The loss of the tumour-suppressor miR-145 results in the shorter disease-free survival of prostate cancer patients

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Background: Prostate cancer (PCa) is characterised by great heterogeneity of the disease progression rate. Tumours range from insignificant and not life threatening to high risk for relapse ones. Consequently, a large number of patients undergo unnecessary treatment. miR-145 is a well-documented tumour suppressor and its expression, which is regulated by the p53 pathway, has been found to be decreased in the majority of human malignancies. The aim of our study was to evaluate the clinical utility of miR-145 for the prognostication of PCa.

Methods: Total RNA was isolated from 137 prostate tissue specimens obtained from 73 radical prostatectomy-treated PCa patients and 64 transurethral- or open prostatectomy-treated benign prostate hyperplasia (BPH) patients. Following polyadenylation and reverse transcription, miR-145 levels were determined by quantitative real-time PCR assay, using SNORD48 (RNU48) for normalisation purposes.

Results: Downregulated miR-145 expression was found in PCa compared with BPH patients. The reduction of miR-145 expression in PCa was correlated with higher Gleason score, advanced clinical stage, larger tumour diameter and higher prostate-specific antigen (PSA) and follow-up PSA levels. In addition, higher risk for biochemical recurrence and significantly shorter disease-free survival (DFS) was found for the PCa patients expressing lower miR-145. Focusing on ‘low- and intermediate-recurrence risk’ PCa patients, miR-145 loss was revealed to be a reliable predictor of biochemical relapse and poor DFS independent from Gleason score, clinical stage, PSA and patients’ age.

Conclusion: The loss of the tumour-suppressor miR-145 increases the risk for disease progression and predicts the poor survival of PCa patients.
tumours is responsible for the diversity of outcomes of patients in the same risk group (Freedland, 2011) and the emerging need of novel biomarkers (Sardana et al, 2008).

MicroRNAs (miRNAs) are a rapidly growing family of small (~22nt) endogenous non-coding RNA molecules able to regulate gene expression at the post-transcriptional level (Bartel, 2004). The mature miRNA binds usually the 3′-untranslated region (3′-UTR) of the miRNAs through complete or partially complementarity, resulting to the translational repression or/and the degradation of the target. Taking into account that miRNAs regulate gene expression, it becomes clear that the upstream regulation of their own levels is crucial for cellular homeostasis and behaviour. The deregulation of miRNA levels has been revealed as a hallmark of the majority of human malignancies (Calin and Croce, 2006). Consequently, the study of miRNAs is prominent in cancer-related research nowadays, aiming to clarify their role during tumourigenesis and to examine their clinical efficacy (Bartels and Tsongalis, 2013; Croce, 2009; Freedland, 2011). Downregulated miR-145 levels has been documented in PCa as well as by the methylation status of its promoter (Porkka et al, 2011). Inhibition of cell growth, in terms of cell cycle arrest and apoptosis, (miR-145) has a well- characterised tumour-suppressor regulatory mechanism and to examine their clinical efficacy (Bartels and Tsongalis, 2013; Croce, 2009).

Although the tumour-suppressor role of miR-145 is well documented, there is no complete evaluation of its clinical utility for PCa patients. The aim of this study was the analysis of the miR-145 expression profile in prostate tumours, the correlation of its levels with the clinicopathological features of the disease and the evaluation of its prognostic significance for the patients.

MATERIALS AND METHODS

Study population. For the purpose of our study, 137 consecutive tissue samples were obtained from 73 patients diagnosed with PCa (Table 1) and 64 patients with benign prostate hyperplasia (BPH) at the ‘Laiko’ General Hospital, Athens, Greece. Transurethral or open prostatectomy was performed in BPH patients, whereas PCa patients underwent radical retropubic prostatectomy. Following the removal of the prostate gland from the PCa patients, a tissue sample of approximately 200 mg was sectioned from the peripheral zone based on the preoperative features of the biopsy and on the Gleason score, Clinical stage, PSA, DRE and age

