Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 derived miniRNA as a novel plasma-based biomarker for diagnosing prostate cancer

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Abstract Examining plasma RNA is an emerging non-invasive diagnosis technique. However, whether tumour-derived long non-coding RNAs (lncRNAs) in plasma can be used as a novel approach to detect human prostate cancer (PCa) has not yet been established. The study was divided into three parts: (1) the characteristics of PCa-related lncRNA fragments were systematically studied in the plasma or serum of 25 patients; (2) the source of the circulating lncRNA fragments was explored in vitro and in vivo; and (3) the diagnostic performance of metastasis associated in lung adenocarcinoma transcript 1 (MALAT-1) derived (MD) miniRNA was validated in an independent cohort of 192 patients. The expression levels of lncRNAs were measured by quantitative real time polymerase chain reaction (qRT-PCR). The MD-miniRNA copies were calculated using a standard curve in an area under the ROC curve (AUC)-receiver operating characteristic (ROC) analysis. Genome-wide profiling revealed that MALAT-1 and prostate cancer gene 3 (PCA3) are overexpressed in PCa tissues. Plasma lncRNAs probably exist in the form of fragments in a stable form. MD-miniRNA enters cell culture medium at measurable levels, and MD-miniRNA derived from human PCa xenografts actually enters the circulation in vivo and can be measured to distinguish xenografted mice.
1. Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males worldwide.\(^1\) Although serum prostate specific antigen (PSA) has been used to identify an increasing number of patients, the PSA technique has a number of limitations.\(^2,3\) New PCa-specific biomarkers that can complement and improve the current PCa detection strategies are urgently needed.

Emerging evidence indicates that long non-coding RNAs (lncRNAs), which are generally defined as transcripts longer than 200 nucleotides (nt), are dysregulated and play important roles in tumourigenesis and tumour progression.\(^4-11\) One type of lncRNAs transcribed from the introns of known genes correlates with the degree of differentiation of PCa,\(^3\) and one lncRNA, \(PCAT-1\), was identified as a prostate-specific regulator of cell proliferation.\(^11\) Another such lncRNA is \(PCGEM1\),\(^10\) which is a prostate tissue-specific and PCa-associated lncRNA gene. One of the well-characterised lncRNAs is \(differential display 3\) (\(DD3\)), or \(prostate cancer gene 3\) (\(PCA3\)). This lncRNA has great potential for reducing the number of unnecessary biopsies and provides an opportunity to establish the utility of molecular diagnostics in clinical practice.\(^12,13\) Recent reports have demonstrated that a novel lncRNA, metastasis associated in lung adenocarcinoma transcript 1 (\(MALAT-1\)), can predict metastasis and survival in early-stage non-small cell lung cancer.\(^4\) Further study found that \(MALAT-1\) is over-expressed in human hepatocellular carcinoma (HCCs), and in breast, pancreatic, lung, colon and prostate cancers.\(^7\)

Recently, RNA-Seq was developed as an approach to transcriptome profiling that uses deep-sequencing technologies. It has recently provided a far more precise approach for qualitative and quantification lncRNA measurement than other methods.\(^14\) In our previous study,\(^15\) we analysed the transcriptomes of 14 pairs of PCa and matched adjacent normal tissues from patients who underwent radical prostatectomy by RNA-Seq and identified 406 PCa-associated lncRNA transcripts, including two well-known lncRNAs, \(PCA3\) and \(MALAT-1\).

