Expression of microRNAs following radiation therapy and association with severity of radiotherapy-induced toxicity among patients with prostate adenocarcinoma: A systematic review and meta-analysis

JAGTAR SINGH[1], THANUJA THACHIL[2], SEMA MISIR[3], DILER US ALTAY[4], SERAP OZER YAMAN[5], GURPREET SINGH[6], MATHEW SUJI EAPEN[7], KIELAN DARCY McALINDEN[7], NINA PETROVIC[8,9] and SUKHWINDER SINGH SOHAL[7]

[1]Genesis Care Pty Ltd., Alexandria, New South Wales 2015; [2]Austin Radiation Oncology Centre, Ballarat Central, Victoria 3350, Australia; [3]Department of Biochemistry, Faculty of Pharmacy, Campus Sivas Cumhuriyet University, Sivas 58140; [4]Department of Nutrition and Dietetics, Faculty of Health Sciences, Ordu University Cumhuriyet Campus, Ordu 52200; [5]Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon 61080, Turkey; [6]Department of Mental Health, Monash Health, Clayton, Melbourne, Victoria 3168; [7]Respiratory Translational Research Group, Department of Laboratory Medicine, School of Health Sciences, College of Health and Medicine, University of Tasmania, Launceston, Tasmania 7250, Australia; [8]Laboratory for Radiobiology and Molecular Genetics, VINČA 'Institute of Nuclear Sciences-National Institute of The Republic of Serbia', University of Belgrade; [9]Institute for Oncology and Radiology, Belgrade 11000, Serbia

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Abstract. Radiation-induced normal tissue toxicity is a common acute and chronic outcome of radiotherapy (RT) for prostate cancer (PCa). There are currently no existing pre-assessments before treatment to predict acute and late RT-induced toxicity. Novel predictive blood biomarkers in radiation oncology may improve treatment decision-making and therapeutic monitoring for patients with PCa. A comprehensive systematic search of microRNA (miRNA/miR) profiling studies published in PubMed, Science Direct and Google Scholar was performed. The present systematic review, supported by meta-analysis, summarises key findings and discusses the results of prospective clinical studies investigating miRNA expression levels and their association with RT-induced toxicity in PCa. Out of seven clinical studies, six highlighted levels of 10 miRNAs changing in plasma, serum or peripheral blood mononuclear cells during RT. The post-RT expression levels of miRNA-132-5p, miRNA-1-3p, miRNA-410 and miRNA-221 were increased, and miRNA-23a-3p expression was decreased among patients with low-grade RT-induced toxicity. Furthermore, in patients with high-grade RT toxicity, miRNA-197-3p, miRNA-151a-5p, miRNA-18b-5p, miRNA-99a and miRNA-21 expression was increased, while miRNA-132-5p expression was decreased. The present miRNA meta-analysis could be the focus of future studies to identify their potential clinical value as PCa biomarkers and therapeutic mediators in RT-induced toxicity. The variations in miRNA levels post-RT could be used as predictive biomarkers of RT-induced toxicity. However, further extensive validation is required to establish the relationship between miRNA expression levels and RT-induced toxicity in PCa and to confirm their predictive value.

Correspondence to: Dr Jagtar Singh, Genesis Care Pty Ltd., 41-43 Bourke Road, Alexandria, New South Wales 2015, Australia
E-mail: jagtar.singh@genesiscare.com

Key words: prostate cancer, radiotherapy, radiotherapy-induced toxicity, plasma or serum, microRNA expression

Introduction

Prostate cancer (PCa) is the second most frequently diagnosed malignancy (excluding non-melanoma skin cancer) and is the fifth leading cause of cancer-related mortality in men worldwide (1,2). According to the Global Cancer Observatory statistics for 2020, >1.4 million men were newly diagnosed with PCa and 375,304 associated deaths were recorded in 2020 (2). However, the ability to diagnose and determine the PCa stage is restricted and insufficiently specific when pre-screening methods such as prostate-specific antigen are used (3).