| Variable | No. of patients | miR-145-negative | miR-145-positive | P-value |
|----------|-----------------|------------------|------------------|---------|
| **Gleason score** | | | | |
| 5 | 2 | 0 (0.0) | 2 (100.0) | 0.013b |
| 6 | 22 | 5 (22.7) | 17 (77.3) | |
| 7 (3 + 4) | 25 | 10 (40.0) | 15 (60.0) | |
| 7 (4 + 3) | 14 | 9 (64.3) | 5 (35.7) | |
| 8 | 7 | 5 (71.4) | 2 (28.6) | |
| 9 | 3 | 3 (100.0) | 0 (0.0) | |
| **Clinical stage** | | | | |
| < pT2a | 17 | 4 (23.5) | 13 (76.5) | 0.047b |
| pT2a | 18 | 6 (33.3) | 12 (66.7) | |
| pT2c | 10 | 5 (50.0) | 5 (50.0) | |
| pT3a | 15 | 7 (46.7) | 8 (53.3) | |
| pT3b | 13 | 10 (76.9) | 3 (23.1) | |
| **PSA (ng ml⁻¹)** | | | | |
| < 4.0 | 7 | 0 (0.0) | 7 (100.0) | <0.001b |
| 4.0–10.0 | 14 | 12 (85.7) | 2 (14.3) | |
| > 10.0 | 22 | 18 (81.8) | 4 (18.2) | |
| Unknown | 1 | | | |
| **DRE** | | | | 0.145b |
| Positive | 44 | 23 (52.3) | 21 (47.7) | |
| Unknown | 2 | | | |
| **Age (years)** | | | | 0.887b |
| < 65 | 36 | 15 (41.7) | 21 (58.3) | |
| 65–74 | 32 | 15 (46.9) | 17 (53.1) | |
| > 75 | 4 | 2 (50.0) | 2 (50.0) | |
| Unknown | 1 | | | |

Abbreviations: DRE = digital rectal examination; PCa = prostate cancer; RO = relative quantification.

**Calculated by Fisher’s exact test.**

For the evaluation of miR-145 prognostic significance, 62 PCa patients were successfully followed-up, whereas 11 patients were macroscopic findings. Thereafter, the tissue sample was divided into two mirror-image specimens, one of which was tested by a pathologist for the presence of malignancy, whereas the remaining one was immediately frozen in liquid nitrogen and stored at −80 °C until the analysis. Patients who had received hormonal therapy or radiotherapy before the surgery were not included in our study. Our study was approved by the ethics committee of ‘Laiko General Hospital’ and performed with respect to the ethical standards of the Declaration of Helsinki, as revised in 2008.

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miR-145 loss indicates PCA patients' poor outcome

National Comprehensive Cancer Network (NCCN) guidelines for PCa were used for the stratification of PCA patients according to their recurrence risk (Mohler et al., 2010). Biochemical relapse was defined by two consecutive measurements of serum PSA ≥ 0.2 ng mL⁻¹ (Boccon-Gibod et al., 2004). Disease-free survival (DFS) was defined as the interval between the radical prostatectomy and the time of biochemical relapse, or the time period following the surgery and the most recent measurement of serum PSA for the patients who did not present biochemical recurrence. As pre-treatment PSA and the most recent measurement of serum PSA for the patients time of biochemical relapse, or the time period between the surgery and the pulverisation of 50–120 mg of prostate tissue, total RNA was defined by two consecutive measurements of serum PSA.

Extraction and polyadenylation of total RNA. Following the pulverisation of 50–120 mg of prostate tissue, total RNA was isolated using TRI-REAGENT (Molecular Research Center, Inc., Cincinnati, OH, USA). Polyadenylation at the 3'-end of the RNAs was performed in a 15 μl reaction containing 1 μg of total RNA, 800 μM ATP and 1 U of E. coli Poly(A) Polymerase (New England Biolabs, Ipswich, MA, USA), at 37 °C for 60 min (Shi and Chiang, 2005; Mavridis et al., 2013). Total RNA concentration and purity were determined spectrophotometrically at 260 and 280 nm, whereas RNA integrity was evaluated by agarose gel electrophoresis.

First-strand cDNA synthesis. The polyadenylated total RNA was thereafter used as template for the first-strand cDNA synthesis. Reverse transcription was carried out in a 20 μl reaction using 50 U MMLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA), 40 U recombinant ribonuclease inhibitor (Invitrogen) and 0.25 μM poly(T) adapter 5’-GGGAGCAAGAATATAAGCAGTCACTA TAGTTTTTTTTTTTTTNN-3’ (V = G, A, C and N = G, A, T, C) as reaction primer, at 37 °C for 60 min (Shi and Chiang, 2005; Mavridis et al., 2013).

