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Original article

Tumor expression of miR-34a-3p is an independent predictor of recurrence in non−muscle-invasive bladder cancer and promising additional factor to improve predictive value of EORTC nomogram

Jaroslav Juracek, M.Sc.[a], Michal Stanik, M.D., Ph.D.[b,*], Petra Vesela, B.S.[a], Lenka Radova, Ph.D.[a], Jan Dolezel, M.D., Ph.D.[b], Marek Svoboda, M.D., Ph.D.[c], Ondrej Slaby, Ph.D.[a,c,*]

[a] *Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
[b] *Department of Urologic Oncology, Masaryk Memorial Cancer Institute, Brno, Czech Republic*
[c] *Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Brno, Czech Republic*

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Abstract

Objectives: Non−muscle-invasive bladder cancer (NMIBC) is a heterogeneous disease characterized by a high primary tumor recurrence rate. Current prognostic systems used for predicting recurrence in individual patients have limitations and do not consider the biological background of this tumor type. Our study aimed to find microRNAs (miRNAs) associated with NMIBC recurrence.

Methods: Seventy-eight NMIBC patients were prospectively enrolled and divided into exploratory and validation cohorts. Out of these patients, 32 developed recurrence within 18 months after surgery, while 46 did not show any sign of recurrence after 30 months. Expression profiles of 2,578 miRNAs were obtained using Affymetrix miRNA microarrays and candidate miRNAs validated using the individual quantitative reverse-transcription polymerase chain reaction (qRT−PCR).

Results: The expression profiling revealed a set of 137 miRNAs differentially expressed between NMIBC patients with and without recurrence ($P < 0.05$). In the validation phase, miR-34a-3p had a significantly higher expression in tumors of NMIBC patients without recurrence ($P = 0.0155$). Decreased expression of miR-34a-3p was associated with significantly shorter recurrence-free survival ($P = 0.009$). Cox regression analysis confirmed that miR-34a-3p is an independent biomarker associated with a lower risk of recurrence (hazard ratio (HR) = 0.3184, 95% confidence interval = 0.003−0.681, $P = 0.0258$). Combination of miR-34a-3p and European Organization for Research and Treatment of Cancer risk score into one predictive model enabled to predict individual risk of recurrence with high statistical significance and analytical performance ($P < 0.0001$; area under curve = 0.8368; sensitivity 83%, and specificity 75%).

Conclusions: Our data suggest that miR-34a-3p is an independent biomarker of NMIBC recurrence and a promising candidate for further independent validations as an additional factor to improve predictive value of European Organization for Research and Treatment of Cancer nomogram.

Keywords: Non−muscle invasive bladder cancer; Recurrence; microRNA; miR-34-3p

1. Introduction

Approximately 80% of bladder cancers are non−muscle-invasive bladder cancers (NMIBC). This type of cancer affects various patients and includes pathological stages Ta (noninvasive papillary carcinomas confining to the epithelium or mucosa), T1 (tumors invading subepithelial connective tissue), and carcinoma in situ (flat tumors, nonpapillary carcinomas confined to the urothelium) [1]. Treatment involves local therapy combining transurethral resection of the bladder tumor (TURT) with either intravesical immunotherapy or chemotherapy [2]. Despite relatively good curability and favorable prognosis of NMIBC, 30% to 80% of cases recur, and within 5 years approximately 20% of these tumors progress to muscle-invasive form [3].

*Corresponding author. Tel.: 00420543136902 (O.S.); +420543131111 (M.S.).
E-mail addresses: stanik@mou.cz (M. Stanik), on.slaby@gmail.com (O. Slaby).

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Main parameters used for prediction of recurrence and progression are stage, grade, tumor size, prior recurrence rate, presence of CIS, and multiplicity [4]. Predictive models combining these prognostic factors may be used to stratify patients into risk groups, helpful in the clinical management of bladder cancer. Generally, the probability of recurrence or progression of NMIBC can be estimated using nomograms, provided for instance, by the European Organization for Research and Treatment of Cancer (EORTC) or the Club Urológico Español de Tratamiento Oncológico [3,5]. Although clinically valuable, such tools have significant limitations. They were developed based on the retrospective data in which tumors were staged and graded according to older versions of the TNM classification and the world health organization (WHO) grading system, or based on the inconsistencies in the therapy of enrolled subjects. Moreover, risk nomograms disregard the biological background of an individual tumor, and therefore do not always accurately reflect the clinical outcome. To estimate a risk of recurrence in an individual patient, an effective biomarker is needed. It would also help to find the best treatment and to monitor the disease's progress, in order to avoid tumor recurrence and progression.

