MicroRNA-205, a novel regulator of the anti-apoptotic protein Bcl2, is downregulated in prostate cancer

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Abstract. Decreased expression of the microRNA miR-205 has been observed in multiple tumour types due to its role in the epithelial to mesenchymal transition, which promotes metastasis. We determined the expression of miR-205 in 111 archival samples of prostate carcinoma and found it to be strongly reduced in most samples, with a median expression level of 16% in comparison to benign tissue from the same patient. Lower miR-205 expression correlated significantly with tumour size and miR-205 levels decreased with increasing Gleason score from 7a=3+4 to 8=4+4. In addition, we describe the anti-apoptotic protein BCL2 as a target of miR-205, relevant for prostate cancer due to its role in prognosis of primary tumours and in the appearance of androgen independence. The repression of BCL2 by miR-205 was confirmed using reporter assays and western blotting. BCL2 mRNA expression in the same collective of prostate cancer tissue samples was associated with higher Gleason score and extracapsular extension of the tumour (pT3). Consistent with its anti-apoptotic target BCL2, miR-205 promoted apoptosis in prostate cancer cells in response to DNA damage by cisplatin and doxorubicin in the prostate cancer cell lines PC3 and LnCap. MiR-205 also inhibited proliferation in these cell lines.

Introduction

The expression of miR-205, transcribed from the MIR205HG gene on chromosome 1q, is specific for epithelial tissues (reviewed in ref. 1). Loss of expression of this microRNA in cancer has been found to be involved in the epithelial to mesenchymal transition (EMT), a reversible series of molecular changes that lead to the conversion of polarised immobile epithelial cells to more motile mesenchymal cells. EMT is required for embryonic development, but also occurs in epithelial tumours, leading to metastasis. Other microRNAs that are repressed during the EMT are the members of the miR-200 family, namely miR-200a, miR-200b, miR-200c, miR-141 and miR-429. The decreased expression of these miRNAs indirectly downregulates E-cadherin expression through the increased expression of ZEB1 and ZEB2, which repress the CDH1 gene (2), reviewed in (1). MiR-205 has been found to be downregulated in different tumour types, including breast cancer (3), head and neck cancer (4) and invasive bladder cancer, in contrast to non-invasive tumours (5). Microarray based screens of prostate cancer tissue also indicated a consistent downregulation of miR-205 (6-8). Nevertheless, upregulation of miR-205 in cancer has also been observed, e.g., in endometrial cancer (9,10), esophageal cancer (11) and non-small cell lung cancer (12). In the tumour types where increased expression of miR-205 occurs, it appears not to decrease cell growth, but it does still inhibit the EMT through repression of ZEB2 (11) and inhibits metastasis (13).

Another confirmed target of this microRNA is the transcription factor E2F1 (14,15), which is involved in cell division and apoptosis. E2F1, in a negative feedback loop, appears to upregulate miR205 expression (16). In melanoma cell lines, miR-205 was also found to be positively regulated by full length p73 and repressed by the N-terminally truncated p73 isoform DNp73. Although p53 binds to the same sites as p73, it did not significantly regulate miR-205 in this system (16). In contrast, in breast cancer cell lines, a robust upregulation by p53 was seen. These authors did not test for the effects of p73, though (15). Positive regulation by DNp63, which also binds to the same sites at the promoter as p53 and p73, has also been described (17,18). In breast cancer it has recently been shown that overexpression of ErbB2 represses miR-205 leading to increased growth in the soft-agar assay (19), but whether this is a direct regulation remains elusive. Another possibility is silencing by methylation of the miR-205 promoter. This hypothesis is further supported by the fact that the CpG island located upstream of the first exon of miR205HG was found to be methylated in multiple prostate cancer cell lines (20) and

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5'Aza treatment was able to increase miR-205 expression in breast cancer cell lines (15).

In the present study, we investigated the downregulation of miR-205 in prostate cancer samples. The levels of miR-205 correlated significantly inversely with tumour size and decreased from Gleason score 7a=3+4 to 8=4+4. Additionally, we identified the anti-apoptotic gene BCL2 as a target of miR-205 in prostate cancer. We also correlated BCL2 expression to miR-205 levels in prostate cancer tissues. BCL2 is a potentially relevant miR-205 target specifically in prostate cancer, due to its known association to prognosis and disease progression (21,22).

Materials and methods

Patient collective. A series of 111 formalin-fixed paraffin-embedded (FFPE) prostatectomy specimens with primary prostate adenocarcinoma (PCa) from the Department of Pathology, Ruhr University Bochum, diagnosed between 2009 and 2011 were collected for the study. The composition of the cohort concerning Gleason grade, age, PSA levels and stage is shown in Table I. The PCa specimens were subjected to histological examination by an expert pathologist for confirmation of the Gleason grading, WHO classification of the tumour and staging according to the tumour-node-metastasis system. No patients had distant metastases at the time of diagnosis. The study was approved by the local Ethics Committee (Votum no. 3991-11).