Radiation therapy or radiotherapy (RT) is a standard treatment provided to patients with locally advanced PCa (4,5), with 50-60% of the total patients relying on this treatment (6). However, patients may experience off-target adverse effects of RT-induced toxicity, harming the surrounding normal tissues (7). RT toxicity is classified as acute or early if it occurs within 3 months of RT completion and is usually resolved within

4-6 weeks post-treatment (8,9). Chronic or late toxicities last several months and even years post-completion of RT and may induce permanent tissue changes (8,10,11). In PCa, symptoms associated with genitourinary (GU) and gastrointestinal (GI) toxicity are common in early RT-induced toxicity and are validated through scoring criteria based on Common Terminology Criteria for Adverse Events (12). The most frequent and acute effect following RT is inflammation, leading to tissue damage and late side effects such as fibrosis (13). Patients with PCa often experience fatigue, prostate atrophy, physiological complications of the urogenital tract, such as bladder and/or erectile dysfunction, urinary incontinence, infertility, diarrhoea, and rectal bleeding, and rarely secondary tumour development, which influences their quality of life (14). However, current pre-treatment assessments cannot be used to predict acute or late RT-induced toxicity (15). Therefore, novel biomarkers are required in RT oncology to predict RT-induced toxicity and improve decision-making, treatment and therapy monitoring of patients with PCa.

There have been some investigations on the possible use of microRNAs (miRNAs/miRs) as biomarkers to predict RT-induced toxicity, including RT-induced dermatitis for breast cancer and esophagitis for non-small cell lung cancer (16,17). miRNAs are a class of small non-coding RNA molecules (21-25 nucleotides) involved in post-transcriptional regulation. miRNAs are important during the DNA damage response and regulate the expression of various genes (18). Thus, they serve a vital role in physiological cellular processes such as cell cycle regulation (19), apoptosis (20) and cancer metastasis (21). In addition, they are stable in different biological samples, such as plasma and serum, under appropriate storage conditions and can be used as an efficient diagnostic marker from liquid biopsies (22). Therefore, in numerous types of cancer (23), including PCa (24), they are available for sampling from bodily fluid-liquid biopsies (25). These inherent features of miRNAs make them attractive candidates for minimally invasive biomarkers in PCa. Several studies have used quantitative PCR (qPCR) or RNA array methodologies to investigate miRNA expression before and after RT exposure in patients with PCa (26-31). Several previous studies have reported that there could be variations in miRNA expression levels in response to RT (32-35). However, few prospective studies clinically investigate miRNAs in blood samples to predict the severity of RT-induced toxicity based on miRNA expression in patients with PCa (26,29-31).

The identification of circulating miRNAs induced by RT may aid in the development of a radiation biomarker for use in clinical diagnostic procedures in the future. Therefore, the present study aimed to examine the latest literature on the impact of RT on the circulating miRNA profile in the blood of patients with PCa. In addition, the present study aimed to demonstrate the association of miRNA expression levels with RT-induced toxicity, and to provide a valuable understanding of carefully selected miRNAs.

Materials and methods

Search strategy. The clinical studies investigating miRNA expression and the association with RT-induced toxicity in PCa were identified using electronic databases [PubMed (https://pubmed.ncbi.nlm.nih.gov/), Science Direct (https://www.sciencedirect.com/) and Google Scholar (https://scholar.google.com.au/)]. Furthermore, reference lists of relevant studies were assessed to identify further appropriate studies. The systematic search for miRNA studies was carried out using the following key words: Prostate cancer, plasma, serum, miRNA expression, side effects of RT, RT-induced toxicity, genitourinary and gastrointestinal toxicity.

Selection (inclusion and exclusion) criteria. Titles and abstracts of relevant studies were evaluated for their contents, ensuring adherence to both inclusion and exclusion criteria for the systematic review. The inclusion criteria were: i) Studies investigating miRNA expression in patients with PCa only; ii) studies investigating the patient's blood plasma or serum and peripheral blood mononuclear cells (PBMCs) for miRNAs; iii) the study recorded the sample size, sampling methods, diagnostic methods, patient characteristics and clinicopathological outcome; and iv) studies analysed the association of miRNA expression levels with RT-induced toxicity. The exclusion criteria for the systematic review were: i) Studies investigating miRNA expression in other types of cancer; ii) editorials, commentaries and review articles; iii) studies investigating miRNA expression in animal samples and *in vitro* cell lines; and iv) non-English language published studies.

Study review methods and outcome measure. The relevant published articles were retrieved in January 2023 and June 2023 and imported into an Endnote X21 database (36). Analogous articles were identified and deleted using the duplicate function in Endnote. Furthermore, the article titles and abstracts were carefully screened to avoid irrelevant studies. Only studies describing multivariable-adjusted hazard ratios were considered. Studies that reported crude or unadjusted outcome measures among patients treated with RT were excluded.