MicroRNAs (miRNA) are a class of endogenous, short noncoding, and single stranded RNAs that regulate gene expression at the posttranscriptional level, by binding to the 3′untranslated region of target mRNAs [6]. Deregulation of a miRNA level plays an important role in many human disorders, including cancer, in which miRNAs have emerged as highly tissue-specific biomarkers with potential clinical applicability as diagnostic biomarkers or prognostic predictors [7,8]. In bladder cancer, distinct miRNA alterations can characterize pathobiology and the course of the disease, thus having potential implications for prognosis prediction [9]. In this study, we aimed to find miRNAs associated with recurrence of primary NMIBC, which thus could serve as independent prognostic factors of recurrence.

2. Material and methods

2.1. Patients

The study included 78 primary NMIBC patients undergoing endoscopic treatment with TURT at the Department of Urologic Oncology, Masaryk Memorial Cancer Institute between September 2013 and May 2016. The study was approved by the local ethical committee of Masaryk Memorial Cancer Institute, and all the participants signed informed consent forms.

Out of the 78 patients approached, 32 patients (25 males, 7 females, median age 71) presented recurrence within first 18 months after primary tumor resection, while 46 patients (38 males, 8 females, median age 76) showed no sign of recurrence within follow-ups longer than 30 months. Grading and staging were assigned according to the WHO criteria and TNM classification. The tumors were assigned the following grades: 29 low−grade pTa, 16 high−grade pTa, 12 PUNLMP pTa, 2 high-grade pTa+pTis, 5 low−grade pT1, 10 high−grade pT1, and 4 high-grade T1+pTis. EORTC risk score was calculated accordingly to standard guidelines [3]. Table 1 summarizes the clinical-pathological characteristics of the cohort.

2.2. Tissue sample processing and RNA extraction

Tumor tissue was extracted during TURT, placed in RNAlater Stabilization Solution (Invitrogen by Thermo Fisher Scientific, Waltham, MA) and stored at −80°C until analyzed. Before RNA isolation, the tumor tissue samples were thawed and placed into homogenization tubes containing Ceramic Beads (Qiagen, Hilden, Germany) and Lysis/Binding Buffer (Thermo Fisher Scientific, Waltham, MA). Tissue disintegration was performed using Precellys Evolution tissue homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). Total RNA enriched for fraction of small RNA was isolated using mirVana miRNA isolation kit (Invitrogen by Thermo Fisher Scientific, Waltham,

Table 1
Clinicopathological characteristics of study subjects. IQR stands for an interquartile range

	Exploratory phase		Validation phase	
	Patients with recurrence	Patients without recurrence	Patients with recurrence	Patients without recurrence
No. of patients	7	8	25	38
Median age (IQR)	71 (64−76)	76 (66−80)	73 (67−75)	74 (68−81)
Sex ratio (M:F)	5:2	7:1	20:5	31:7
Pathological stage				
Ta	4	6	16	31
Ta + Cis	1	0	1	0
T1	2	2	4	7
T1 + Cis	0	0	4	0
Grade				
PUNLMP	0	0	1	11
Low-grade	3	3	12	16
High-grade	4	5	12	11

MA), according to the manufacturer's recommendations. The quality and quantity of RNA were determined using the NanoDropTM 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

2.3. MiRNA expression profiling

Expression levels of miRNAs in 7 NMIBC patients, who presented early recurrence and 8 NMIBC patients without recurrence were detected and quantified by the GeneChip miRNA 4.0 Array (Affymetrix by Thermo Fisher Scientific, Waltham, MA), according to the manufacture's protocol. The Affymetrix raw data were normalized and statistically evaluated in R [10] using the Bioconductor package. For further validation, we selected miRNAs matching the predefined criteria (fold change $>$ 10, average signal $>$ 2.0, $P < 0.05$).