Determination of miR-205 and BCL2 levels in patient samples. The histopathological tumour regions of interest were micro-dissected with the corresponding HE slide as a guide (tumour was marked with a pen on the HE slide) and used for RNA extraction using the miRNA FFPE kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

For the detection of miRNA levels, 500 ng total RNA was transcribed using the miScript reverse transcription kit (Qiagen); qRT-PCR was carried out with 25 ng cDNA per reaction using the miScript SYBR green PCR system (Qiagen) with the miR-205 primer (#MS00003780) on an Eppendorf Realplex4 cycler (Eppendorf, Hamburg, Germany). *RNU6B* (U6 small nuclear RNA 2, #MS00014000) was used for the normalization to total RNA (23). For the detection of *BCL2* in FFPE samples and cell culture, RNA was transcribed to cDNA using the High Capacity cDNA system (Life Technologies, Darmstadt, Germany). qRT-PCR was carried out with the TaqMan system (primer set #Hs99999018_m1) with 2X Universal Master Mix (Life Technologies). Template cDNA (25 ng) was used per PCR. 18S rRNA (#Hs99999901_s1) was used as internal control.

Cell culture. The prostate cancer cell lines PC3 and LnCap and the osteosarcoma cell line U2-OS were obtained from ATCC (Manassas, VA, USA). PC3 and LnCap cells were cultivated in RPMI-1640 (Pan Biotech, Aidenach, Germany) and U2-OS was cultivated in DMEM high glucose (Pan Biotech), both supplemented with 10% fetal calf serum (Hyclone-Thermo Scientific, Bonn, Germany), 100 U/ml penicillin and 100 μg/ml streptomycin (PAN Biotech); cisplatin and doxorubicin were obtained from Sigma-Aldrich (Taufkirchen, Germany).

Table I. Characteristics of the patient collective.

Cohort feature	Median	Range	N
Age (years)	63.5	47.00-75.5	111
Tumour size (cm^3)	2.30	0.16-18.00	111
PSA (ng/ml)	7.70	2.3-38	104
Tumour characteristics		**No. of samples (%)**	
Node-positive (N1) cases		6 (5.41)	
Distant metastasis (M1)		0 (0)	
Lymphatic vessel invasion (L1)		8 (7.21)	
Vascular invasion (V1)		1 (0.90)	
Perineural invasion (Pn1)		65 (78.38)	
Gleason sum			
3+3		14 (12.61)	
3+4		47 (42.34)	
4+3		37 (33.33)	
4+4		13 (11.71)	
Stage			
pT2a		10 (8.93)	
pT2b		1 (0.89)	
pT2c		72 (96.29)	
pT3a		14 (12.50)	
pT3b		13 (11.61)	
pT4		1 (0.89)	

Transfection of prostate cell lines with miRNA mimics. PC3 cells were seeded at $5x10^5$ per well and LnCap were seeded at $8x10^5$ cells per well in 6-well plates in their normal medium and transfected in suspension with 10 nM miRNA mimics (Qiagen) or AllStars negative control siRNA, labelled with AlexaFluor-647 (Qiagen), using 6 μl HiPerFect (Qiagen). The transfection efficiency was determined by measuring the fluorescence of the AllStars oligos by flow cytometry and was between 90 and 96%. Whenever the assay required, the cells were treated with cisplatin or doxorubicin 72 h after transfection.

Reporter assay. Fragments of the 3' untranslated region (UTR) of E2F1 (262 bp), E2F5 (389 bp) and BCL2 (438 bp) containing the potential miR-205 binding sites were cloned into pGL3 as 3'UTR of the firefly luciferase gene by PCR. U2-OS cells were transiently transfected in triplicates with 40 ng of the pGL3 construct, 10 ng of the expression vector for renilla luciferase pRL-CMV (Promega, Mannheim, Germany) and 10 nmol of miRNA mimics (Qiagen), using the transfection reagent Attractene according to the manufacturer's instructions (Qiagen). Cells were harvested 30 h after transfection and activities of firefly and renilla luciferase were measured using the Dual-Luciferase reporter assay system (Promega). The resulting luminescence was measured with a Tecan M200 microplate reader (Tecan, Crailsheim, Germany).