In the light of the low specificity of the serum PSA test, efforts to develop more specific minimally invasive tests to detect and monitor PCa have never ceased. Emerging biomarkers show promise for the early detection of PCa, including prostate-specific membrane antigen (PSMA),\(^16\) hepsin,\(^17\) \(a\)-methylacyl-CoA racemase (AMACR)\(^18\) and so on. And more importantly, the urine \(TMPRSS2:ERG\) fusion transcript test provides new insights into the role of gene fusion as a non-invasive biomarker for diagnosing PCa.\(^19\) This urine gene fusion test, in combination with urine \(PCA3\), improves the utility of serum PSA for predicting PCa risk and clinically relevant cancer on biopsy. Furthermore, although much evidence indicates that circulating microRNAs are promising biomarkers for diagnosing cancer and other diseases,\(^20,21\) lncRNAs have not yet been studied for this purpose. Here, we show that lncRNA fragments are detectable in human plasma in a remarkably stable form. In addition, our results suggest that plasma MD-miniRNA levels may distinguish PCa from controls.

2. Methods

2.1. Clinical samples

All of the clinical samples were obtained from the Shanghai Changhai Hospital (Shanghai, China). All of the subjects gave informed consent. This project was approved by the Clinical Research Ethics Committee of Shanghai Changhai Hospital.

Prostate tumours and their matched adjacent normal tissues were obtained from 14 patients and were defined as the discovery cohort for the RNA-seq. Ten PCa/adjacent normal tissue pairs and a set of 10 tumours and 15 benign prostatic hyperplasia (BPH) samples were selected for the validation. Detailed information about the discovery and validation sets is summarised in Supplementary Table 1.

Twenty-five hospitalised patients with various diseases (Supplementary Table 2) were recruited from the department of urology. One hundred and ninety two consecutive patients, including 87 PCa patients (positive prostate biopsy), 82 patients with negative prostate biopsies (PSA > 4 ng/ml) and 23 healthy controls (PSA < 4 ng/ml) were enrolled in the study from May 2011 to December 2011. Their detailed information is summarised in Supplementary Table 3. The plasma
from another 10 PCa patients was collected before and 7 d after surgical resection (Supplementary Table 3). All of the samples were stored at −80°C until further processing. All of the PCa patients were newly diagnosed and treatment naïve. The final diagnoses of these patients were based on a histological evaluation of PCa biopsy specimens. Tumour status was histological assessed using surgically resected tissue specimens following prostatectomy if possible.

2.2. Sample processing and total RNA isolation

The total RNA was isolated from the tissues using TRIzol reagent (Invitrogen, United States of America (USA)). The plasma and serum sample processing and total RNA isolation have been previously described.20 In brief, whole blood samples were collected and processed within 1 h for plasma collection. For serum collection, the samples were allowed to clot at room temperature for a minimum of 30 min and a maximum of 2 h. All samples were centrifuged at 1800g for 15 min at 4°C in a refrigerated microfuge. The plasma/serum was transferred to a fresh tube, leaving behind a fixed 0.5 cm layer of plasma supernatant to avoid disturbing the pellet. The total RNA was extracted from 350 μl of plasma/serum using a mirVana PARIS Kit (Ambion 1556, USA) strictly in accordance with the manufacturer’s protocol. The RNA was then eluted with 50 μl of Ambion elution solution. The average volume of eluate recovered from each column was 45 μl. The amount of total RNA extracted from 1 ml of plasma/serum is approximately less than 500 ng. Samples whose total RNA were higher than 500 ng extracted from 1 ml of plasma/sera were culled out lest genomic DNA contamination. To rule out genomic DNA contamination, we also did experiments to spike-in increasing amounts of genomic DNA as a positive control in the quantitative real time polymerase chain reaction (qRT-PCR). Compared to the standard curve of spiked-in genomic DNA (Supplementary Fig. 1), we removed the samples which were contaminated by genomic DNA.

2.3. Cell culture

All cell lines were cultured at 37 °C in a 5% CO2 incubator. LNCap-AD, 22Rv1, PC-3, DU145 and C4-2 cell lines were maintained in RPMI medium 1640 (Gibco, Invitrogen, USA) supplemented with 10% fetal bovine serum, 2 mM l-glutamine and 25 mM 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES). LNCaP-AI cell line was maintained in phenol-red free RPMI medium 1640 (Gibco, Invitrogen, USA) supplemented with 10% charcoal stripped fetal bovine serum, 300 mg/L l-glutamine, 2000 mg/L glucose and 2000 mg/L NaHCO3. The normal prostate PWR-1E cell line was cultured in keratinocyte serum free medium (Gibco, Invitrogen, USA).