Quantitative real-time PCR (qPCR). A SYBR-Green fluorescent-based qPCR assay was applied for the quantification of miR-145 levels (Shi and Chiang, 2005; Mavridis et al., 2013). Specific forward primers for the miR-145 and the small nuclear RNA, C/D box 48 (SNORD48), also known as RNU48, were designed according to their published sequences (NCBI Reference Sequence: NR_029686.1 and NR_002745.1, respectively) and in silico specificity analysis. Specifically, the combination of the 5’-CCAGT TTTCCAGGAATTCCTA-3’ forward primer for miR-145 or the 5’-TGAATGTAGCCACGGATTCT-3’ forward primer for SNORD48 with the universal reverse primer 5’-GGGAGCAAGAATATAAGCAGTCACTA-3’, which hybrides the above mentioned poly(T) adapter, amplifies a 65 bp miR-145- or a 105 bp SNORD48-specific amplicon, respectively (Supplementary Figure 1).

The qPCR was performed in the 7500 Real-Time PCR System using the sequence detection software (Applied Biosystems, Carlsbad, CA, USA). The 10 μl reaction mixture consists of Kapa SYBR Fast Universal 2X qPCR Master Mix (Kapa Biosystems Inc., Woburn, MA, USA), 200 nM of each PCR primer and 0.2 ng of cDNA. The thermal protocol consists of a 3-min polymerase activation step at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and the primer annealing and extension step at 60 °C for 1 min.

Melting curve analysis and agarose gel electrophoresis were performed following the amplification in order to distinguish the accumulation of the specific reaction products from nonspecific ones or primer-dimers. Calibration curves for miR-145 and SNORD48 amplification were generated by a validation experiment using as template serial dilutions of a control cDNA covering eight orders of magnitude (10⁻¹⁰ ng cDNA) (Supplementary Figure 1). The linear increases of miR-145 (y = −3.35x + 13.93; r² = 0.999) and SNORD48 (y = −3.53x + 18.88; r² = 0.996) amplification indicate the 90.5% and 92.1% reaction efficiencies, respectively, as well as the absence of PCR inhibition by the template.

The expression analysis of miR-145 was carried out using the 2⁻ΔΔCt relative quantification (RQ) method (Livak and Schmittgen, 2001). Duplicate reactions were performed for each tested sample and the average Ct (Avg. Ct) was calculated for the quantification analysis. SNORD48 was used as an endogenous reference control and the LNCaP PCa cell line as our assay calibrator. More precisely, using the formula ΔCt = Avg. CtmiR-145 - Avg.CSNORD48, the miR-145 expression of each tested sample was normalised to the SNORD48 endogenous reference control of the sample. Thereafter, the normalised miR-145 expression levels of the tested samples were quantified relative to the expression of the calibrator (RQ unitscalibrator = 1), using the formula RQ unitssample = 2⁻ΔΔCt, where ΔΔCt = ΔCtsample - ΔCtcalibrator. No template controls, reverse transcriptase-negative controls, Poly(A) polymerase-negative controls and DNA template controls generated undetectable Ct. The intra-assay %CV was calculated to be 3.7%.

Statistical analysis. The analysis of the differences in miR-145 levels between PCa and BPH patients was performed by the non-parametric Mann–Whitney U-test. To evaluate the ability of miR-145 to discriminate PCa patients from BPH ones, logistic regression and ROC analysis were used. Univariate and multivariate logistic regression models were used for the calculation of the odds ratio (OR) of log₂miR-145, serum PSA levels, patient’s age and digital rectal examination (DRE). The ROC curve was developed by plotting %sensitivity versus (100%-specificity) and the area under curve (AUC) was calculated by the Hanley and McNeil method.

To evaluate the miR-145 expression differences between the different Gleason score or clinical stage groups of patients, the non-parametric Mann–Whitney U and Kruskal–Wallis tests were used appropriately. Using the X-tile algorithm, the 2.97 × 10⁻³ RQ units of miR-145 (equal to the 45th percentile of the PCA patients’ cohort) was adopted as an optimal cutoff value to classify PCa patients into miR-145 (+) and miR-145 (−) cohorts, expressing higher and lower miR-145 levels, respectively. The distribution of miR-145 (+) and miR-145 (−) patients to Gleason score, clinical stage, serum PSA levels, DRE and patients’ age was analysed using the χ² and Fisher’s exact tests. The correlation of miR-145 levels with tumour diameter, pre-treatment PSA and follow-up PSA levels of PCa patients was determined by the Spearman’s correlation coefficient (rS).