MTT assay. Cells were plated at 10,000 (PC3) or 5,000 (LnCap) per well in 24-well plates. One, three, five and seven days after plating, respectively, 100 μl of 5 mg/ml MTT (thiazolyl blue tetrazolium bromide, Carl Roth, Karlsruhe, Germany) in PBS was added per well; cells were lysed after 4 h by addition of 250 μl triplex solution (10% SDS; 5% isobutanol, 0.012 M HCl). Absorbance was measured at 562 nm.

Apoptosis assays. Caspase activities were measured using the Caspase Glo 3/7 assay (Promega), following the manufacturer's instructions. Values were corrected for differences in cell numbers by doing an MTT assay in parallel.

The mitochondrial membrane potential was determined in LnCap by JC-1 (Axxora, Lörrach, Germany) staining. JC-1 was added to the cells at a final concentration of 5 μg/ml, incubated for 10 min at 37˚C, after which the cells were trypsinized and washed extensively with PBS. In PC3, the mitochondrial membrane potential was instead measured using the FlowCellect™ MitoPotential Red kit (Millipore, Schwalbach, Germany), following the manufacturer's instructions. In both cases, treatments were performed in triplicates; fluorescence was measured on a Guava easyCyte 8HT flow cytometer (Millipore).

For the determination of the sub-G1 fraction, prostate carcinoma cells were transfected with miR-205 mimics or AllStars control oligos, trypsinized, plated at $5x10^4$ cells per well in 6-well plates in triplicates the next day and treated with doxorubicin or cisplatin 48 h after transfection. Forty-eight hours after treatment with doxorubicin and cisplatin, respectively, cells were harvested by trypsinisation and combined with the floating cells. Cells were fixed in 70% ice-cold ethanol and stained in 0.1% Triton X-100 (Carl Roth), 0.5 mg/ml RNaseA (Sigma-Aldrich) and 60 μg/ml propidium iodide (MP Biochemicals, Illkirch, France) in PBS. The sub-G1 fraction of propidium iodide stained cells were measured on a Guava easyCyte 8HT flow cytometer (Millipore).

Western blotting. Cells were harvested by scraping into ice-cold RIPA buffer (1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 50 mM Tris-HCl pH 8.0) containing EDTA-free complete protease inhibitors (Roche, Mannheim, Germany). Protein concentration was measured using the BCA assay (Thermo Scientific) according to the manufacturer's instructions. Incubation with primary antibodies anti-BCL2 (Cell Signaling, Frankfurt, Germany) or anti-α-tubulin (clone DM1A, Thermo Fisher, Bonn, Germany) was done overnight at 4˚C. Blots were incubated with horseradish peroxidase labelled secondary antibodies (Cell Signaling) and the signal was detected using ECL (Thermo Scientific). Changes in protein expression were quantified using ImageJ Software (V.1.43u; NIH; USA). By normalizing integrated density values (IDV) of the protein bands of interest were normalized to the corresponding tubulin IDVs.

Statistics. Comparisons between pairs of values were done using the t-test; the Welch t-test was applied if the F-test indicated a significant difference between the variances. Statistical inferences were derived from single experiments. For statistical testing on expression levels of miR-205 and *BCL2*, data were log2 transformed, to allow the use of normal distribution-based tests and Pearson's correlation coefficient. For *post hoc* testing between individual pairs of conditions, the Scheffé test was applied. Statistical analysis was carried out by the Statistica 10 program (StatSoft, Tulsa, OK, USA).

