in prostate cancer development or progression, which may prove useful for future diagnostic and/or therapeutic approaches.

[1] Hermani et al. (2005) Clin. Cancer Res. 11, 5146-52
[2] Hermani et al. (2006) Exp. Cell Res. 312, 184-97

Allelic silencing at 13q14.3: a novel oncogenic mechanism

Daniel Mertens[1], Stephan Wolf[1], Cora Mund[2], Dirk Kienle [3], Sibylle Ohl[1], Petra Schroeter[1], Frank Lyko[2], Hartmut Döhner[3], Stephan Stilgenbauer[3], Peter Lichter[1]

1) Department of Molecular Genetics and 2) Department of Epigenetics, DKFZ, Heidelberg, Germany; 3) Department of Internal Medicine III, University of Ulm, Germany

INTRODUCTION: Genomic material from chromosomal band 13q14.3 distal to RB1 is recurrently lost in a variety of human neoplasms. Lack of point mutations in candidate tumor suppressor genes and downregulation of these genes in tumors indicate an epigenetic pathomechanism localized in the critical region.
AIM: Characterization of the epigenetic tumor suppressor mechanism localized in 13q14.3.
METHODS: Candidate tumor suppressor genes are down regulated by more than a factor of two in tumors with loss of one copy of the critical region. In addition, the presence of large non-coding RNA genes in 13q14.3 is reminiscent of imprinted regions where only one gene copy is active. Therefore we tested candidate tumor suppressor genes for monoallelic expression in healthy probands using single nucleotide polymorphisms and sequencing of RT-PCR products. Genotyping parents of these probands allowed allocation of the parental origin of either gene copy. In addition, we performed FISH experiments to measure replication timing of the two copies of the critical region to find out whether they are functionally different. As transcriptional activity and replication timing are effectuated by chromatin packaging, we used combined bisulfite-restriction (COBRA) analyses and bisulfite sequencing to assess DNA methylation of the critical region. Treatment of cultured cells with inhibitors of DNA-methyltransferases and histone-deacetylases allowed functional correlation of chromatin modification with expression of candidate tumor suppressor genes localized in the critical region.
RESULTS: In line with an imprinting mechanism, we find that the two copies of the critical region replicate asynchronously, suggesting differential chromatin packaging of the two copies of 13q14.3. In addition, we could detect monoallelic silencing of genes localized in the critical region and expression of one gene copy only. However, expression originated from either the maternal or paternal copy, excluding an imprinting mechanism. DNA methylation analyses showed one of the CpG islands of the region to be methylated. Demethylation of DNA and histone hyperacetylation induced biallelic expression, while replication timing was not affected.
CONCLUSIONS: We propose that differential replication timing represents an early epigenetic mark that distinguishes the two copies of 13q14.3, resulting in differential chromatin packaging and monoallelic expression. This has profound effects for the tumor suppressor mechanism localized in 13q14.3: Deletion of the single active copy of the region at 13q14.3, which is detected in more than 50% of CLL tumors, or point mutations only in the active gene copies will suffice for complete loss of tumor suppressor function, as the remaining gene copies are epigentically silenced. Thus, we provide a model for the pathomechanism of 13q14.3 in CLL by the interaction of genetic lesions and epigenetic silencing.

Development of Array-based Assay for High-resolution DNA-Methylation Profiling of B-cell Chronic Lymphocytic Leukemia

E.A. Moskalyov[1,2], V. Beier[1], A.T. Eprintsev[2], I.A. Vorobjev[3], E.A. Nikitin[3], J.D. Hoheisel[1]
[1] Division of Functional Genome Analysis, Deutsches Krebsforschungszentrum, Heidelberg, Germany,
[2] Biology Faculty, Department of Biochemistry, Voronezh State University, Voronezh, Russia,
[3] Hematology Research Center of Russia, Moscow, Russia.

Background

Genomic DNA-methylation in mammals represents a normal molecular process of methyl-group addition to cytosine next to guanosine (CG-dinucleotide). DNA-methylation pattern of a given cell is supposed to dictate the realisation of genomic information. In cancer, considerable change of DNA-methylation patterns occurs, namely global hypomethylation and local hypermethylation, latter being involved in transcriptional silencing in various tumor types. B-cell chronic lymphocytic leukemia (B-CLL) represents a highly heterogeneous uncurable malignancy with no obvious genetic hallmark and high overall DNA-methylation degree. Analysis of DNA-methylation patterns is crucial for understanding underlying pathomechanisms of the malignacy. Besides, DNA-methylation patterns are considered to be promising molecular markers for early cancer diagnosis and prognosis. However, only individual CG-dinucleotides can be characterised by means of routinely used methods. Thus, high-resolution analysis of DNA-methylation is needed.

Purpose

Development of array-based approach for DNA-methylation profiling of B-CLL. Analysis of methylation patterns with respect to clinical data.

Materials and Methods

Genomic DNA from tumor samples is treated with sodium bisulfite, what leads to conversion of unmethylated cytosines to uracils, whereas methylated cytosines remain unconverted. After PCR-amplification of candidate regions, methylation patterns can be detected in the same way as single nucleotide polymorphisms (SNPs). Mixture of respective PCR-products of a tumor template is hybridised on *in situ* synthesised oligonucleotide microarray (*febit biotech*, Heidelberg, Germany). Discrimination occurs by means of detection of the difference in stability between full-match and mismatch binding, the former being a more stable structure. The proportion of intensities coming from full-match and mismatch oligonucleotides reflects the proportion of methylated DNA-targets relative to the total number of templates.