The significance of miR-145 expression for the DFS of PCa patients was assessed with Cox proportional hazards regression analysis as well as Kaplan–Meier survival analysis using the log-rank test. Hazard ratio (HR) was calculated for miR-145, Gleason score, clinical stage, serum PSA levels, patient’s age and DRE, using both univariate and multivariate regression models. Kaplan–Meier survival curves were generated by plotting the %DFS probability versus the time period following the radical prostatectomy.

RESULTS

miR-145 expression is downregulated in PCa patients. Statistically significant (P = 0.037) underexpression of miR-145 was detected in the PCa tissues (range: 38.36–1.67 × 10⁷ RQ units;
median: $3.80 \times 10^3$ RQ units) compared with the BPH ones (range: $898.57 \pm 3.37 \times 10^3$ RQ units; median: $6.07 \times 10^3$ RQ units). ROC analysis (AUC: 0.604; 95% CI: 0.509–0.698; $P = 0.037$) illustrated that miR-145 loss in PCa can discriminate the malignant from the benign specimens. Moreover, the univariate logistic regression highlighted a statistically significant ($P = 0.020$) lower risk of suffering from PCa for the patients with high miR-145 levels (OR: 0.484; 95% CI: 0.263–0.892). Multivariate logistic regression models showed that the abovementioned discriminatory value of miR-145 (OR: 0.917; 95% CI: 0.427–1.968; $P = 0.824$) is not independent from the PSA serum concentration, patients’ age and DRE.

The decreased miR-145 expression correlates with higher Gleason score, late-stage tumours, larger tumour diameter and higher PSA and follow-up PSA serum levels. The downregulation of miR-145 levels in PCa patients was further associated with higher Gleason score tumours (Figure 1A). miR-145 expression was significantly ($P = 0.006$) reduced in the groups with Gleason score $\geq 8$ (median: $1.78 \times 10^3$ RQ units) and Gleason score 7 (median: $2.95 \times 10^3$ RQ units) compared with the group with Gleason score $\leq 6$ (median: $9.52 \times 10^3$ RQ units). Analysing miR-145 as bivariate (Table 1), 80.0% of the patients with Gleason score $\geq 8$ were characterised as miR-145 $(-)$ compared with the significantly lower 48.7% and 20.8% of the patients groups with Gleason score 7 and Gleason score $\leq 6$, respectively ($P = 0.004$).

Analysis of the miR-145 levels in relation to the clinical stage of the patients demonstrated the downregulation of miR-145 expression ($P = 0.026$) as the disease progresses to late-stage tumours (Figure 1B). Classifying patients into $\leq\text{pT}2\text{c}$ and $\geq\text{pT}3\text{a}$ cohorts, significantly lower miR-145 expression ($P = 0.023$) was detected in the $\geq\text{pT}3\text{a}$ (median: $2.26 \times 10^3$ RQ units) compared with the $\leq\text{pT}2\text{c}$ group (median: $6.88 \times 10^3$ RQ units; Figure 1C), resulting to the characterisation as miR-145 $(-)$ of the 66.7% of $\geq\text{pT}3\text{a}$ patients compared with the significant lower percentage of 33.3% of the $\leq\text{pT}2\text{c}$ cohort ($P = 0.030$; Table 1).

Apart from the most significant prognostic factors of Gleason score and clinical stage, the loss of miR-145 was found to correlate with higher pre-treatment PSA and follow-up PSA serum levels as well as with tumours of larger diameter (Figure 2). As pre-treatment PSA was used as the last measurement prior the radical prostatectomy, whereas follow-up PSA was used as the most recent measurement of the non-relapsed patients or the PSA measurement at the time of biochemical recurrence of the relapsed ones. A statistically significant negative correlation was highlighted between the miR-145 levels and the pre-treatment PSA of PCa patients with larger tumour diameter ($r_s = -0.403$, $P < 0.001$; Figure 2A) or $\chi^2$ test ($P < 0.001$; Table 1). Moreover, the reduction of miR-145 expression levels were also associated with higher follow-up PSA ($r_s = -0.296$, $P = 0.020$; Figure 2B) as well as with larger tumour diameter ($r_s = -0.422$, $P = 0.025$; Figure 2C).