Data extraction. Two reviewers independently extracted the following data from eligible studies related to PCa: i) General information (first author, publication year, method of patient recruitment and sampling methods); ii) clinical characteristics such as T-stage, age, treatment option, number of patients and follow-up period; iii) clinical outcomes: Biochemical recurrence, side effects of RT or RT-induced toxicity; and iv) diagnostic methods: miRNA array and reverse transcription-qPCR (RT-qPCR).

Quality assessment. The Quality Assessment of Diagnostic Accuracy Studies-2 tool was used to evaluate the quality of the included studies. Every assessment question received a score of 'yes', 'no' or 'unclear' (37). The case selection process, index test, reference standard, case procedure and progress are all included in this assessment. 'Yes (1)', 'No (-1)' and 'Unclear (0)' are the scores. Lastly, the overall score indicates the calibre of the research in the following ways: 8-14 denotes high-quality literature, while 0-7 denotes low-quality literature with a high likelihood of bias.

Furthermore, to further assess the quality of the retrieved studies, the articles were evaluated based on the following principles: i) Studies included the clinical characteristics

of participants and blood samples in a detailed description; ii) studies that met the inclusion and exclusion criteria for participants; iii) studies reported disease course stage and starting point among all the participants; iv) studies described the association between clinical characteristics and outcomes; and v) studies considered other factors that influence the predictive result.

Meta-analysis. The Comprehensive Meta-Analysis programme was used to compare the effect sizes of selected miRNA studies with the groups of RT-induced PCa toxicity. 'Hedge's g' was used to determine the effect size due to the difference in sampling and measurement tools in the calculations (38). In meta-analysis studies, fixed effects or random effects models are used according to heterogeneity (39). The fixed effects model is applied when the effect sizes of the studies included in the meta-analysis do not change, whereas the random effects model is applied when the effect sizes differ between studies (39).

The effect size can be classified as a strong effect size if it is >0.80 and a weak effect size if it is <0.20. According to this classification, $d \leq 0.20$ is considered a weak effect size, $0.20 < d < 0.80$ is considered a medium effect size and $d \geq 0.80$ is considered a strong effect size (40). Cochran's Q statistics, P-value and I^2 tests were used to test the heterogeneity of effect sizes. In the heterogeneity assessment, if the heterogeneity rate (I^2) is <25%, it is absent; 25-50% is considered low; 51-75% is considered moderate; and >75% is considered high (41).

The asymmetry of the funnel plot was tested using linear regression to assess publication bias. The funnel plot did not exhibit any noticeable asymmetry. The closer the regression line is to 90˚, and the smaller the angle between it and the diagnostic odds ratio (DOR) axis, the less likely it is to exhibit bias. The angle in this figure is extremely near to 90˚, which suggests that there is no discernible publication bias and that the findings of the meta-analysis are trustworthy.

Results

Study search. The literature search identified 175 studies: 41 from PubMed, 21 from Science Direct and 113 from Google Scholar. Of these 175 studies, 88 were excluded following the title review and 87 studies were selected at the first screening stage. At the second screening stage, 63 studies were removed following abstract examination and 24 were selected. At the eligibility criteria stage, 17 studies were removed for the following reasons: Outcomes not evaluated (n=3), systematic review articles (n=10), duplication of study groups (n=2), a relationship of RT with miRNA levels was not considered (n=1) and a study on animal samples (n=1). Ultimately, after eligibility consideration, seven articles were selected, and Fig. 1 shows the literature search and selection strategy as a flowchart.

miRNA expression levels in response to RT. Few clinical studies have evaluated miRNA levels of interest in blood samples (serum or plasma) collected from pre-RT baseline, during a fractionated RT course, and through to follow-up (Table I) (26-31,42). Of the seven studies, six highlighted modified peripheral blood lymphocyte, plasma and serum miRNA expression levels in the group of patients with PCa post-RT (26-31,42). Zedan *et al* (42) observed significantly lower miRNA-93 and miRNA-221 levels in the follow-up samples compared with baseline samples (P=0.006 and $P \leq 0.001$, respectively). Furthermore, miRNA-93 downregulation was more significant in the RT subgroup (P=0.018) than in the radical prostatectomy (RP) subgroup (P=0.030). Conversely, miRNA-221 plasma levels were more downregulated in the RP subgroup ($P \leq 0.001$) than in the RT subgroup (P=0.028) (42).