Results

33 genes are currently involved in the study, representing following functional categories: *apoptosis (DAPK1, BID, STK17A, STK17B, BAX, BCL2, BAD, TP53, TWIST2, RAD9, APAF1), proliferation (CDKN2B, BUB1, CDKN3, UBE2C, CENPE), cytokinesis (FSCN1, ZYX), cell signalling (SOCS1, ZAP70), Wnt-pathway (SFRP1, SFRP2, SFRP3, DKK1, DKK2, DKK3, PYGO1, PYGO2), adhesion (CDH1)*, as well as *CALCA, GRM7, TERT* and *BCL6*. In most cases, regions surrounding transcriptional start points are chosen for analysis. First preliminary data from B-CLL samples suggest the presence of hypermethylation in *APAF1, BAX, CALCA, CDH1, CDKN2B, DAPK1, DKK3, FSCN1, GRM7, PYGO1, SFRP1, SFRP2, SFRP3, ZYX*. DNA-hypermethylation of E-cadherin coding gene *CDH1* is confirmed with

bisulfite sequencing. Validation of microarray data as well as study of DNA methylation degree and frequency is in process.

This project is supported with a scholarship for young scientists from German Academic Exchange Service (DAAD).

The preliminary analysis of chromosome abnormality in breast phyllodes tumors by comparative genomic hybridization

Yun NIU [*] Shu-hua LV [*] Qing-jie LIU [**] Xiao-wei WANG [**] Yan CHEN [**] Xiu-min DING [*]
* Breast Cancer Research Key laboratory of National (Education Ministry), Tianjin Cancer Hospital and Institute, Tianjin Medical University, Tianjin, 300060, China.
** Chinese National Human Genome Center, Beijing, 100176, China.

【Abstract】

Objective To analyze the DNA sequence copy number change in breast phyllodes tumors (PTs) from the benign to the malignant and to explore the pathogenesis as well as to provide a molecular cytogenetic marker with clinical and pathological diagnosis. **Methods** Comparative genomic hybridization (CGH) was used to analyze chromosomal copy number changes in 9 cases with benign PTs or borderline PTs or malignant PTs. **Results** Average chromosome copy number changes detected in benign, borderline and malignant PTs were 8, 19, 25 regions, respectively. The genomic DNA changes（genomic gains, loss and amplification）were found and were analyzed in those tumors. The frequent changes were gain of 1q (7/9) , followed by gain of 1p （6/9） and 9q（6/9）and loss of 9q and 17q（6/9）. The study did not reveal any loss on 3p. **Conclusion** Cumulative alterations in chromosome play a role in the development and malignancy of breast phyllodes tumors. It is suggested to identify the significance of the involved genomic DNA gain and loss regions in PTs.

Key Words: Comparative genomic hybridization; Chromosome abnormality; Breast phyllodes tumors

---

E-mail: yunniu2000@126.com **Fax:** 0086-22-23359984 **Tel:** 0086-13821228780
Address: Breast Cancer Research Key laboratory of National (Education Ministry), Tianjin Cancer Hospital and Institute, Tianjin Medical University, HuanHuXi Rord, TiYuanBei, HeXi District, Tianjin, 300060,China

Functional Oncogenomics in Mantle Cell Lymphoma

Armin Pscherer[1], Julia Schliwka[1], Kathrin Wildenberger[1], Antoaneta Mincheva[1], Carsten Schwaenen[2], Hartmut Döhner[2], Stephan Stilgenbauer[2] and Peter Lichter[1]

[1] Department Molecular Genetics, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

[2] Department of Internal Medicine III, Medical School Ulm, Robert-Koch-Str. 8, 89081 Ulm, Germany

Abstract

A broad range of malignant diseases, such as mantle cell lymphoma (MCL), is associated with complex genomic alterations, demanding multi-modal functional testing of candidate genes. Therefore we have developed a bidirectional targeted transgenesis tool, which allows well-controlled modulation of individual gene activities within a cellular MCL system.

This versatile Cre/lox-based transgenesis system permits functional analysis of virtually any candidate gene: for tumor suppressor genes by complementation of respective genomic DNA or for oncogenes by inactivation via integrated shRNA coding plasmids. Complementation by genomic DNA ensures wild-type regulated gene expression whereas genomic integration of shRNA coding inserts by an advanced RNAi-strategy mediates specific knock-down of gene expression. Site-specific genomic integration of an unmodified BAC, which contains the *CDKN2A/B* genes absent in the MCL model system, restored CDKN2A/B expression resulting in the inhibition of cell proliferation. CCND1, strongly overexpressed in the model system, was downregulated via shRNA expression, again inhibiting proliferation. Notably, this site-specific shRNA-strategy circumvents interferon-response induced when using other RNAi gene knock-down methods.

Furthermore we use the presented transgenesis system to compensate MCL cells for deleted or downregulated miRNAs and additionally as a screening tool on a proteome level to experimentally identify target genes, which are regulated by a distinct miRNA.

(FASEB J. in press)