The downregulation of miR-145 expression correlates with the shorter DFS of PCa patients. Elevated risk for biochemical recurrence was designated for the miR-145 $(-)$ PCa patients by the univariate Cox proportional regression analysis ($HR: 2.533$, $P = 0.010$), highlighting their shorter DFS period (Table 2). Along with miR-145, the univariate analysis indicated the unfavourable clinical value of Gleason score, clinical stage and PSA serum levels for the outcome of PCa patients.

The prognostic significance of miR-145 for radical prostatectomy PCa patients was further assessed with Kaplan–Meier survival analysis (Figure 3). Shorter DFS period was demonstrated for miR-145 $(-)$ patients (average DFS: 24.52 months) compared with miR-145 $(+)$ (average DFS: 40.69 months) group ($P = 0.007$; Figure 3A). Overall, these findings strengthen the case for an unfavourable outcome, in terms of shorter DFS, of PCa patients with downregulated miR-145 expression.

miR-145 loss represents an independent unfavourable prognostic marker for the DFS of low- and intermediate-risk PCa patients treated with radical prostatectomy. Considering
the need for more accurate prediction of the low- and intermediate-recurrence risk patients’ outcome, PCa patients were further grouped according to their recurrence risk using NCCN guidelines for PCa (Mohler et al, 2010).

A significantly higher risk for biochemical relapse was indicated by the univariate Cox proportional analysis (Table 3) for the miR-145 (−) patients of the ‘low- and intermediate-recurrence risk’ cohort (HR: 3.211; 95% CI: 1.071–9.622; \(P = 0.037\)). For the same patients’ group, Gleason score, clinical stage, PSA serum levels and DRE failed to have a statistically significant prognostic value for the biochemical recurrence of the patients. This unfavourable outcome of the miR-145 (−) patients was revealed to be independent from patients’ Gleason score, clinical stage, PSA levels, age and DRE, as indicated by the multivariate models (HR: 4.467; 95% CI: 1.268–15.736; \(P = 0.020\)). The abovementioned independent predictive significance of miR-145 for the ‘low- and intermediate-recurrence risk’ patients’ outcome was further verified and confirmed by multivariate Cox regression analysis (Table 3) for the ‘intermediate-recurrence risk’ cohort (HR: 4.425; 95% CI: 1.112–17.613; \(P = 0.035\)).

The higher risk for biochemical recurrence of the miR-145 (−) ‘low- and intermediate-recurrence risk’ patients was clearly illustrated by the Kaplan–Meier survival plots. Significantly shorter DFS expectancy (\(P = 0.027\)) is attributed to the miR-145 (−) patients (average DFS: 28.00 months) in relation to the miR-145 (+) ones (average DFS: 47.36 months; Figure 3B). In addition, poor DFS of the patients expressing lower miR-145 levels was indicated by Kaplan–Meier DFS analysis (\(P = 0.020\)) for the intermediate-recurrence risk patients group (Figure 3C). Evaluating the prognostic efficacy of miR-145 for the ‘high- and very high-recurrence risk’ PCa patients, Kaplan–Meier survival analysis pointed out similar DFS intervals for the miR-145 (−) and miR-145 (+) patients (Figure 3D). Summarising, miR-145 proved to be an independent unfavourable marker for the disease progression and the DFS expectancy of the ‘low- and intermediate-recurrence risk’ PCa patients.

**DISCUSSION**

PCa represents a malignancy with good response to treatment, and therefore, guidelines and clinical practice support the active treatment of PCa in the local or regional disease stages (Heidenreich et al, 2011; Siegel et al, 2012). However, a significant portion of the patients undergo treatment without any benefits. The results from population-based PCa screening trials revealed that the reduction of PCa-specific mortality from PSA screening suffers from high risk of overdiagnosis, leading to treatment of miR-145 loss indicates PCa patients’ poor outcome