2.4. Xenograft experiments

All of the animal experiments were performed in accordance with institutional animal welfare guidelines. In brief, 22RV1 cells suspended in a solution of ice-cold 50% basement membrane matrix (BD Matrigel) in Hank’s Balanced Salt Solution (HBSS) (GIBCO) were subcutaneously injected into 5- to 6-week-old male athymic nude mice (nu/nu). An equal number of control mice received mock injections of 200 μl of 50% Matrigel in HBSS. After 4 weeks, the mice were euthanised and their blood was collected in EDTA (ethylenediaminetetraacetic acid) vacutainer tubes by cardiac puncture.

2.5. Northern blot

Total RNA was extracted from cells with Trizol reagent (Invitrogen). RNAs were separated by 1% agarose containing formaldehyde. 30 μg of RNA samples was loaded on the gel. Electrophoresis was carried out at 4 °C and constant 100 V for 1 h in 1× 3-(N-morpholino)propanesulfonic acid (MOPS) buffer. The portion of the gel containing the RNAs was transferred to a nylon membrane (Millipore, Bedford, MA, USA) by electrophoresis at constant 100 V for 45 min. The nylon membrane was then baked for 30 min at 80°C.

Pre-hybridisation was performed at 65 °C for 6 h in 15 ml of solution (10× Denhardt’s solution, 6× single-strand conformation (SSC) and 0.5% sodium dodecyl sulfate (SDS)). Hybridisation was performed at 65 °C for 6 h in 15 ml of the same solution, in the presence of a MALAT-1 RNA probe (5’-GCCCAACAGGAA-CAAGTCTCAATTTTTAAAGGCTCGATG- GAAAAATTCTCAATCTGAAATCCCTAGG- GAAG-3’), labelled with digoxigenin at its 5’ end. Membranes were washed at 65 °C in a 2% SSPE, 0.5% SDS solution for 20 min, hybridised with anti-digoxigenin-AP and visualised with CDP-star, following standard procedures (Roche, Switzerland). Signals were quantified using a Fuji Bioimager Bas2000. Membrane was treated with degradation buffer (0.2 M NaOH, 0.5% SDS) at 37 °C for 0.5 h to degrade the MALAT-1 probe, and then hybridized with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe (5’- CTGATGCCCCCATGTTGTCATGGGTTGA-3’) as an internal control.

2.6. lncRNA fragment quantification by qRT-PCR

qRT-PCR was performed using SYBR® Premix Ex Taq™ (Perfect Real Time) (Takara:DRR081A) with an Applied Biosystems Step One Plus. The gene-specific sequence information for the qRT-PCR primers is given
in Supplementary Table 4. In brief, a fixed volume of RNA elute (2.5 μl) from a given volume of starting plasma (350 μl) was reverse transcribed to cDNA using a PrimeScript® RT reagent Kit (Perfect Real Time) (Takara:RR037A) according to the manufacturer’s instructions. Then 2 μl of the cDNA solution was amplified using 10 μl SYBR® Premix Ex Taq™ (Perfect Real Time) (Takara:DRR081A), 2 μl primers, 0.4 μl ROX Reference Dye (50×) and nuclease-free H2O in a final volume of 20 μl. qRT-PCR was performed according to the manufacturer’s recommended cycling conditions. No amplification of the signal was obtained when nuclease-free water was added instead of cDNA. The data were analysed with StepOne Software version v2.1 (Applied BioSystems, USA). The reactions for generating a standard curve were run in eight replicates. The remaining reactions were run in triplicate.

