Effect of YB-1 on the Regulation of Micro RNA Expression in Drug-sensitive and Drug-resistant Gastric Carcinoma Cells

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Abstract. *The multifunctional Y-Box protein 1 (YB-1) exerts positive and negative regulatory effects on gene expression by different mechanisms. Since transcription can be controlled by micro RNAs (miRNAs), YB-1 could also cause effects on gene expression by regulation of cellular miRNAs. To test this hypothesis, a previously established and well-characterized cell model derived from drug-sensitive (EPG85-257P/tetR/YB-1) and multidrug-resistant (EPG85-257RDB/tetR/YB-1) gastric carcinoma cells, in which the expression of YB-1 can be inhibited by tetracycline-dependent triggering of the RNA interference (RNAi) pathway, was investigated concerning their miRNA expression profiles in the presence and absence of YB-1. Microarray hybridizations demonstrated that six miRNAs (miR-96*, miR-210, miR-503, miR-623, miR-1275, miR-1290) were up-regulated more than 1.5-fold in drug-sensitive cells following YB-1 inhibition, but no differences in miRNA expression could be detected in multidrug-resistant cells. Independent validation of these findings by quantitative real-time revesre transcriptase polymerase chain reaction did not confirm these effects. Likewise, an in silico analysis of potential regulatory effects of the miRNAs on their target genes did not support the potential miRNA regulatory effects of YB-1. In conclusion, the data provide evidence that YB-1 has no direct influence on global miRNA expression pattern in different variants of gastric carcinoma cells and, therewith, does not control gene expression by regulation of miRNAs.*

Y-Box protein 1 (YB-1) is a 42 kDa protein consisting of 322 amino acid residues. The YB-1-encoding gene consists of 8 exons spanning 400 kb and is localized on chromosome 1p34. YB-1 was first identified as a protein which interacts with the Y-box in the promoter region of human MHC class II complex genes (1). The Y-box is an inverted version of the classical CCAAT-box, well known as a component of many eukaryotic genes.

YB-1 is member of the family of cold shock domain (CSD) proteins. The CSD of YB-1 is 43% identical to that of bacteria, but has not yet been shown to be involved in cold-shock response. YB-1 has pleiotropic biological functions in the regulation of transcription, translation, DNA repair and in cellular response to various stress factors including radiation and drug treatment as well as viral infection (2, 3). It consists of three different domains: (i) the *N*-terminal domain (A/P domain) which varies in CSD proteins, (ii) the highly conserved CSD, and (iii) the *C*-terminal domain (CTD). The A/P domain is involved in the regulation of transcription (2). The CSD can bind to nucleic acids such as double-stranded (ds) and single-stranded (ss) DNA, RNA and oligonucleotides. The domain contains two conserved ribonucleoprotein particle (RNP) motifs which play a role in intracellular transport processes, as well as in the control of translation. The CTD consists of alternate acidic and alkaline amino acid residues (B/A repeats) which contribute to protein-protein interactions. B/A repeats are a common feature of many proteins which are translocalized between the cytoplasm and the nucleus following interaction with ribonucleoprotein (RNP) complexes (4). Furthermore, within the CTD, two regions were identified which are involved in the regulation of the subcellular distribution of YB-1: (i) the noncanonical nuclear localization signal (NLS), and (ii) the cytoplasmic retention site (CRS) (5).

YB-1 has been shown to be expressed in many malignancies, but whether or not YB-1 plays a causative role in the development of cancer is unknown at this time (3). In a transgenic mouse model, it was demonstrated that overexpression of YB-1 resulted in the development of invasive breast cancer (6). In specimens prepared from tumor tissue, it was demonstrated that YB-1 can be involved in the regulation of oncogenes, *e.g.* overexpression of HER2 in breast cancer (7), and other cancer-associated factors, such as the multidrug resistance (MDR)-associated drug extrusion pump MDR1/P-glycoprotein (MDR1/P-gp) (8). Furthermore, YB-1 was demonstrated to be an independent prognostic and predictive marker in these tumors (7, 9).