Similarly, a pilot study also observed the effect of RT on miRNAs and identified elevated levels of miRNA-21 in the post-RT group compared with the pre-RT group (P=0.043) (26). Rana *et al* (31) reported that six miRNAs, including miRNA-132-5p (upregulated; P=0.001), miRNA-23a-3p (downregulated; P=0.020), miRNA-1-3p (upregulated; P=0.047), miRNA-197-3p (upregulated; P=0.017), miRNA-151a-5p (upregulated; P=0.031) and miRNA-18b-5p (upregulated; P=0.020), showed variation in expression post-RT compared with pre-RT. In an additional study, miRNA-410 and miRNA-221 expression levels were also altered in post-RT compared with pre-RT blood samples (29). Another study by Someya *et al* (30) also found statistically significant differences in the expression of miRNA-199a in post-RT samples. Upregulation of hsa-let-7a-5p and hsa-miRNA-21-5p was identified after RT, and the difference was significant only in the high-risk group (P=0.037) (28). Upregulation of two miRNAs, hsa-let-7a-5p (fold change, 2.24) and hsa-miRNA-21-5p (fold change, 1.77), was observed to be potentially induced by RT (28).

Out of seven studies, one study indicated no significant variation (P>0.05) in plasma miR-223 and miR-126 expression levels between the RT-treated and control groups (27). The lack of variation and significant differences in miRNA expression may indicate that these miRNAs are not tumour-specific in serum/plasma.

miRNAs as biomarkers for RT-induced toxicity. The included studies reported the possible association between miRNA expression levels and RT-induced toxicity in patients with PCa. Out of the seven studies, four indicated an association between miRNA expression levels and RT-induced toxicity (26,29-31). The studies investigating blood-based miRNA biomarkers and RT-induced toxicity are summarised in Table II.

In the low-toxicity group, miRNA-410 and miRNA-221 expression levels were significantly increased after RT and associated with grade 1-2 acute GI toxicity (P=0.020 and P=0.013, respectively) (29). In addition, three miRNAs exhibited variation in expression post-RT compared with pre-RT. miRNA-132-5p (upregulated; P=0.001) and miRNA-1-3p (upregulated; P=0.047) were associated with low RT-induced toxicity, while the expression levels of miRNA-23a-3p (downregulated; P=0.020) were decreased in the low RT-induced toxicity group (31).

Furthermore, in the high-toxicity group, miRNA-21 expression levels were higher among patients with acute GU RT-induced toxicity than among those without GU radiotoxicity (P=0.068); however, this difference was not statistically significant (26). Furthermore, miRNA-99a and miRNA-221 expression levels were elevated in the high-toxicity group