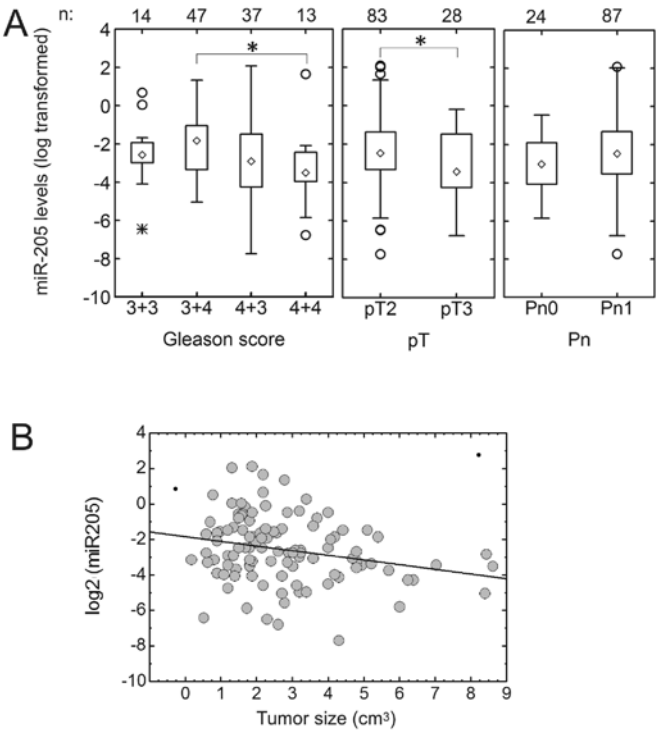

Figure 1. MiR-205 is downregulated in prostate cancer. (A) Relative miR-205 levels in comparison to matched benign tissue, in relation to Gleason score, pT category and perineural invasion status (Pn). ◇, Median; □, 25-75%; ↕, non-outlier range; ○, outliers; *p<0.05. (B) Correlation between miR-205 expression (log transformed) and tumour size by perineural invasion status. Two outliers (16 and 18 cm^3) were excluded according to the two-sided normal test. The regression line was calculated on all samples combined.

Results

MiR-205 is downregulated in prostate cancer. We determined the expression of miR-205 by qRT-PCR in 111 FFPE specimens of prostate carcinoma. The miR-205 expression was found to be strongly reduced in 102 of 111 analyzed prostate cancer samples, with a median relative expression level of 0.160 in comparison to benign tissue from the same patient (interquartile range, IQR, 0.085-0.350). MiR-205 expression levels decreased with increasing Gleason score from 7a=3+4 to 8=4+4, but Gleason score 6=3+3 samples showed lower expression than Gleason score 7a samples (median expression levels: Gleason 3+6, 0.168; Gleason 7a, 0.303; Gleason 7b, 0.150; Gleason 8, 0.087; p=0.050 by ANOVA on log transformed data) (Fig. 1A). Its expression was also higher in tumours confined to the prostate (pT2) than in those that extend beyond the prostate (pT3) (p=0.0425). However, differences in miR-205 expression between perineural invasion categories were not significant (Fig. 1A). Only 9 of 111 samples showed an increased expression of miRNA 205 in tumour tissue in comparison to the benign controls; these were all relatively small tumours (<4 cm^3). The inverse correlation

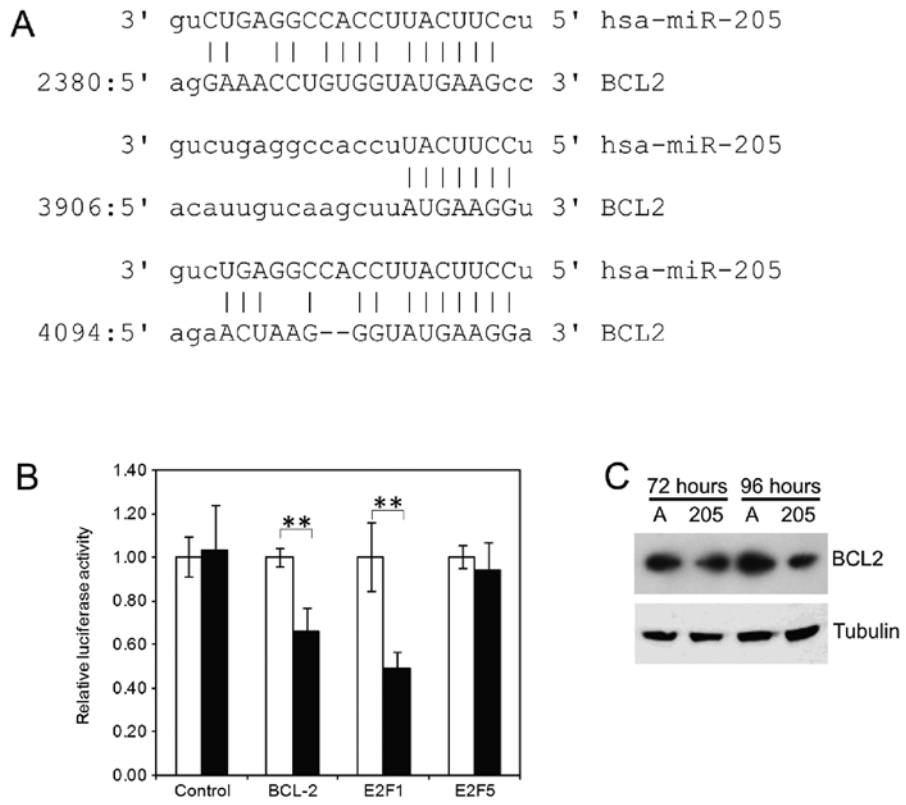

Figure 2. BCL2 as a miR-205 target. (A) Potential miR-205 binding sites in the 3'UTR of *BCL2* mRNA. (B) Reporter assay for repression of luciferase activity after miR-205 binding. A fragment of the 3'UTRs of *BCL2*, *E2F1* and *E2F5* containing the potential binding sites was cloned into the reporter vector. Luciferase activity after transfection of miR-205 mimics (black bars) is expressed in comparison to AllStars control (white bars); $^{**}p<0.01$. (C) Decrease of BCL2 signal in the prostate cancer cell line PC3 by western blotting after transfection with miR-205 oligos in comparison to control transfected cells. 205, transfected with miR-205 mimics; A, transfected with AllStars control oligos.

between miR-205 expression and tumour size was significant (r=-0.234, p=0.0087) (Fig. 1B).