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**Table 2. Cox proportional regression analysis for the prediction of the PCa patients’ DFS**

| Covariant    | Univariate analysis | Multivariate analysis |
|--------------|---------------------|----------------------|
|              | HR                  | 95% CI               | \(P\)-value\a \b | HR                  | 95% CI               | \(P\)-value\a \b |
| miR-145      | Positive            | 1.00                 | 1.00               | 1.00                 | 1.00                 |
|              | Negative            | 2.533                | 1.249–5.137        | 0.010                | 1.264                | 0.489–3.273          | 0.629               |
| Gleason score| 2.244               | 1.407–3.578          | 0.001              | 1.660                | 0.944–2.921          | 0.078               |
| Clinical stage| 1.504             | 1.178–1.922         | 0.001              | 1.151                | 0.818–1.621          | 0.419               |
| PSA          | 1.092               | 1.035–1.151         | 0.001              | 1.034                | 0.957–1.117          | 0.397               |
| Age          | 0.996               | 0.944–1.051         | 0.877              | 0.983                | 0.930–1.039          | 0.548               |
| DRE          | 2.285               | 1.104–4.727         | 0.026              | 1.535                | 0.679–3.469          | 0.303               |

**Abbreviations:** CI = confidence interval; DFS = disease-free survival; DRE = digital rectal examination; HR = hazard ratio; PCa = prostate cancer.  
\a CI of the estimated HR. 
\b Test for trend.
clinically insignificant prostate tumours (Andriole et al, 2009; Schroder et al, 2009). Moreover, because of the heterogeneity of the disease, a big number of patients undergo useless invasive treatment because of the rapid progression and the immediate relapse of the disease (Trapasso et al, 1994; Zincke et al, 1994). The evolving role of miRNAs during tumourigenesis and cancer progression offers a big pool of candidates for the improvement of PCa patients’ prognosis, risk assessment and treatment decisions (Schefer et al, 2010b; Fendler et al, 2011).

Using the BPH patients’ cohort as control group, decreased miR-145 expression was detected in the tissue specimens from PCa patients. ROC analysis highlighted the ability of miR-145 expression levels to discriminate the malignant from the benign specimens of the gland, whereas logistic regression analysis pointed out a greater risk of suffering from PCa for patients with reduced miR-145 levels. The loss of miR-145 expression in PCa appeared to follow the progression of the disease to late-stage and poorly differentiated tumours. Underexpressed miR-145 levels were detected in high Gleason score tumours compared with well-differentiated ones. Moreover, the patients in advanced stages were found to express lower miR-145 levels in relation to those suffering from early-stage tumours. Overall, these data support the tumour-suppressor function of miR-145 for PCa cell growth and invasion and point out the relatively higher risk of suffering from late-stage and poorly differentiate tumours for the patients expressing lower miR-145 levels.

Downregulated miR-145 levels have been documented in the majority of the studied malignancies so far. miR-145 has been proposed to be a crucial modulator of Akt and KRas pathways’ tumourigenic role in cell microenvironment. The p53 (Sachdeva et al, 2009; Suzuki et al, 2009) and FoxO (Gan et al, 2010) transcription factors have been found to induce miR-145 expression. Thereafter, miR-145 silences MYC (c-Myc) leading to cell cycle arrest and apoptosis. In PCa, mutations of p53 and hypermethylation of the MIR145 promoter region, preventing p53 binding, were found to be responsible for the reduced miR-145 expression (Suh et al, 2011). In addition, miR-145 was found to further enhance activation of the p53 pathway and the expression of p53 transcriptional targets BBC3 (PUMA) and CDKN1A (P21), highlighting the existence of a tumour-suppressor loop between p53 and miR-145 (Spizzo et al, 2010). Focusing on KRas pathway, the repression of miR-145, through RREB1 transcription factor, revealed to be necessary for the KRas-mediated oncogenic cell transformation (Kent et al, 2010).

The tumour-suppressor role of miR-145 for PCa and the correlation of miR-145 loss with high Gleason score and late-stage tumours, prompted us to evaluate its significance for the prognostication of the disease. The loss of miR-145 was indeed further associated with higher PSA serum levels prior the surgery and during the follow-up period, as well as with larger tumour diameter. The Cox proportional regression analysis was further confirmed the elevated risk for biochemical recurrence for the radical prostatectomy-treated patients with lower miR-145. Moreover, Kaplan–Meier survival plot strongly illustrated the significantly shorter DFS of the miR-145 (−) patients compared with miR-145 (+) ones. Overall these findings support the unfavourable prognostic value of miR-145 loss for PCa, which was found to correlated with higher Gleason score, advance clinical stage, higher risk for biochemical recurrence and shorter DFS.