2.4. Quantitative reverse transcription PCR

To determine prognostic potentials of selected miRNAs, quantitative reverse transcription PCR (qRT-PCR) was used in 25 NMIBC patients who presented early recurrence

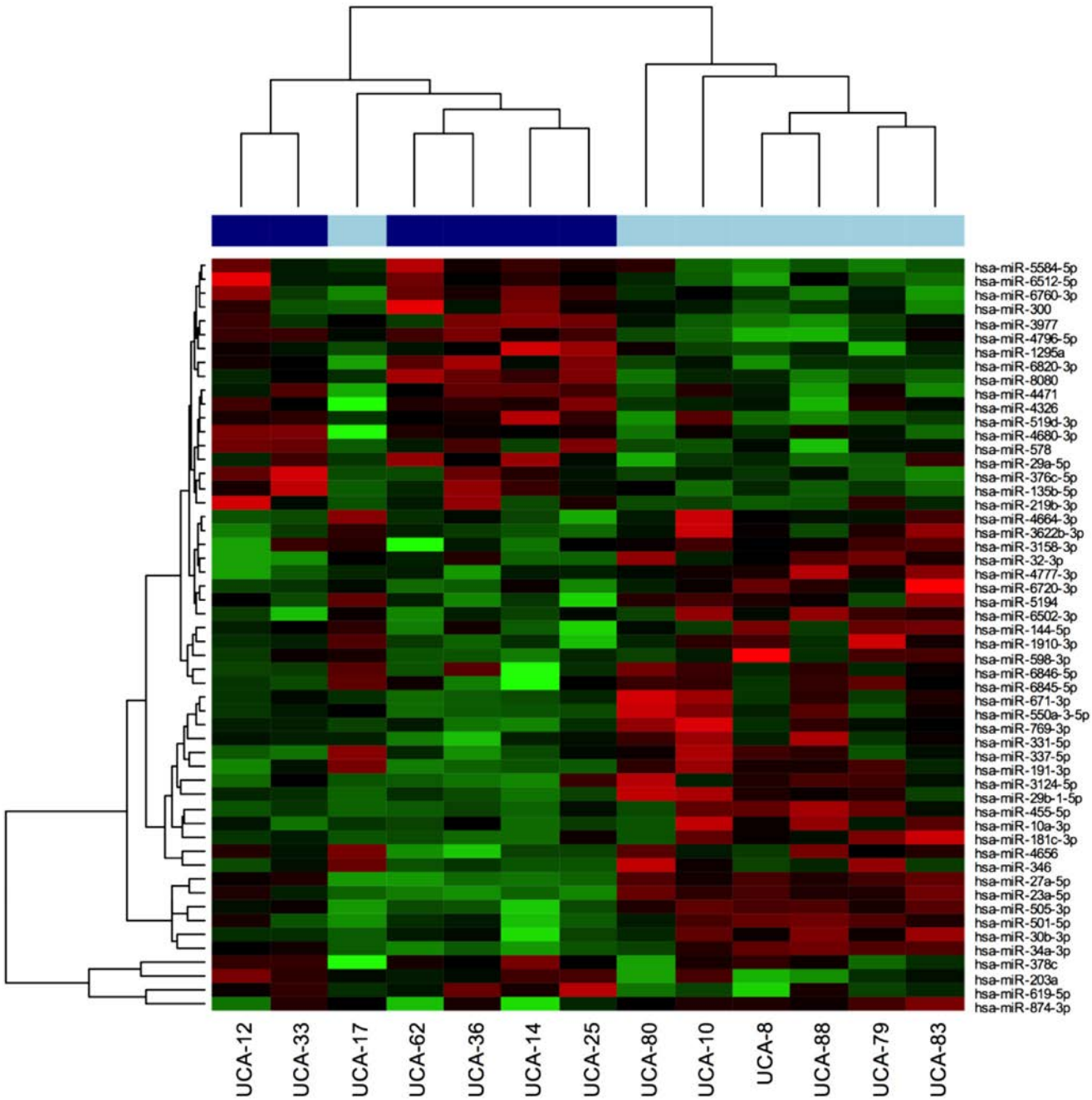

Fig. 1. A hierarchical clustergram representing miRNAs differentially expressed between the NMIBC patients who developed recurrence (dark blue color) and those who did not (light blue color) ($P < 0.025$).

and 38 NMIBC patients who showed no sign of recurrence. Expression level analysis was carried out accordingly to the standard TaqMan MicroRNA Assay protocol (miR-34a-3p: 002316, miR-619-5p: 467223_mat, miR-874-3p: 002268; miR-203a-3p: 464144_mat; miR-27a-5p: 002445; miR-505-3p: 001049; miR-23a-3p: 000399; Thermo Fisher Scientific, Waltham, MA) on Roche LightCycler 480 PCR system. To normalize the measurements, the same amount of total RNA entering the reverse transcription and PCR reaction was used. The average expression of the miRNAs analyzed was normalized using hsa-let-7b, which was selected as a reference gene based on the microarray data and GenEx software. Relative expression was calculated by the $2^{-\Delta Ct}$ method. To enable interplate comparisons and eliminate contaminations, we used interplate controls and nontemplate negative controls for all the qRT-PCR measurements.