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Different ways have been proposed in which YB-1 may regulate gene expression, including induction of changes in DNA conformation (10), direct interaction with promoter sequences (11), or interaction with other transcription factors such as p53 (12) or AP-2 (13). Since in recent years, it has become clear that genes are not exclusively regulated by transcription factors but can also be regulated by micro RNAs (miRNAs) (14, 15), YB-1 may also cause gene regulatory effects *via* regulation of cellular miRNAs. To test this hypothesis, namely that YB-1 has a direct influence on the global expression signatures of miRNAs, a previously established and well-characterized cell model (16) in which the expression of YB-1 can be down-regulated by tetracycline-dependent triggering of the RNA interference (RNAi) pathway was investigated concerning the miRNA expression pattern in the presence and absence of YB-1.

Materials and Methods

Tetracycline-inducible anti-YB-1 shRNA-expressing cancer cell models and cell culture. Medium for human carcinoma cells was Leibovitz L-15 medium (Biowhittaker, Walkersville, MD) supplemented by 10% fetal calf serum (FCS) (GIBCO/BRL, Grand Island, NY), 1 mM L-glutamine, 6.25 mg/l fetuin, 80 IE/l insulin, 2.5 mg/ml transferrin, 0.5 g/l glucose, 1.1 g/l $NaHCO_3$, and 1% minimal essential vitamins. The cancer cells were cultivated in a humidified atmosphere of 5% CO_2 at 37˚C. Establishment and characterization of the tetracycline-inducible anti YB-1 shRNA expressing cell variants EPG85-257P/tetR/YB-1 and EPG85-257RDB/tetR/YB-1 was described in detail previously (16). The shRNA expressing cell lines were derived from the human gastric carcinoma cell line EPG85-257P (17) and its classical multidrug-resistant, MDR1/P-gp-positive derivative EPG85-257RDB which was established by *in vitro* exposure to daunorubicin (Daunoblastin®; Pfizer Pharma GmbH, Berlin, Germany) (18). These cell models are stably transfected with the tetracycline repressor encoding expression vector pcDNA6/TR (Invitrogen, Carlsbad, CA, USA) and the expression vector pTER (19) containing a modified RNA polymerase III-dependent H1-RNA promoter with a tetracycline operator region driving the expression of a biological highly active shRNA directed against the YB-1 encoding mRNA. In cells engineered to express the bacterial tetracycline repressor, anti-YB-1 shRNAs are not synthesized in the absence of tetracycline. For maintenance of tetracycline repressor expression and inducible anti YB-1 shRNA expression, the medium was supplemented with 10 μg/ml blasticidin (Invitrogen) and 200 μg/ml Zeocin (Invitrogen); 2 μg/ml tetracycline (doxycycline, Invitrogen) were used for induction of anti-YB1 shRNA expression.

Northern blot. For confirmation of shRNA-mediated down-regulation of the YB-1-specific mRNA, Northern blot analysis was performed by applying standard procedures as described previously (16). Blots were hybridized with 25 ng of a YB-1 or aldolase encoding cDNA fragment labeled with [^{32}P] dCTP by random primed labeling (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). As control for equal RNA loading, the membranes were stripped and re-hybridized with a fructose-bisphosphate aldolase-specific cDNA probe.

Western blot. For confirmation of shRNA-mediated down-regulation of YB-1 protein, Western blot analysis was performed as described previously (16). YB-1 was detected using polyclonal rabbit antibodies against YB-1 (Cell Signaling Technology, Inc., Danvers, MA, USA) at 1:1,000. As a loading control, mouse monoclonal antibody (mAb) directed against actin (mAb 1501R; Chemicon, Temecula, CA, USA) was used at 1:5,000. Primary antibody incubation of 2 hours was followed by incubation with peroxidase-conjugated mouse anti-rabbit IgG (1:10,000) (A-1949; Sigma, St. Louis, MO, USA) and the protein-antibody complexes were visualized by chemiluminescence developing reagents (ECL system; GE Healthcare Bio-Sciences Corp.). Each Western blot is representative of at least three independent experiments.