We thereafter hypothesised that miR-145 expression may also serve the prognostication of the low- and intermediate-risk group of patients. The poor outcome of the miR-145 (−) patients was
confirmed for the ‘low- and intermediate-recurrence risk’ cohort as well as for the ‘intermediate-recurrence risk’ group alone. The Cox proportional regression analysis highlighted the higher risk for biochemical relapse of the miR-145 (−) patients. Moreover, this unfavourable prognostic significance was revealed to be independent from the patients’ Gleason score, clinical stage, PSA levels and age. In addition, significantly shorter DFS was indicated for the miR-145 (−) patients, compared with miR-145 (+) patients of the same cohorts. Focusing on ‘high- and very high-risk’ PCa patients group, similar DFS expectancy was indicated for the patients independently from the loss of miR-145, supporting that the accumulation of deregulated molecular pathways in late-stage and dedifferentiated tumours overcomes the tumour-suppressor role of miR-145; taking this into consideration, it would be intriguing to design a comprehensive large-scale analysis of high-and very high-risk PCa patients. These data clearly denote the independent and unfavourable nature of the miR-145 loss for the outcome of the intermediate-risk of radical prostatectomy-treated patients and support the use of miR-145 expression for the stratification of these patients’ cohort according to their DFS expectancy. Future studies, focusing on low-recurrence risk PCa patients, will further reinforce the prognostic significance of miR-145 for the outcome prediction of low-risk group of patients and their proper management.

Table 3. Cox proportional regression analysis for the prediction of the ‘low- and intermediate-recurrence risk’ and the ‘intermediate-recurrence risk’ PCa patients’ DFS

| Covariant       | Low- and intermediate-recurrence risk PCa patients | Intermediate-recurrence risk PCa patients |
|-----------------|-------------------------------------------------|------------------------------------------|
|                 | Univariate analysis                             | Multivariate analysis                    |
|                 | HR  | 95% CIa | P-valueb | HR  | 95% CIa | P-valueb |
| miR-145         |     |         |          |     |         |          |
| Positive        | 1.00 | 1.00    | 0.00     | 1.00 | 1.00    | 0.00     |
| Negative        | 3.211 | 3.211   | 0.002    | 4.425 | 4.425   | 0.0035   |
| Gleason score   | 1.067 | 1.067   | 0.003    | 1.225 | 1.225   | 0.0035   |
| Clinical stage  | 0.711 | 0.711   | 0.003    | 0.392 | 0.392   | 0.0035   |
| PSA             | 1.101 | 1.101   | 0.003    | 1.102 | 1.102   | 0.0035   |
| Age             | 1.024 | 1.024   | 0.003    | 0.069 | 0.069   | 0.0035   |
| DRE             | 1.166 | 1.166   | 0.003    | 2.820 | 2.820   | 0.0035   |

Abbreviations: CI = confidence interval; DFS = disease-free survival; DRE = digital rectal examination; HR = hazard ratio; PCa = prostate cancer.

The Cox proportional regression analysis for the ‘low- and intermediate-recurrence risk’ cohort as well as for the ‘intermediate-recurrence risk’ group alone. The Cox proportional regression analysis highlighted the higher risk for biochemical relapse of the miR-145 (−) patients. Moreover, this unfavourable prognostic significance was revealed to be independent from the patients’ Gleason score, clinical stage, PSA levels and age. In addition, significantly shorter DFS was indicated for the miR-145 (−) patients, compared with miR-145 (+) patients of the same cohorts. Focusing on ‘high- and very high-risk’ PCa patients group, similar DFS expectancy was indicated for the patients independently from the loss of miR-145, supporting that the accumulation of deregulated molecular pathways in late-stage and dedifferentiated tumours overcomes the tumour-suppressor role of miR-145; taking this into consideration, it would be intriguing to design a comprehensive large-scale analysis of high-and very high-risk PCa patients. These data clearly denote the independent and unfavourable nature of the miR-145 loss for the outcome of the intermediate-risk of radical prostatectomy-treated patients and support the use of miR-145 expression for the stratification of these patients’ cohort according to their DFS expectancy. Future studies, focusing on low-recurrence risk PCa patients, will further reinforce the prognostic significance of miR-145 for the outcome prediction of low-risk group of patients and their proper management.

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