2.5. Statistical analysis

Differences between the groups (patients with and without recurrence) in the miRNA levels were compared using the nonparametric Mann–Whitney *U* test. Kaplan-Meier survival analysis was used to analyze correlation between miRNA levels and RFS (recurrence-free survival) of NMIBC patients. The optimal cut-off value of miRNA expression and analytical performance (AUC [area under curve]; sensitivity and specificity) in the discrimination of NMIBC patients with and without recurrence were determined using receiver operating characteristic (ROC) analysis. This statistical analysis was performed with GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA www.graphpad.com). For all the analyses, a significance level of 0.05 was used.

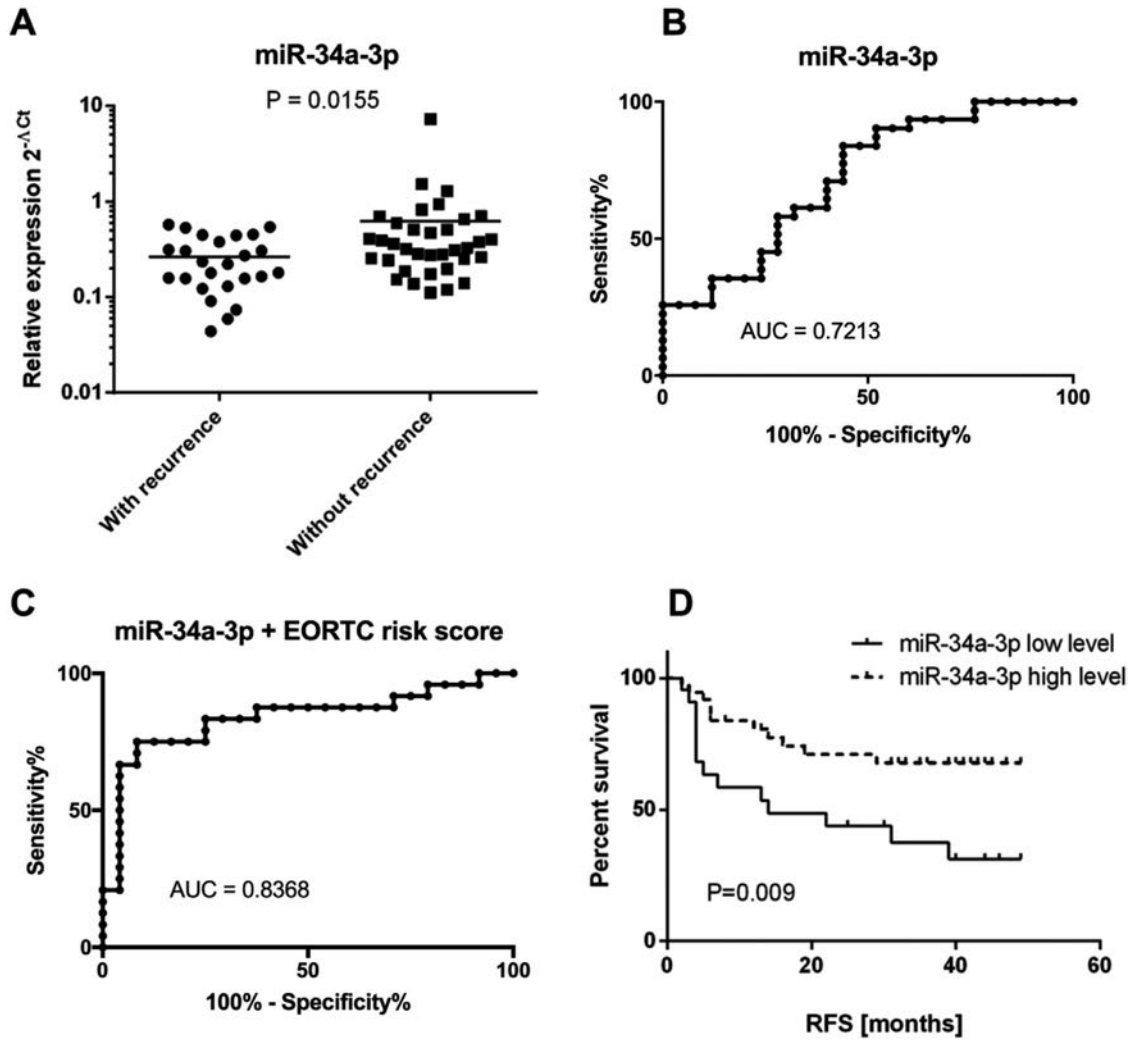

Fig. 2. An independent validation of miR-34a-3p as a predictor of recurrence in NMIBC patients. (A) Expression levels of miR-34a-3p in the NMIBC patients with and without recurrence (Mann-Whitney U test, $P = 0.0155$). (B) ROC analyses using the expression of miR-34a-3p as a discriminator between the patients with and without recurrence ($P = 0.0155$). (C) ROC analyses using the model based on combination of the miR-34a-3p expression and EORTC risk score as a discriminator between the patients with and without recurrence ($P < 0.0001$). (D) Kaplan-Meier survival analysis of miR-34a-3p. NMIBC patients with a lower level of miR-34a-3p have shorter recurrence-free survival (RFS; $P = 0.009$).

3. Results

To identify miRNAs associated with the recurrence status of NMIBC patients, we used a whole-genome miRNA profiling, which enabled us to detect 2,578 human mature miRNAs in 15 NMIBC samples. Subsequent Linear Models for Microarray Data (LIMMA) and hierarchical clustering (HCL) analysis revealed 137 miRNAs differentially expressed in the tumors of NMIBC patients with and without recurrence ($P < 0.05$) (Fig. 1), among which 58 were significantly up-regulated and 79 down-regulated in patients who presented recurrence (see Supplementary Table S1). Using the predefined selection criteria (fold change > 10, average signal > 2.0, $P < 0.05$), we selected seven candidate miRNAs (miR-34a-3p, miR-619-5p, miR-874-3p, miR-203a-3p, miR-27a-5p, miR-505-3p, and miR-23a-3p) for independent validation.