Global miRNA expression profiling. For global miRNA expression profiling, RNA was extracted from cells that were treated for 5 days with 2 μg/ml tetracycline, or not treated using miRNEASY kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. RNA quality and abundance were determined using a Bioanalyzer 2100 (Agilent Technologies Inc., St. Clara, USA). Two miRCURY LNA™ microRNA arrays (v. 9.2 and v. 11.0; Exiqon, Vedbaek Denmark,) were performed. The two microarrays performed differ in their experimental set up. For direct comparison array analysis treated and untreated samples of one cell line are hybridized to the same microarray slide and directly compared by normalizing the miRNA expression in the treated sample with the respective expression level in the untreated sample. The common reference microarray analysis was performed by hybridizing all samples to a common reference sample and analyzed by normalizing the miRNA expression level of each sample to the common reference and subsequently comparing the treated and untreated samples. Labeling was performed with the miRCURY™ LNA Array Power labeling kit (Exiqon) following the manufacturers' instructions. To quantify the signals, the arrays were scanned and image analysis was performed by Exiqon. Data were normalized by using spike-in controls. Hybridization signals were subjected to background correction using Normexp plus offset value 10, a convolution model described by Ritchie *et al*. (20). Obtained data were normalized by LOESS.

Isolation of small RNAs and quantitative real-time RT-PCR for detection of miRNAs. Quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) quantification of miRNA expression was performed on a Stratagene Mx3005P QPCR System (Agilent Technologies). RNA was extracted from cells following 5-day exposure to 2 μg/ml tetracycline using the miRVana miRNA Isolation Kit (Applied Biosystems, Foster City, CA, USA). RNA was quantified by UV spectroscopy and cDNA was synthesized from 500 ng RNA using the QuantiMir RT Kit (System Biosciences, CA, USA). Real-time PCR reaction mix included 15 μl RT^2 SYBR Green/ROX qPCR Master Mix (SABiosciences Corp., MD, USA), 1 μl gene-specific forward primer, 1 μl Universal Reverse Primer supplied with the Master Mix and 1 μl cDNA. Reactions were incubated in a 96-well optical plate at 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 s and 60˚C for 1 min. The threshold cycle (Ct) was determined using default threshold settings. All experiments were carried out in triplicates and repeated three times. RNU48 was used as an endogenous control to normalize miRNA expression. Data were then analyzed using the $2^{-\Delta\Delta C}$T method (21). Oligodeoxynucleotide primer sequences for miRNA qRT-PCR are shown in Table I.

Table I. *Oligodeoxynucleotide primers used for quantitative real time RT-PCR.*

miRNA	Oligodeoxynucleotide primer (forward)†
miR-96*	TTT GGC ACT AGC ACA TTT TTG CT
miR-210	CTG TGC GTG TGA CAG CGG CTG A
miR-503	TAG CAG CGG GAA CAG TTC TGC AG
miR-623	ATC CCT TGC AGG GGC TGT TGG GT
miR-1275	GTG GGG GAG AGG CTG TC
miR-1290	TGG ATT TTT GGA TCA GGG A
RNU48	TCT GAG TGT GTC GCT GAT GC

†An universal reverse oligodeoxynucleotide primer was used to quantify small RNA expression levels. Amplification product sizes ranged from 50 to 100 bp.

In silico analysis of microarray-predicted YB-1-regulated miRNAs. For assessment of potential biological consequences of the YB-1-dependent regulation of six miRNAs, two features of the miRNAs were analyzed *in silico*: (i) regulation of potential target genes, and (ii) potential association with signal transduction pathways.

Potential targets of the six miRNAs were screened for genes that have been identified by global mRNA profiling using microarrays (22) as differentially regulated following YB-1 knock-down in the same cell models. For miRNA target prediction, the MiRanda algorithm (version 3.0) (23) as supplied by miRBase (24) was used. Putative target genes were taken into consideration when they were more than 2-fold up- or down-regulated following YB-1 repression.