The anti-apoptotic gene BCL2 is a target of miR-205. The reduced expression of miR-205 in prostate cancer indicates that its targets may aid tumour progression. From the targets suggested by the miRGen program (www.diana.pcbi.upenn.edu/miRGen/html), we selected *BCL2* as a novel candidate. BCL2 appears interesting in this context, as its overexpression in prostate cancer is a known marker for poor prognosis (24).

In the ~5-kb long 3'UTR, 3 potential miR-205 binding sites are found, one weak (6-mer + a) centrally located at position 2380 and two potentially stronger sites near the 3' end of the 3'UTR at positions 3906 (7-mer-m8) and 4094 (7-mer-1a) (Fig. 2A), both with a ΔG above -13.40 kCal/mol (25). For a random sequence of this length, a ΔG of -13.40 kCal/mol would be expected (26). Additionally, these sites are located relatively close together and near the 3' end of a long 3'UTR, which are also typical characteristics of functional miRNA binding sites (27).

The fragment containing these two most 3' terminal sites was cloned into the pGL3 vector as 3'UTR for the luciferase gene (28). A reporter assay was done in U2-OS cells with miR-205 or control oligos to test for reduced luciferase activity after binding the microRNA. The osteosarcoma cell line U2-OS was used for this assay due to its ease of transfection and the stability of the assay in this cell line. As positive controls, equivalent constructs containing miR-205 binding sites in *E2F1* and *E2F5* were used. In comparison to the control oligos, miR-205 caused a 34% reduction in the luciferase signal when the BCL2 UTR was tested. In comparison, the *E2F1* UTR caused a 51% percent reduction in signal intensity and the *E2F5* UTR only 6%. The decrease in luminescence was significant for both *BCL2* and *E2F1* ($p<0.01$, t-test). The control vector did not show significantly reduced luciferase activity in the presence of miR-205 (Fig. 2B).

Next, we analyzed if overexpression of miR-205 leads to a decrease in the BCL2 protein levels of the prostate cancer cell line PC3. We observed a decrease in the BCL2 level by western blotting at 72 and 96 h after transfection with miR-205 mimics, in comparison those transfected with control oligos (Fig. 2C).

BCL2 expression in comparison to miR-205 levels in patient samples. Having confirmed *BCL2* as a miR-205 target, we determined the expression of BCL2 at the mRNA level by TaqMan assay in the series of archival samples for which miR-205 levels were measured by qRT-PCR. The quality of the RNA was sufficient for reliable PCR results in 84 of 111 samples. The mRNA levels of *BCL2* have been found to correlate well with its protein level (29), allowing for the use of qRT-PCR to measure *BCL2* expression. *BCL2* is strongly expressed in the basal cells of benign glands (24,30), making the comparison to benign tissue difficult for this