2.7. Normalisation of experimental qRT-PCR data

The lncRNA expression levels in the tissue/cell samples were normalised to β-actin. The relative expression was calculated using the equation: ΔCt = Ct(target) – Ct(β-actin); Folds = 2^(-ΔCt(tumour) – ΔCt(normal)). Our study is the first to establish the existence of lncRNA fragments in plasma; consequently, no established endogenous plasma mRNA control values for normalising the plasma or serum samples exist. We therefore chose to use a fixed volume of RNA elute (2 μl) from a given volume of starting plasma (350 μl) as the input for the RT reaction. The Ct values of the lncRNA fragments were determined using 40 cycles of SYBR qRT-PCR. We then applied the standard-curve quantitation method, which has been previously described,22 to measure the cDNA copy number of the MD-miniRNA. The cDNA copy number of a particular template was extrapolated from the standard curve (Supplementary Fig. 2) using StepOne Software version v2.1 (Applied BioSystems, USA).

2.8. Statistical analysis

The significance of the tissue, cell, plasma, and serum lncRNA levels was determined by the Mann–Whitney, Wilcoxon, χ², and Kruskal–Wallis tests as appropriate. MD-miniRNA receiver operating characteristic (ROC) curves were constructed to discriminate among different groups of patients. The area under the ROC curve (AUC) was used to assess the predictive power. The sensitivity and specificity were calculated according to the standard formulas. All of the p-values were two-sided and p < 0.05 was considered to be statistically significant. All of the statistical calculations were performed using the SPSS software (version 15.0).

Fig. 1. Validation of prostate cancer (PCa)-related long non-coding RNAs (lncRNAs) in PCa tissues. (A) SYBR quantitative real time polymerase chain reaction (qRT-PCR) was used to assess the expression levels of the indicated lncRNAs in an additional set of 10 pairs of PCa and adjacent normal tissue samples. (B) A scatter plot shows the lncRNA expression levels in 10 PCa and 15 benign prostatic hyperplasia (BPH) tissue samples.
3. Results

3.1. Discovery of PCa-related lncRNA transcripts

To discover the lncRNAs whose expressions are dysregulated in PCa tissues, we compared 14 PCa tissues to their matched adjacent normal tissues using RNA-Seq. Using this method, 406 differentially expressed lncRNAs were identified, including PCA3 and MALAT-1. To validate these results, qRT-PCR was used to assess the expression levels of these two lncRNAs in an additional set of 10 pairs of PCa and adjacent normal tissues. Comparing the PCA3 and MALAT-1 expression levels in the 10 paired tissue samples revealed that PCA3 and MALAT-1 were highly expressed in most of the PCa tissues (Fig. 1A). The results are consistent with our previous study, and Lin et al. A scatter plot of the expression levels of these two lncRNAs in 10 PCa tissue samples and 15 BPH tissue samples showed a remarkable difference between the two groups (Fig. 1B). The dashed line at the 100% sensitivity threshold indicates that PCA3 can distinguish PCa from BPH with 80% specificity, and that MALAT-1 can distinguish at 100% specificity.

3.2. The general characterisation of endogenous lncRNAs and their fragments in plasma

To determine whether plasma lncRNAs can serve as potential biomarkers for detecting PCa, we first explored the existence pattern of plasma MALAT-1. We designed primers for nine amplicons that were found every 1000 bp over the complete transcript. All of the MALAT-1 fragment expression levels were determined from eight PCa plasma samples using qRT-PCR. Interestingly, we found that expression levels were significantly different among different fragments (Fig. 2A). PCA3 was also studied (data not shown). Although there are no available reports that elucidate this phenomenon, the more highly expressed fragments may play a more important role. We then selected...
MALAT-1 and PCA3 fragments with the highest plasma expression levels for further study. The fragments were named MALAT-1 derived miniRNA (MD-miniRNA) and PCA3 derived miniRNA (PD-miniRNA). Because of the low diagnostic power of PD-miniRNA derived from our subsequent examination (Supplementary Fig. 3), PD-miniRNA was only used to demonstrate the general characterisation of the lncRNA fragments in human plasma. In addition, we sequenced the PCR product with the highest plasma expression which was named MD-miniRNA (Fig. 2B). Thus, we proved that the amplicon is indeed the intended fragment derived from MALAT-1.