Validation was performed on the cohort consisted of 25 NMIBC patients with recurrence and 38 patients with primary NMIBC without recurrence. Out of the 7 validated miRNAs, only miR-34a-3p confirmed to be associated with a lower risk of recurrence, showing significantly higher expression ($P = 0.0155$; fold change (FC) = 2.4; AUC = 0.7213; sensitivity 71% and specificity 60%; Fig. 2A and B) in the NMIBC patients without recurrence (Table 2). Independently, we have evaluated the association of EORTC risk score (EORTC-RS) with the development of recurrence and have confirmed only statistical trend ($P = 0.0832$; AUC = 0.6433; sensitivity 63% and specificity 61%). Consequently, miR-34a-3p and EORTC-RS were combined in one model and individual risk was calculated according to the formula RiskModel = −0.7228 + 5.8495*miR-34a-3p − 0.41525* EORTC-RS). By use of cut-off = −0.0775, application of this RiskModel reached high statistical significance and analytical performance ($P < 0.0001$; AUC = 0.8368; sensitivity 83% and specificity 75%; Fig. 2C). Kaplan-Meier survival analysis revealed that a decreased expression level of miR-34a-3p was also associated with significantly shorter recurrence-free survival (RFS) of NMIBC patients (the log-rank test, $P = 0.009$) (Fig. 2D). The other examined miRNAs showed no association with RFS. In addition, we did not observe any other significant associations of miRNA levels and clinical-pathological features of NMIBC patients, such as tumor grade, TNM stage, tumor size, or risk group based on an EORTC risk nomogram. In multivariate analysis using the Cox proportional hazards model, the only significant prognostic factor was expression level of miR-34a-3p (HR = 0.3184, 95% confidence interval = 0.003−0.681, $P = 0.0258$; Table 3).

4. Discussion

Although current prognostic systems used for predicting recurrence and progression in individual patients with NMIBC are clinically valuable, they have serious limitations [3]. Even novel classification models [11], which improved evaluation of a NMIBC recurrence risk, show inconsistent results across the worldwide population. The main reason is that these methods use retrospective data, including tumors, classified using different treatment protocols and older versions of the TNM classification and the WHO grading system. Especially intravesical Bacillus Calmette−Guérin vaccine (BCG) therapy combined with ongoing maintenance therapy significantly reduces the risk of recurrence in NMIBC patients [12] and can influence survival data. Most importantly, these classifiers do not comprise the molecular feature of the disease. Thus, they can fail to distinguish tumors that have identical histopathological characteristics, but which belong to distinct molecular subgroups [13−15] with different aggressiveness and clinical risks. Although, several studies have evaluated gene signature to predict the progression of NMIBC [16,17], to date there are no validated tissue or liquid-based biomarkers to complement or replace clinical-pathologic risk stratification.