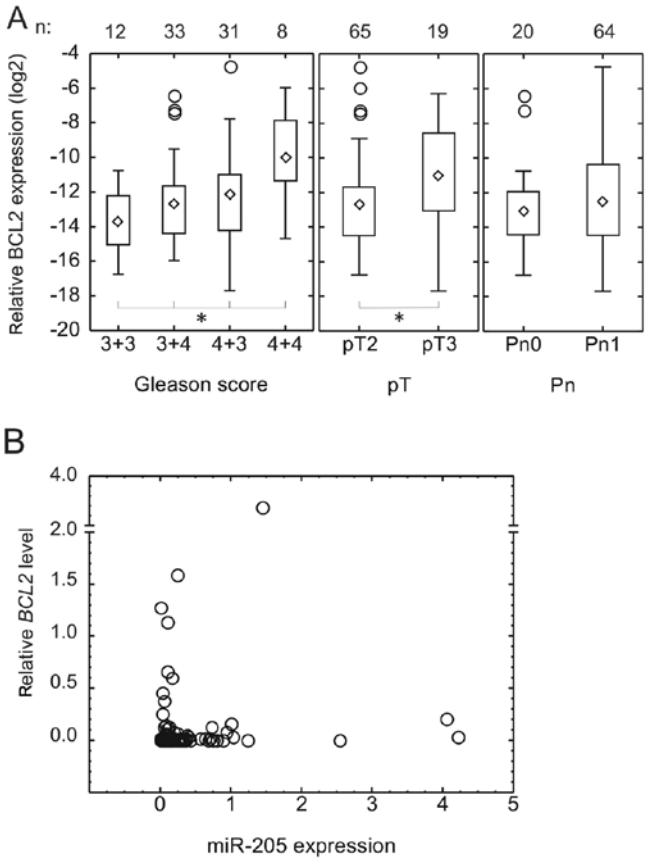

Figure 3. BCL2 expression in samples of prostate cancer in relation to clinical parameters and miR-205 expression. (A) *BCL2* levels normalized to 18S expression (log2 transformed) in relation to Gleason score, pT category and perineural invasion status. ◇, Median; □, 25-75%; ↕, non-outlier range; ○, outliers; ∗, extremes. *p<0.05. (B) Relation between *BCL2* expression (log transformed, relative to 18S) and miR-205 expression (log transformed).

gene. Therefore, we calculated *BCL2* expression levels as ΔCt relative to 18S levels in tumour tissue. *BCL2* mRNA expression increased significantly with increasing Gleason grade (median expression relative to 18S: Gleason 3+3, 0.0074% (n=12); Gleason 3+4, 0.0148% (n=33); Gleason 4+3, 0.022% (n=31); Gleason 4+4, 0.100% (n=8); p=0.173. Expression was also stronger in tumours with extension beyond the prostate (pT3, median, 0.0488%) in comparison to those confined to the prostate (pT2, median, 0.0148%) (p=0.0399). There was no significant difference between those with (Pn1, median, 0.0172%) and without perineural invasion (Pn0, median, 0.0118%) (Fig. 3A). *BCL2* levels did not correlate to tumour size either.

Although there was no linear correlation between miR-205 and BCL2 expression, all the samples except one with high BCL2 expression, had low (<30% of benign) miR-205 expression (Fig. 3B).

MiR-205 promotes apoptosis in prostate cancer cells. Having confirmed anti-apoptotic gene *BCL2* as a miR-205 target, we investigated the effect of miR-205 overexpression on the induction of cell death in prostate cancer cells. We measured caspase 3/7 activity in cells transfected with miR-205 mimics after treatment for 24 h with 0.1 μg/ml doxorubicin (0.1724 μM), 4 and 10 μM cisplatin (PC3) or 10 and 20 μM (LnCap) cisplatin. A consistent increase in caspase activity was observed in cells transfected with miR-205 in comparison to control oligos in both cell lines in combination with cisplatin treatment (Fig. 4A). Cisplatin (4 μM) does not induce significant cell death in LnCap cells, therefore higher doses were used in this cell line (Fig. 4B and data not shown). Doxorubicin only caused a significant difference in PC3 cells in this assay.

As a marker for apoptosis, we determined the sub-G1 fraction by flow cytometry 48 h after genotoxic treatment. We observed a significant increase in the fraction of apoptotic cells both in control cells and cells treated with doxorubicin or 4 μM cisplatin (Fig. 4C) in PC3. LnCap shows the strongest increase in apoptosis at 10 μM cisplatin concentration in combination with miRNA205 mimics. In these cells there was no increase in the spontaneous apoptosis rate after miR-205 transfection, in contrast to PC3. Apoptosis is also characterized by loss of mitochondrial membrane potential (ΔΨM), which can be measured through a change in the fluorescence spectrum of the JC-1 dye. Loss of ΔΨM was significantly increased in LnCap cells transfected with miR-205 after treatment with 10 μM cisplatin, as compared to control transfected cells, confirming the results for caspase 3/7 activation (Fig. 4B).

MiR-205 inhibits cell division in prostate cancer cells. We next determined the effect of miR-205 overexpression on cell growth. For this, PC3 and LnCap cells were transfected with either AllStars negative control siRNA or miR-205 mimics and then plated out at low densities. An MTT assay was performed at the starting point and at 3, 5 and 7 days thereafter. We observed a significantly lower cell number in the miR-205 transfected cells (p<0.05), in comparison to control transfected cells at 7 days (Fig. 5) in both cell lines.

Discussion

In this study, we describe the downregulation of the microRNA miR-205 in prostate cancer, which is known to be involved in the epithelial to mesenchymal transition (1,20). In our series of samples, expression of miR-205 was reduced in 76 of 84 prostate cancer specimens, with a median relative expression level of 18.0% in tumour tissue compared to benign tissue of the same patient. This finding is in accordance with earlier data from microarray experiments (7,8) and from qRT-PCR in a smaller series of patients (31). The level of miR-205 correlated inversely with tumour size, which fits with the growth inhibitory effects we observed for this microRNA. Moreover, there was a trend for miR-205 expression to decrease with increasing Gleason grade from 7a (3+4) to 8 (4+4). This is noteworthy as it corresponds to the worse prognosis of patients with primarily Gleason grade 4 as opposed to primarily Gleason grade 3 (32).