The predicted miRNA targets found to be up- or down-regulated by YB-1 according to the microarray mRNA expression studies were analyzed for their association with signal transduction pathways according to the Kyoto Encyclopedia of Genes and Genomes (KEEG) (http:www.genome.jp/kegg/). In particular the affiliation with the Jak/STAT-, MAPK-, TGF-β- and the Wnt-pathways was investigated since components of these pathways have been predicted by global mRNA expression analysis to be regulated by YB-1 (22).

Results

Confirmation of shRNA-mediated down-regulation of YB-1. Northern blot (Figure 1A) and Western blot (Figure 1B) experiments confirmed that tetracycline treatment of stable anti-YB-1 shRNA expression vector-transfected cell lines resulted in a down-regulation of YB-1-specific bands as observed previously (16).

Global miRNA expression profiling. The miRNA expression profiling analyses identified six different miRNAs to be more than 1.5-fold up-regulated in EPG85-257P/tetR/YB-1 cells in the absence of YB-1. Direct comparison microarray analysis demonstrated miR-96* (1.52-fold), miR-210 (1.81-fold), miR-503 (1.60-fold) and miR-623 (1.63-fold) as being up-regulated; common reference microarray analysis found miR-1275 (1.65-fold) and miR-1290 (1.87-fold) expression to be elevated. No miRNA was differentially regulated in EPG85-257RDB/tetR/YB-1 cells following YB-1 knock-down.

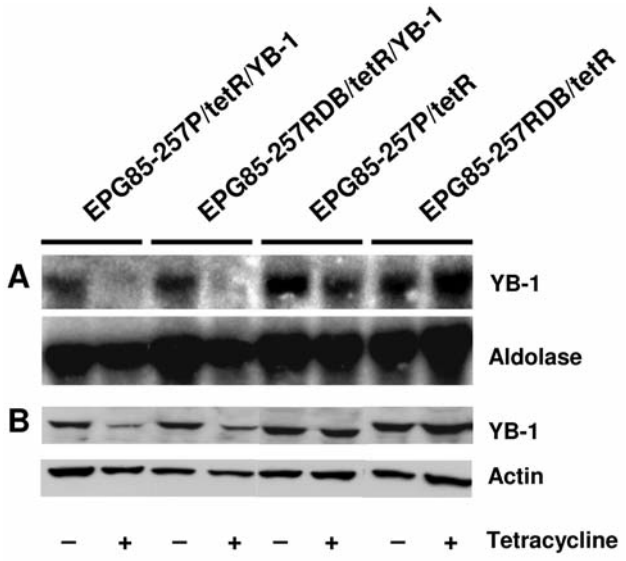

Figure 1. *ShRNA-mediated decrease of YB-1 mRNA and protein expression in human gastric carcinoma cells. A, Northern blot analysis depicting silencing of YB-1 mRNA expression following treatment with tetracycline. As loading control, the Northern blot membranes were probed using an aldolase-specific cDNA. B, Western blot analysis of YB-1 expression as result of tetracycline treatment in gastric carcinoma cells. As control for equivalent protein loading, the filter was incubated with a mouse mAb directed against actin. EPG85-257P/tetR, parental EPG85-257P cells expressing the tetracycline repressor; EPG85-257RDB/tetR, multidrug-resistant EPG85-257RDB cells expressing the tetracycline repressor; EPG85-257P/tetR/YB-1, EPG85-257P/tetR cells containing the anti-YB-1 shRNA-encoding tetracycline-dependent expression vector; EPG85-257RDB/tetR/YB-1, EPG85-257RDB/tetR cells containing the anti-YB-1 shRNA-encoding tetracycline-dependent expression vector.*

Verification of YB-1-dependent miRNA regulation. For independent validation of the global miRNA expression data obtained by microarray analyses, a quantitative real-time RT-PCR procedure was performed. For this approach, RNA isolated from EPG85-257P/tetR/YB-1, EPG85-257P/tetR, EPG85-257RDB/tetR/YB-1, and EPG85-257RDB/tetR cells grown in the presence or absence of tetracycline was isolated and analyzed in three independent experiments. The quantitative analysis demonstrates that none of the analyzed miRNAs showed statistically significant differences in expression levels following knock-down of YB-1. A representative example is displayed in Figure 2.