Therefore, this study aimed to identify miRNAs associated with recurrence of NMIBC. A limited number of studies have focused on miRNAs with prognostic significance in NMIBC. Most of them mainly dealt with extensively studied cancer-related miRNAs [18,19], like Zaravinos et al., who demonstrated that miR-21 and miR-378 might serve as independent prognostic factors for bladder cancer

Table 2
Validation of candidate miRNAs differentially expressed between NMIBC patients with and without recurrence. IQR stands for an interquartile range

	Median expression (IQR)			
	Recurrence	No recurrence	Fold change	*P* value
miR-34a-3p	**0.221 (0.157−0.379)**	**0.328 (0.247−0.551)**	**2.40**	**0.0155**
miR-619-5p	0.702 (0.204−2.445)	0.930 (0.343−2.369)	1.35	0.7126
miR-874-3p	0.047 (0.028−0.067)	0.040 (0.028−0.052)	1.05	0.5440
miR-203a-3p	1.996 (0.835-7.047)	3.871 (0.886−12.231)	1.40	0.5216
miR-27a-5p	0.020 (0.013−0.033)	0.032 (0.015−0.052)	0.51	0.1414
miR-505-3p	0.032 (0.022−0.053)	0.030 (0.014−0.047)	1.07	0.6151
miR-23a-3p	0.001 (0.0003−0.0012)	0.001 (0.0003−0.0014)	0.12	0.4253

miRNA with P value lower than 0.05 is bolded.

Table 3
Univariate and multivariate Cox analysis of recurrence-free survival for NMIBC patients

	Univariate		Multivariate		
	Variable	*P*value	HR	95% CI	*P* value
Sex	male vs. female	0.9537	0.6724	0.159–2.330	0.4692
Age	> 65 vs. < 65	0.9694	1.1016	0.929–1.024	0.3151
Grade	PUNLMP vs. LG vs. HG	0.0255	3.7458	0.881–11.765	0.0771
Stage	Ta vs. T1	0.1208	2.6832	0.483–8.851	0.3281
miR-34a-3p	high vs. low	**0.0134**	**0.3184**	**0.003–0.681**	**0.0258**
EORTC risk group	high (score > 5) vs. low/intermediate (score ≤ 5)	0.1803	0.2576	0.039–1.597	0.1426

miRNA with P value lower than 0.05 is bolded.

recurrence [20]. Similarly, Puerta-Gil et al. reported shorter RFS in NMIBC patients with higher miR-143 and miR-222 levels [21]. These studies, however, lack an exploratory approach based on global miRNA profiling. Jiang et al. took a more suitable approach for prognostic miRNAs identification. They performed genome-wide serum miRNA analysis by next-generation sequencing and validated miR-152 as independently associated with tumor recurrence in NMIBC [22]. In the same way, analysis of miRNA expression signatures in urine revealed that miR-22-3p and miR-200a-3p were independently associated with RFS of NMIBC [23].

To the best of our knowledge, however, no study has focused directly on tumor miRNA profiling used to detect biomarkers enabling one to estimate NMIBC recurrence. To fill this gap, we have analyzed 2,578 human mature miRNAs by microarray technology in clinically well-defined NMIBC patients. For the exploratory phase and subsequent validation, we used tumor tissue samples from a uniform group of NMIBC patients, who presented recurrence within first 18 months after primary lesion extraction. The comparative group consisted of NMIBC patients who showed no sign of recurrence within the follow-up longer than 30 months. From 137 miRNAs differentially expressed between NMIBC patients with and without primary tumor recurrence, we selected four candidate miRNAs. Among them, only miR-203a-3p has been described to play an important role in the molecular pathology of bladder cancer [24]. Another miRNA studied was miR-619-5p, among whose target genes is CD109 (glycosylphosphatidylinositol-anchored glycoprotein) [25]. In another study, expression level of CD109 was elevated in the basal layer of non-invasive urothelial carcinomas and correlated with the pathological grades and tumor stages of urothelial carcinomas [26]. The remaining two miRNAs we studied (miR-34a-3p and miR-874-3p) have not been studied in the context of bladder carcinoma.