The role of miR-205 in cancer development depends on the tissue type, as it has been found to be either overexpressed or downregulated, depending on the tumour origin. Its functions include the prevention of epithelial to mesenchymal transition, which is associated with metastasis, through repression of ZEB1 and ZEB2 and consequent high E-cadherin levels (reviewed in ref. 1). On the other hand, several of its described targets, such as *E2F1* (14), PTEN (33),

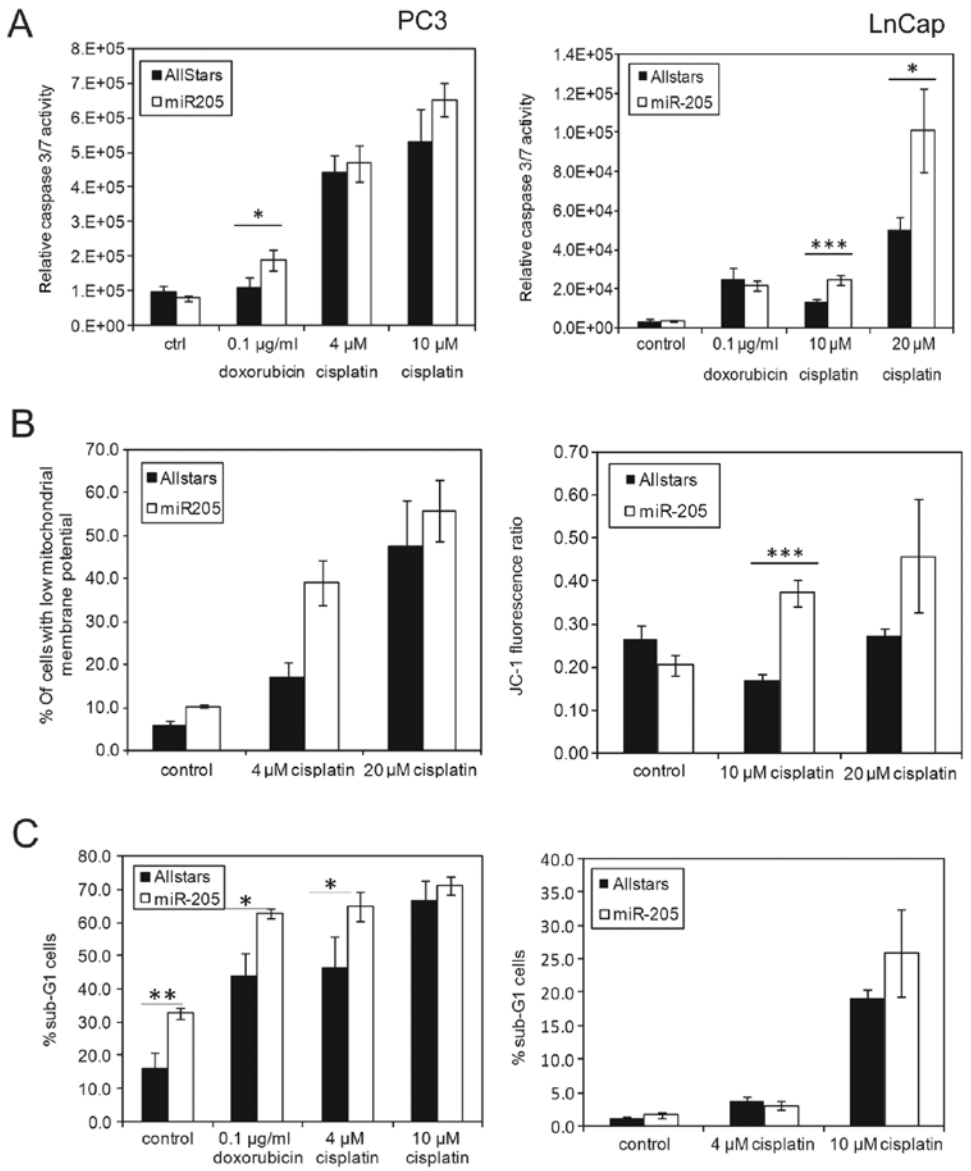

Figure 4. MiR-205 increases apoptosis after DNA damage in PC3 and LnCap cells. (A) Induction of caspase 3/7 activity in PC3 (left panel) and LnCap (right panel) cells transfected with miR-205 mimics or AllStars control oligos after treatment with DNA damaging agents. Cells were treated for 24 h for PC3 and 48 h for LnCap before measurement. (B) Loss of mitochondrial membrane potential upon cisplatin treatment is enhanced after overexpression of miR-205 in PC3 cells (left panel) and LnCap cells (right panel). (C) Increase in sub-G1 fraction in PC3 cells (left panel) and LnCap cells (right panel) transfected with miR-205 or control oligos were treated for 48 h with doxorubicin or cisplatin at the indicated concentrations. There was a consistent increase in the sub-G1 fraction after miR-205 transfection. *Significant difference with $p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