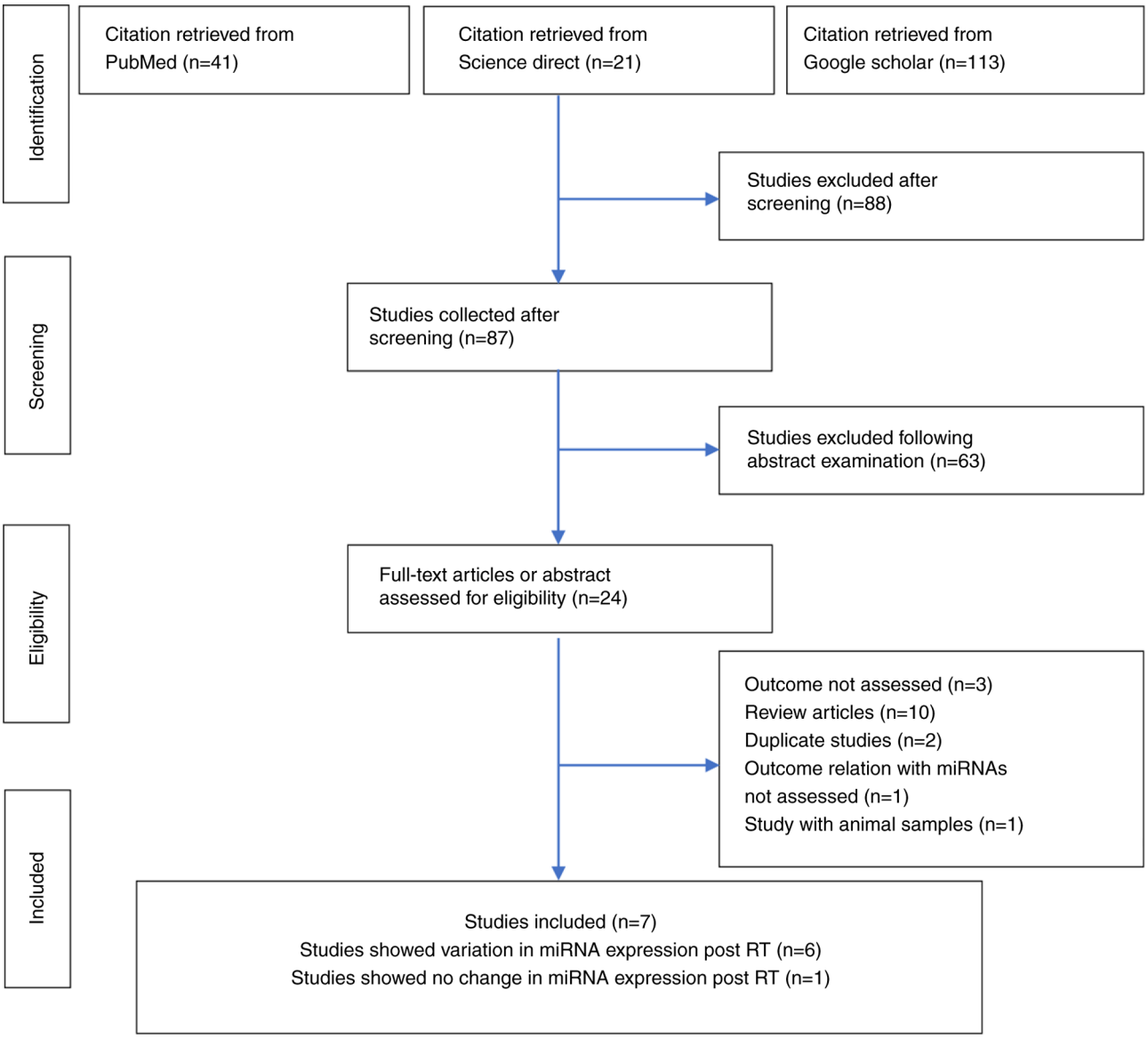


Figure 1. Flow diagram of studies included. miRNA, microRNA; RT, radiotherapy.

(P=0.006 and P=0.050, respectively) (29). In the RT-induced grade 2-3 rectal bleeding group, miRNA-99a expression was higher (P=0.013) after RT (30). Another study reported that miRNA-197-3p (upregulated; P=0.017), miRNA-151a-5p (upregulated; P=0.031) and miRNA-18b-5p (upregulated; P=0.020) expression levels were elevated in post-RT samples compared with pre-RT samples and showed significant association with high RT-induced toxicity (Table III) (31). The study also reported that miRNA-132-5p (downregulated; P=0.003) expression levels were decreased and were associated with the high-toxicity group (31).

Meta-analysis results. Fig. 2 shows the summary results for RT-induced toxicity in PCa. Some miRNAs exhibited altered expression in patients with PCa, including miRNA-221, miRNA-126, miRNA-99a, miRNA-146a, miRNA-125b and miRNA-21. Using the meta-analysis method, a statistically significant signature of two upregulated miRNAs (miRNA-21 and miRNA125b) in RT-induced toxicity in PCa compared with healthy controls was identified. Good performance for RT-induced toxicity in PCa was observed for miRNA21 (95% CI, 0.214-1.076; P=0.003) and miRNA125b (95% CI, 0.139-0.806; P=0.005) expression.

The effect size obtained in the meta-analysis was 0.236 for the random-effects model. As a result of the heterogeneity test, the Q value was estimated as 12.109 and the obtained value was statistically significant (P=0.033). The data obtained in this study were found to be heterogeneous based on the Q test. The I^2 value, which is another indicator for heterogeneity, was 58.710%. This value was high, also indicating heterogeneity. As a result of heterogeneity, the average effect size (point estimate) estimated according to the random effects model was 0.236, and it was determined that there was a moderate effect in the present study according to the Cohen (1988) classification (Fig. 2) (40).

For this, the results of three publication bias tests (Orwin error protection coefficient, Kendall's tau and Egger regression) should also be reported. Orwin's fail-safe N value was

Table I. Observational clinical studies investigating miRNA expression levels following RT in patients with PCa.