Out of these 4 miRNAs, only miR-34a-3p confirmed to have a significantly higher expression level in NMIBC patients without recurrence. Moreover, decreased expression of miR-34a-3p was associated with significantly shorter RFS of NMIBC patients. Subsequent Cox proportional hazards regression confirmed that miR-34a-3p was an independent marker of recurrence. Even though miR-34a-3p was not associated with bladder cancer, there is an evidence that mature miR-34a-3p is generated together with miR-34a-5p [27], which is highly involved in the tumorigenesis of bladder cancer and seems to be associated with a reduced risk of bladder cancer recurrence [28]. Moreover, the list of target genes of miR-34a-3p contains, among others, THBS1, β-catenin, and especially MDM2 [27,29], the master regulator of the tumor suppressor p53. Considering that the loss of p53 function is the most common genetic alteration in bladder cancer, both miR-34a-5p and miR-34a-3p could play important roles in the origin and progression of the disease.

This study has some potential limitations, and still many issues must be addressed to establish miR-34a-3p as a prognostic tool in NMIBC. Since different prognostic miRNAs have been described in other reports—for example, miR-21, miR-143, miR-155, miR-200, miR-214, and miR-222 were reported at least by 2 studies [30]—a model based on their combination might increase prognostic values of tissue miRNAs as prognostic biomarkers in NMIBC. Although the expression level of miR-34a-3p significantly differs between NMIBC patients with and without recurrence, its analytical performance alone is not enough to prognose recurrence. Therefore, as suggested above, we expect this biomarker to be used in combination with other miRNAs as well as the routinely used prognostic nomograms, like the EORTC risk nomogram. Due to a small number of patients in our study, further independent studies are needed to confirm the prognostic potential of miR-34a-3p.

In conclusion, this study was the first to focus on the identification of tumor miRNAs associated with NMIBC recurrence. We have identified and validated miR-34a-3p, for the first time studied in the context of bladder carcinoma as an independent biomarker of recurrence in NMIBC patients and promising additional factor to improve predictive value of EORTC nomogram.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.urolonc.2018.10.014.

References

[1] Sexton WJ, Wiegand LR, Correa JJ, Politis C, Dickinson SI, Kang LC. Bladder cancer: a review of non-muscle invasive disease. Cancer Control 2010;17:256–68.

[2] Aldousari S, Kassouf W. Update on the management of non-muscle invasive bladder cancer. Can Urol Assoc J 2010;4:56–64.

[3] Babjuk M, Burger M, Zigeuner R, Shariat SF, van Rhijn BW, Compérat E, et al. EAU guidelines on non−muscle-invasive urothelial carcinoma of the bladder: update 2013. Eur Urol 2013;64:639–53.

[4] van Rhijn BW, Burger M, Lotan Y, Solsona E, Stief CG, Sylvester RJ, et al. Recurrence and progression of disease in non−muscle-invasive bladder cancer: from epidemiology to treatment strategy. Eur Urol 2009;56:430–42.

[5] Fernandez-Gomez J, Solsona E, Unda M, Martinez-Piñeiro L, Gonzalez M, Hernandez R, et al. Prognostic factors in patients with non−muscle-invasive bladder cancer treated with bacillus Calmette-Guérin: Multivariate analysis of data from four randomized CUETO trials. Eur Urol 2008;53:992–1002.

[6] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.

[7] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257–61.

[8] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834.

[9] Catto JW, Miah S, Owen HC, Bryant H, Myers K, Dudziec E, et al. Distinct microRNA alterations characterize high-and low-grade bladder cancer. Cancer Res 2009;69:8472–81.

[10] R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2014. http://www.R-project.org/.

[11] Ieda T, Muto S, Shimizu F, Taguri M, Yanada S, Kitamura K, et al. Development and validation of a novel recurrence risk stratification for initial non-muscle invasive bladder cancer in Asia. EBioMedicine 2016;12:98–104.

[12] Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Böhle A, Palou-Redorta J, et al. EAU guidelines on non−muscle-invasive urothelial carcinoma of the bladder, the 2011 update. Eur Urol 2011;59:997–1008.