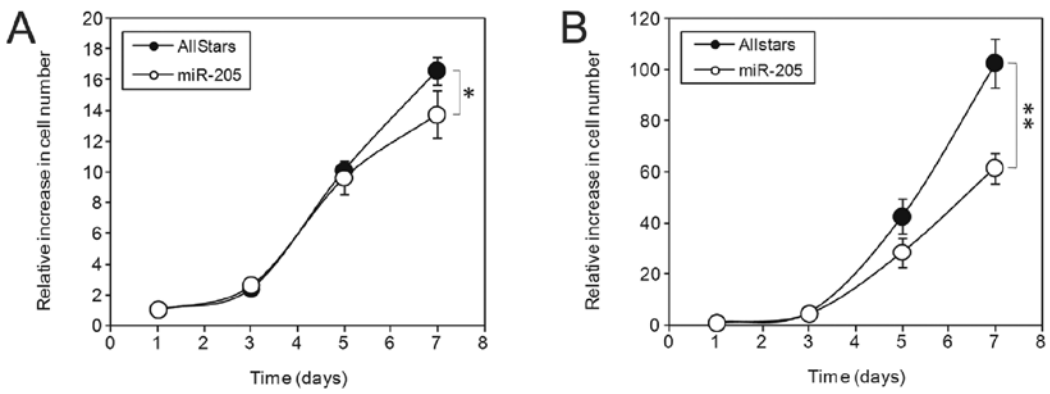

Figure 5. MiR-205 reduces cell growth in PC3 and LnCap cells. PC3 (A) and LnCap (B) cells were transfected with miR-205 or control RNA oligos. A MTT assay was done 1, 3, 5 and 7 days post-transfection. The differences between the cell number at day seven were significant for both cell lines (LnCap, $^{**}p=0.0065$; and PC3, $^{*}p=0.0486$).

BCL2L2 (BCL-W) (20) and *PKCε* (31) are involved in cell growth and survival, whereas *VEGFA* (13) is an important factor in angiogenesis.

In the present study, we describe the anti-apoptotic protein BCL2 as a novel target of miR-205. This protein is likely an important target in prostate cancer, as high BCL2 expression in primary prostate cancer is a marker for poor prognosis, with an increased risk for recurrence (21,22,24,34). Biochemical recurrence during anti-androgen therapy is frequently associated with increased BCL2 expression (35,36). Loss of miR-205 may play a role in androgen independence, as one study reported reduced expression only in androgen-independent tumours (7), which would then correspond to higher BCL2 levels.

The regulation of BCL2 expression in prostate cancer is complex. It is a known target of several other microRNAs, such as miR-15, miR-16 (37) and the miR-34-family (38,39). A different and independent mechanism for low BCL2 expression in prostate cancer is methylation of the BCL2 promoter (30,40), which would explain why many cases do not express BCL2 at all, whereas those that express it, are strongly positive. Gene rearrangements of BCL2 appear to be rare in prostate cancer (21). Taken together, these factors may explain the poor correlation between *BCL2* levels *in vivo* in our samples, in contrast to the *in vitro* results in PC3 and LnCap cells. Further research will be needed to clarify this issue.

With BCL2 as a target, in combination with the expected effects of previously published targets like E2F1, we found that overexpression of miR-205 in prostate cancer cell lines decreases cell division and increases apoptosis in response to chemotherapeutical agents. Moreover, these data are in accordance with results in different prostate cancer cell lines after treatment with cisplatin and docetaxel. The authors attribute their results to repression of BCL-W (encoded by BCL2L2), which is another anti-apoptotic member of the BH3 family (20). As we confirmed the repression of the anti-apoptotic BCL2 by miR-205, the observed effects may also in part be caused by this protein.

In conclusion, we identified the downregulation of miR-205 in prostate cancer and describe *BCL2* as a novel target of this microRNA, with potential prognostic and therapeutic implications. Increased expression of miR-205 was found to inhibit cell growth and to sensitize cells to apoptosis, caused by chemotherapeutical agents in a *BCL2*-dependent manner.

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