We next sought to investigate the stability of plasma MD-miniRNA and PD-miniRNA. The plasma was treated under harsh circumstances including multiple freeze-thaw cycles, incubation at room temperature for up to 24 h, incubation at –80°C and acid–base treatment. The expression levels of the indicated fragments remained stable when the plasma was subjected to multiple freeze-thaw cycles (Fig. 3A). The incubation time course had minimal effects on the expression of MD-miniRNA and PD-miniRNA (Fig. 3B). Moreover, the fragments showed no significant changes when the plasma storage time was prolonged (Fig. 3C) or when the samples were treated with strong acid and base.
Plasma MD-miniRNA and PD-miniRNA were also subjected to RNA degradation assays. Strikingly, RNAses had hardly any effect on the indicated plasma fragments (Fig. 3E). The rest of eight primer pairs were also used to measure the levels of miniRNA under degradation conditions. Collectively, our results suggested that plasma lncRNA fragments are stable, detectable and RNAse degradation-resistant, which provides a foundation for evaluating them as useful cancer biomarkers.

3.3. Comparison of lncRNA fragment levels between EDTA plasma, heparin plasma and serum

Because clinical blood specimens are usually collected by venipuncture using different standard vacutainer tubes, we initiated a study comparing the lncRNA levels in EDTA plasma, heparin plasma and serum. A pairwise comparison found that the EDTA tubes produced high and stable RNA levels. However, a significant decline in the target lncRNA expression levels occurred in the heparin plasma (Fig. 4). Our results indicated that EDTA, rather than heparin plasma is the preferred plasma treatment for further study of lncRNA fragments as blood-based biomarkers. It is because that heparin can exert an inhibitory effect on reverse transcription and of polymerase chain reaction. Additionally, a pairwise comparison indicated that plasma and serum both effectively maintain the stability of the targeted lncRNA fragments (Fig. 4). Our study suggested that plasma and serum are both suitable for investigating lncRNA fragments as blood-based biomarkers.

3.4. MD-miniRNA is present in cell culture medium

After demonstrating that circulating lncRNA fragments are detectable and stable, we next sought to investigate whether MD-miniRNA is present in cell culture medium. Before carrying out this experiment, qRT-PCR (Fig. 5A) and Northern blot (Fig. 5B) were used to detect MALAT-1 in different seven prostate cell lines which included one human normal prostate cell (PWR-1E) and six human PCa cells (C4-2, PC-3, DU145, LNCap-AD, 22RV1, LNCap-AI);. Then, six human prostate cell lines (PWR-1E, 22RV1, LNCap-AD, LNCap-AI, C4-2, and PC-3) were selected. The cell culture medium was collected 1, 2 and 3 d after cell passage. Sample processing and total RNA isolation were...
performed with the same protocol that was used for the plasma. The MD-miniRNA was detectable in the cell culture medium. The MD-miniRNA expression level steadily increased over time in PCa cell lines, but the expression showed hardly change in normal prostate cell (Fig. 5C).

3.5. PCa-expressed MD-miniRNA is present in plasma of PCa xenografts

We next studied whether MD-miniRNA can enter the circulation at levels sufficient to be detectable as a biomarker for diagnosing PCa. A mouse subcutaneous xenograft model system was used. Blood samples were collected once the tumours were well established (approximately 1 month after injection). The RNA was isolated within 2 h after blood collection. qRT-PCR was used to assess the MD-miniRNA expression levels and demonstrated that the presence of cancer can lead to a significant increase in plasma MD-miniRNA expression (Fig. 5D). In conclusion, xenograft-derived MD-miniRNA can enter the circulation of mice.