In silico analyses of microarray-predicted YB-1-regulated miRNAs. An analysis for identification of potentially differentially regulated target genes of the six microarray-identified miRNAs in a data set of global mRNA microarray analyses (22) demonstrated an YB-1-dependent effect on various genes. In EPG85-257P- and EPG85-257RDB-derived cell models, the expression level of most of the predicted

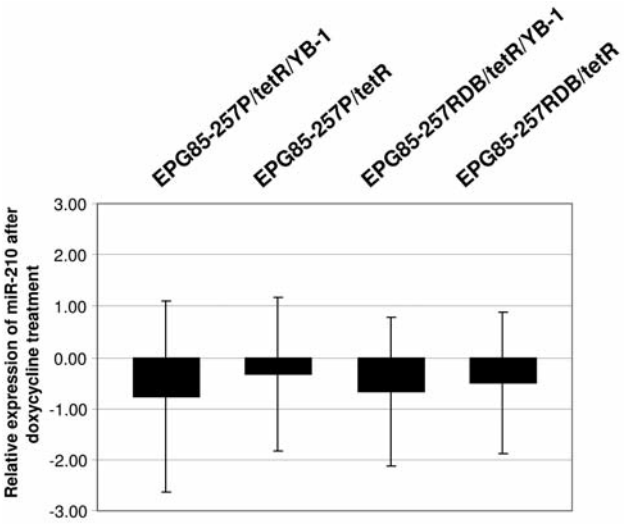

Figure 2. *Expression analysis of miR-210 by quantitative RT-PCR was performed with small RNA isolated from EPG85-257P/tetR/YB-1, EPG85-257RDB/tetR/YB-1, EPG85-257P/tetRandEPG85-257RDB/tetR cells in three independent experiments. The relative expression represents the fold change, which is the ratio of the miRNA expression level in the tetracycline-treated sample to the sample not treated. The mean differential expression was calculated for each cell line. Error bars represent SD.*

target genes decreased following YB-1 knock-down (Table II). In EPG85-257RDB/tetR/YB-1 cells, three members of the MAPK signaling pathway (*FGF18*, fibroblast growth factor 18; *TAOK2*, TAO kinase 2; *ELK1*, member of ETS oncogene family) and a single factor each in Jak/STAT (*IL11RA*, interleukin 11 receptor alpha) and Wnt (*CTBP1*, C-terminal binding protein 1) pathways were regulated by the miRNAs. No component of the TGF-β pathway was regulated. In EPG85-257P/tetR/YB-1 cells, no YB-1-dependent effects on any factor of these pathways were identified.

Discussion

YB-1 was described as being involved in the regulation of gene expression in mammalian cells by different mechanisms (10-13). As yet it has not been reported whether YB-1 also influences cellular gene expression by regulation of the expression of miRNAs. Accordingly, global miRNA expression profiles were analyzed in the presence and absence of YB-1. For this approach, two well-characterized cell models with tetracycline-dependent RNAi-mediated down-regulation of YB-1 were used (16). These cell lines, EPG85-257P/tetR/YB-1 and EPG85-257RDB/tetR/YB-1, were derived from the human gastric carcinoma cell line EPG85-257P (17) and its classical multidrug-resistant, MDR1/P-gp-containing subline EPG85-257RDB (18).