[13] Sjödahl G, Lauss M, Lövgren K, Chebil G, Gudjonsson S, Veerla S, et al. A molecular taxonomy for urothelial carcinoma. Clin Cancer Res 2012;18:3377–86.

[14] Dyrskjøt L, Thykjaer T, Kruhøffer M, Jensen J, Marcussen N, Hamilton-Dutoit S, et al. Identifying distinct classes of bladder carcinoma using microarrays. Nat Genet 2002;33:90–6.

[15] Hedegaard J, Lamy P, Nordentoft I, Algaba F, Høyer S, Ulhøi BP, et al. Comprehensive transcriptional analysis of early-stage urothelial carcinoma. Cancer Cell 2016;30:27–42.

[16] Jeong P, Ha Y, Cho I, Yun S, Yoo E, Kim I, et al. Three-gene signature predicts disease progression of non-muscle invasive bladder cancer. Oncol Lett 2011;2:679–84.

[17] Dyrskjøt L, Reinert T, Algaba F, Christensen E, Nieboer D, Hermann G, et al. Prognostic impact of a 12-gene progression score in non-muscle invasive bladder cancer: a prospective multicenter validation study. Eur Urol 2017;72:461–9.

[18] Zhang X, Zhang Y, Liu X, Fang A, Wang J, Yang Y, et al. Direct quantitative detection for cell-free miR-155 in urine: a potential role in diagnosis and prognosis for non-muscle invasive bladder cancer. Oncotarget 2016;7:3255–66.

[19] Ma L, Xu Z, Xu C, Jiang X. MicroRNA-148a represents an independent prognostic marker in bladder cancer. Tumor Biol 2015;37:7915–20.

[20] Zaravinos A, Radojicic J, Lambrou G, Volanis D, Delakas D, Stathopoulos E, et al. Expression of miRNAs involved in angiogenesis, tumor cell proliferation, tumor suppressor inhibition, epithelial-mesenchymal transition and activation of metastasis in bladder cancer. J Urol 2012;188:615–23.

[21] Puerta-Gil P, García-Baquero R, Jia A, Ocaña S, Alvarez-Múgica M, Alvarez-Ossorio J, et al. miR-143, miR-222, and miR-452 are useful as tumor stratification and noninvasive diagnostic biomarkers for bladder cancer. Am J Pathol 2012;180:1808–15.

[22] Jiang X, Du L, Wang L, Li J, Liu Y, Zheng G, et al. Serum microRNA expression signatures identified from genome-wide microRNA profiling serve as novel noninvasive biomarkers for diagnosis and recurrence of bladder cancer. Int J Cancer 2014;136:854–62.

[23] Du L, Jiang X, Duan W, Wang R, Wang L, Zheng G, et al. Cell-free microRNA expression signatures in urine serve as novel noninvasive biomarkers for diagnosis and recurrence prediction of bladder cancer. Oncotarget 2017;8:40832–42.

[24] Bo J, Yang G, Huo K, Jiang H, Zhang L, Liu D, et al. microRNA-203 suppresses bladder cancer development by repressing bcl-w expression. FEBS J 2011;278:786–92.

[25] Atambayeva S, Niyazova R, Ivashchenko A, Pyrkova A, Pinsky I, Akimniyazova A, et al. The binding sites of miR-619-5p in the mRNAs of human and orthologous genes. BMC Genomics 2017;18:428.

[26] Hagikura M, Murakumo Y, Hasegawa M, Jijiwa M, Hagiwara S, Mii S, et al. Correlation of pathological grade and tumor stage of urothelial carcinomas with CD109 expression. Pathol Int 2010;60:735–43.

[27] Chafik A. miR-34a-5p and miR-34a-3p contribute to the signaling pathway of p53 by targeting overlapping sets of genes. arXiv preprint arXiv:2016:1608.02756.

[28] Andrew AS, Marsit CJ, Schned AR, Seigne JD, Kelsey KT, Moore JH, et al. Expression of tumor suppressive microRNA 34a is associated with a reduced risk of bladder cancer recurrence. Int J Cancer 2015;137:1158–66.

[29] Cha Y, Kim N, Park C, Lee I, Kim H, Yook J. MiRNA-34 intrinsically links p53 tumor suppressor and Wnt signaling. Cell Cycle 2012;11:1273–81.

[30] Xie Y, Ma X, Chen L, Li H, Gu L, Gao Y, et al. MicroRNAs with prognostic significance in bladder cancer: a systematic review and meta-analysis. Sci Rep 2017;7:5619.