3.6. Validation of the source of plasma MD-miniRNA

To further verify that the high level of MD-miniRNA expression is derived from PCa, the MD-miniRNA plasma level was assessed in PCa patients \( n = 10 \) before (pre-Op) and 7 d after (7 d post-Op) surgical removal of the tumour. The MD-miniRNA levels were found to decline significantly following surgery \( (p = 0.022, \text{Wilcoxon test}; \text{Fig. 5E}) \). Serum PSA level \( (p = 0.005, \text{Wilcoxon test}; \text{Fig. 5F}) \) and plasma PD-miniRNA expression level \( (p = 0.037, \text{Wilcoxon test}; \text{Supplementary Fig. 4}) \) were also found to decline from pre-Op to post-Op samples. This finding indicated that plasma MD-miniRNA is derived from prostate tumours.

3.7. Validation of MD-miniRNA as a plasma-based biomarker for diagnosing PCa

To explore the potential role of MD-miniRNA as a biomarker for detecting PCa, particularly to discriminate between patients with positive prostate biopsies and patients with negative prostate biopsies, the MD-miniRNA expression levels in 87 PCa plasma samples (positive biopsy), 82 BPH plasma samples (negative biopsy) and 23 healthy controls (PSA < 4 ng/ml) were measured by qRT-PCR. The MD-miniRNA copies per microlitre plasma were calculated using the MD-miniRNA standard curve. The MD-miniRNA expression levels were significantly elevated in the PCa patients compared to the non-PCa patients \( (p < 0.001; \text{Fig. 6}) \).
Fig. 6A). The MD-miniRNA expression levels of the negative biopsy patients were much lower than those of the PCa patients ($p < 0.001$, Fig. 6B; $p = 0.001$, Fig. 6C).

We next examined the diagnostic performance of MD-miniRNA. An AUC-ROC analysis of using serum PSA (ng/ml) and plasma MD-miniRNA (copies/μl) for PCa diagnosis is summarised in Table 1. Fig. 6D–F shows the diagnostic power of PSA and MD-miniRNA. MD-miniRNA is more effective at distinguishing PCa from non-PCa (AUC-ROC, 0.836) than PSA (AUC-ROC = 0.770), and more effective at discriminating positive biopsies from negative biopsies (AUC-ROC = 0.841) than PSA (AUC-ROC = 0.708), especially when PSA = 4–10 ng/ml (AUC-ROC = 0.767 for MD-miniRNA versus AUC-ROC = 0.446 for PSA). Further analysis of the diagnostic performance of MD-miniRNA revealed that at a cut-off of 867.8 copies of MD-miniRNA per microlitre, the sensitivity was 58.6%, 58.6% and 43.5% and the specificity was 84.8%, 84.1% and 81.6% for discriminating PCa from non-PCa, positive biopsy from negative biopsy (PSA > 4 ng/ml) and positive prostate biopsy from negative prostate biopsy (PSA = 4–10 ng/ml), respectively. According to our results, MD-miniRNA levels are a promising indicator for discriminating between PCa and normal patients, and between positive and negative biopsy patients.

4. Discussion

4.1. lncRNA fragments are stable and detectable in human plasma/serum

Recently, the presence of extracellular or cell-free RNA in circulation has become a promising diagnostic and prognostic tool for human cancers. Although much success has been achieved using circulating mRNAs as potential biomarkers for cancer diagnosis and prognosis, lncRNAs have not yet been studied in this context. This is the first systematic study of the characteristics of lncRNAs and their fragments in plasma. Our study indicated that lncRNAs most likely do not exist in a full-length form, although some remarkably stable fragments are highly expressed and detectable in human plasma. These results are consistent with those reported by Tsui et al. and Ng et al. The molecular mechanism to elucidate the existence patterns of MALAT-1 in plasma remains unclear. Researchers have recently identified a highly conserved small ncRNA mascRNA originating from the 3’ end of mMALAT1 which localises to the cytoplasm. Although plasma MD-miniRNA and this small tRNA-like cytoplasmic mascRNA share the same phenomenon of MALAT-1 existence patterns, MD-miniRNA does not correspond to mascRNA (Supplementary Table 5). In addition, we also proved that EDTA plasma is preferable to heparin plasma for future investigations and that both plasma and serum are good sources of lncRNAs.