Table II. *Differentially regulated target genes of putative YB-1-regulated miRNAs.*

	Up-/down-regulated target genes	
miRNA	EPG85-257P/tetR/YB-1	EPG85-257RDB/tetR/YB-1
miR-96*	6/3	2/11
miR-210	1/8	4/14
miR-503	4/8	4/11
miR-623	7/9	2/12
miR-1275	1/0	3/1
miR-1290	3/6	1/7

Screening for differentially expressed miRNAs in the presence and absence of YB-1 was carried out by microarray technology which was proven to be sensitive and highly specific for simultaneous analysis of the expression of a large number of different miRNAs (25). To improve the methodology, two different strategies of array hybridization were performed using two different microarrays, the direct comparison array and the common reference microarray analysis. In the direct comparison array analysis pairs of samples are labelled with different fluorochromes and simultaneously hybridized to the same array. This design is useful when pairs of samples exist, as in this study analyzing a tetracycline-treated and untreated sample of a cell line. For common reference microarray analysis, all samples of the study are hybridized against a common reference which includes all miRNAs contained in the panel of samples. This design allows direct comparison between all samples and facilitates the separation of technical and biological variation. Although the same RNA samples were used for both miRNA expression analyses, the identified differentially expressed miRNAs differed completely.

Taken together, both microarray analyses identified six miRNAs to be up-regulated more than 1.5-fold in drug-sensitive EPG85-257P/tetR/YB-1 cells following YB-1 inhibition, but no differences in miRNA expression in multidrug-resistant EPG85-257RDB/tetR/YB-1 cells in the absence of YB-1. Independent validation of these findings by quantitative real-time RT-PCR did not confirm any putative YB-1-dependent miRNA regulation. Likewise, the *in silico* analysis of potential regulatory effects of the six miRNAs on their target genes did not support the assumed effect of YB-1 on miRNA regulation.

In conclusion, the data of this study provide evidence that YB-1 appears to have no direct influence on global miRNA expression pattern in different variants of gastric carcinoma cells and, therewith, does not control gene expression by regulation of miRNAs. YB-1 rather seems to regulate transcription either directly or *via* interaction with other cellular factors involved in gene regulation.