4.2. lncRNA fragments are secreted from tumour cells and enter the circulation

What is the source of the circulating lncRNA fragments? Because MALAT-1 is highly expressed in many normal tissue types, the high MD-miniRNA expression in plasma may be due to other organs in the body. However, other study and our present study found that MALAT-1 is over-expressed in PCa tissues and PCa cell lines, so we hypothesised that most lncRNA fragments are secreted from tumour cells and enter the circulation. Our cell experiment demonstrated that MD-miniRNA can be detected in cell culture medium. The xenograft experiment and plasma MD-miniRNA assessing between pre-Op and post-Op further indicated that lncRNA fragments derived from tumour cells can enter the circulation. Although the mechanism by which MD-miniRNA is secreted and enters the bloodstream is unclear, the PCa diagnostic performance of MD-miniRNA deserves further study.

4.3. MD-miniRNA as a blood-based biomarker for PCa detection

The deregulation of lncRNAs has been reported in various human cancers. Several studies have identified and characterised PCA3 as a promising urine-based biomarker to detect PCa. Examining plasma lncRNA
expression levels constitutes a new and promising method for detecting PCAs and other diseases.

In the present study, we identified differentially expressed MALAT-1 using RNA-seq to compare PCA tissues and their matched adjacent normal tissues. We next performed the first published investigation of the diagnostic performance of MD-miniRNA for PCA detection. According to our results, MD-miniRNA might be a promising plasma-based biomarker to discriminate PCA patients from healthy controls, and can predict the biopsy outcomes when PSA is abnormal (PSA > 4 ng/ml). Intriguingly, we have also observed that some normal controls have high MD-miniRNA expression. These normal controls are occasionally acquired from health volunteers whose PSA level is under 4 ng/ml. Although they may be false-positive events, our results indicated that it is necessary to pay more attention to these people. In the previous study, the authors discovered that with PSA levels of 4.0 ng per millilitre or less – levels generally thought to be in the normal range, the rate of biopsy-detected PCAs is 15.2%. Therefore, we believe that those who have high MD-miniRNA expression may have high risk of cancer. These healthy controls need to be further evaluated and given intense follow-up.

In this present era, no other biomarkers could replace PSA for PCA diagnosis. But the emerging new biomarkers could be a complement. PSA is a prostate-specific biomarker, whose expression increases due to either cancer, prostatitis or benign prostate hyperplasia (BPH). Thus, as a diagnostic marker, PSA alone could be very sensitive but with low specificity. Our results demonstrated that MD-miniRNA is a PCA-related biomarker. MD-miniRNA is useful for those patients with elevated PSA, it could help predict prostate biopsy outcome. Although MALAT-1 is a ubiquitously transcribed RNA and its alteration has been found in several other malignancies, the combination of PSA and MD-miniRNA could improve the specificity for the detection of PCAs while maintaining the high sensitivity.

5. Conclusion

Our results showed that plasma MD-miniRNA can be used as a potential non-invasive molecular marker for PCa diagnosis. This is a preliminary study, and is limited by the small sample size. Further large-scale studies are needed to confirm our findings.

Authors’ contributions

S.R., F.W., Y.S., J.S., X.G. and Z.L. designed the experiments. F.W., J.S., Yi S. and W.X. prepared tissue and blood specimens for RNA extraction. J.L., M.W. and W.C. performed the cellular experiments. F.W. and Z.Z. performed the animal experiments. F.W. and J.S. performed the qRT-PCR. F.W., C.X. and J.H. analysed the data. F.W., S.R., Y.S., X.G. and Z.L. drafted and revised the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2013.04.026.

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