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References

1 Didier DK, Schiffenbauer J, Woulfe SL, Zacheis M and Schwartz BD: Characterization of the cDNA encoding a protein binding to the major histocompatibility complex class II Y box. Proc Natl Acad Sci USA *85*: 7322-7326, 1988.
2 Kohno K, Izumi H, Uchiumi T, Ashizuka M and Kuwano M: The pleiotropic functions of the Y-box-binding protein, YB-1. Bioessays *25*: 691-698, 2003.
3 Wu J, Startford AL, Astanehe A and Dunn SE: YB-1 is a transcrition/translation factor that orchestrates the oncogenome by hardwiring signal transduction to gene expression. Translat Oncogenom *2*: 49-65, 2007.
4 Ranjan M, Tafuri SR and Wolffe AP: Masking mRNA from translation in somatic cells. Genes Dev *7*: 1725-1736, 1993.
5 Bader AG, Kang S, Zhao L and Vogt PK: Oncogenic PI3K deregulates transcription and translation. Nat Rev Cancer *5*: 921-929, 2005.
6 Bergmann S, Royer-Pokora B, Fietze E, Jürchott K, Hildebrandt B, Trost D, Leenders F, Claude JC, Theuring F, Bargou R, Dietel M and Royer HD: YB-1 provokes breast cancer through the induction of chromosomal instability that emerges from mitotic failure and centrosome amplification. Cancer Res *65*: 4078-4087, 2005.
7 Fujii T, Kawahara A, Basaki Y, Hattori S, Nakashima K, Nakano K, Shirouzu K, Kohno K, Yanagawa T, Yamana H, Nishio K, Ono M, Kuwano M and Kage M: Expression of HER2 and estrogen receptor alpha depends upon nuclear localization of Y-box binding protein-1 in human breast cancers. Cancer Res *68*: 1504-1512, 2008.
8 Bargou RC, Jürchott K, Wagener C, Bergmann S, Metzner S, Bommert K, Mapara MY, Winzer KJ, Dietel M, Dörken B and Royer HD: Nuclear localization and increased levels of transcription factor YB-1 in primary human breast cancers are associated with intrinsic *MDR1* gene expression. Nat Med *3*: 447-450, 1997.
9 Janz M, Harbeck N, Dettmar P, Berger U, Schmidt A, Jürchott K, Schmitt M and Royer HD: Y-box factor YB-1 predicts drug resistance and patient outcome in breast cancer independent of clinically relevant tumor biologic factors HER2, uPA and PAI-1. Int J Cancer *97*: 278-282, 2002.
10 Norman JT, Lindahl GE, Shakib K, En-Nia A, Yilmaz E and Mertens PR: The Y-box binding protein YB-1 suppresses collagen alpha 1(I) gene transcription *via* an evolutionarily conserved regulatory element in the proximal promoter. J Biol Chem *276*: 29880-29890, 2001.
11 Mertens PR, Harendza S, Pollock AS and Lovett DH: Glomerular mesangial cell-specific transactivation of matrix metalloproteinase 2 transcription is mediated by YB-1. J Biol Chem *272*: 22905-22912, 1997.
12 Okamoto T, Izumi H, Imamura T, Takano H, Ise T, Uchiumi T, Kuwano M and Kohno K: Direct interaction of p53 with the Y-box binding protein, YB-1: a mechanism for regulation of human gene expression. Oncogene *19*: 6194-6202, 2000.
13 Mertens PR, Steinmann K, Alfonso-Jaume MA, En-Nia A, Sun Y and Lovett DH: Combinatorial interactions of p53, activating protein-2, and YB-1 with a single enhancer element regulate gelatinase A expression in neoplastic cells. J Biol Chem *277*: 24875-24882, 2002.
14 He L and Hannon GJ: MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet *5*: 522-531, 2004.
15 Bartel DP: MicroRNAs: target recognition and regulatory functions. Cell *136*: 215-233, 2009.
16 Kaszubiak A, Kupstat A, Müller U, Hausmann R, Holm PS and Lage H: Regulation of MDR1 gene expression in multidrug-resistant cancer cells is independent from YB-1. Biochem Biophys Res Commun *357*: 295-301, 2007.
17 Dietel M, Arps H, Lage H and Niendorf A: Membrane vesicle formation due to acquired mitoxantrone resistance in human gastric carcinoma cell line EPG85-257. Cancer Res *50*: 6100-6106, 1990.
18 Lage H: Molecular analysis of therapy resistance in gastric cancer. Dig Dis *21*: 326-338, 2003.
19 van de Wetering M, Oving I, Muncan V, Tjon Pon Fong M, Brantjes H, van Leenen D, Holstege FCP, Brummelkamp TR, Agami R and Clevers H: Specific inhibition of gene expression using a stably integrated, inducible small-interfering-RNA vector. EMBO Rep *4*: 609-615, 2003.
20 Ritchie ME, Silver J, Oshlack A, Holmes M, Diyagama D, Holloway A and Smyth GK: A comparison of background correction methods for two-colour microarrays. Bioinformatics *23*: 2700-2707, 2007.
21 Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods *25*: 402-408, 2001.
22 Kurucz R, Belian E, Treue D and Lage H: Regulation of mRNA expression in drug-sensitive and drug-resistant gastric carcinoma cells is independent of YB-1 expression. Anticancer Res *30*: 693-700, 2010.
23 Enright AJ, John B, Gaul U, Tuschl T, Sander C and Marks DS: MicroRNA targets in Drosophila. Genome Biol *5*: R1, 2003.
24 Griffiths-Jones S, Saini HK, van Dongen S and Enright AJ: miRBase: tools for microRNA genomics. Nucleic Acids Res *36*: D154-D158, 2008.
25 Babak T, Zhang W, Morris Q, Blencowe BJ and Hughes TR: Probing microRNAs with microarrays: tissue specificity and functional inference. RNA *10*: 1813-1819, 2004.

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