Author/s, year	No. of patients	T-stage	Treatment	Type of sample	miRNA detection methods	miRNAs	Results of collected studies	(Refs.)
Kopcalic *et al*, 2019	15	Localized PCa	RT	PBLs	RT-qPCR	miRNA-21, miRNA-146a and miRNA-155	Significantly higher levels of miRNA-21 in the post-RT samples compared with the baseline samples (P=0.043).	(26)
Bahtiyar *et al*, 2018	25	Localized PCa	RT	Blood plasma	RT-qPCR	miRNA-223 and miRNA-126	No significant differences in expression levels of miRNA-223 and miRNA-126 were observed between the RT treated patients and control groups.	(27)
Malla *et al*, 2018	11	Localized PCa	RT	Blood serum	RT-qPCR	hsa-let-7a-5p, hsa-miRNA-141-3p, hsa-miRNA-145-5p, hsa-miR-21-5p and hsa-miRNA-99b-5p	Upregulation of hsa-let-7a-5p and hsa-miRNA-21-5p was identified after RT; the difference was significant only in the high-risk group (P=0.037). The evaluation of has-let-7a-5p and hsa-miRNA-21-5p revealed different expression levels in both risk groups. Upregulation of two miRNAs, hsa-let-7a-5p (fold change, 2.24) and hsa-miRNA-21-5p (fold change, 1.77), was observed to be potentially induced by RT.	(28)
Someya *et al*, 2018	69	Localized PCa	RT	PBLs	RT-qPCR	miRNA-410, miRNA-221 and miRNA-99a	Expression levels of miRNA-410 and miRNA-221 (P=0.020 and P=0.013, respectively) were altered in post-RT blood samples compared with pre-RT blood samples.	(29)
Someya *et al*, 2015	48	Localized PCa	RT	PBLs	miRNA array and RT-qPCR	miRNA-99a	Statistically significant differences in the expression of miRNA-199a in post-RT samples.	(30)
Rana *et al*, 2019	12	Localized PCa	RT	Blood plasma	RT-qPCR	miRNA-132-5p, miRNA-23a-3p, miRNA-1-3p, miRNA-197-3p, miRNA-151a-5p and miRNA-18b-5p	Six miRNAs exhibited differential expression in post-RT samples compared with pre-RT samples: miRNA-132-5p (upregulated; P=0.001), miRNA-23a-3p (downregulated; P=0.020), miRNA-1-3p (upregulated; P=0.047), miRNA-197-3p (upregulated; P=0.017), miRNA-151a-5p	(31)

Table I. Continued.

Author/s, year	No. of patients	T-stage	Treatment	Type of sample	miRNA detection methods	miRNAs	Results of collected studies	(Refs.)
Zedan *et al*, 2019	149	Local or locally advanced cancer	Radical prostatectomy and RT	Blood plasma	RT-qPCR	miRNA-21, miRNA-93, miRNA-125b and miRNA-221	(upregulated; P=0.031) and miRNA-18b-5p (upregulated; P=0.020). Significantly higher levels of miRNA-21 in the post-RT group compared with the control group (P=0.043). Levels of miRNA-93 and miRNA-221 were significantly lower in the follow-up samples compared with the baseline samples (P=0.006 and P<0.001, respectively). The same observation was recorded for miRNA-125b in the observational cohort (P=0.008). Both miRNA-125b and miRNA-221 were correlated with risk assessment (r=0.23, P=0.015, and r=0.203, P=0.016, respectively) while miRNA-93 showed a tendency towards a significant correlation with the prostatectomy Gleason score (r=0.276; P=0.0576).	(42)

miRNA/miR, microRNA; PBL, peripheral blood lymphocyte; PCa, prostate cancer; RT, radiotherapy; RT-qPCR, reverse transcription-quantitative PCR.

Table II. Studies investigating blood-based miRNA expression levels following RT and RT-induced toxicity.

Author/s, year	No. of patients	Tumour stage	Treatment	miRNAs detection methods	miRNAs	Results and comments	(Refs.)
Kopcalic *et al*, 2019	15	Localized PCa	RT	RT-qPCR	miRNA-21, miRNA-146a and miRNA-155	Higher levels of miRNA-21 were observed in patients with acute GU RT-toxicity than in the group without GU RT-toxicity (P=0.068); however, this difference was not statistically significant. Furthermore, within the group of patients who experienced GU RT-toxicity, significantly higher levels of miRNA-21 were identified in the post-RT group compared with the control group (P=0.046).	(26)
Someya *et al*, 2018	69	Localised PCa	RT	RT-qPCR	Low-toxicity patients miRNA-410 and miRNA-221 High-toxicity patients: miRNA-99a and miRNA-221	miRNA-410 and miRNA-221 expression was significantly associated with grade 1-2 gastrointestinal toxicity. Furthermore, miRNA-99a and miRNA-221 expression levels were elevated in the high-toxicity group (P=0.006 and P=0.050, respectively).	(29)
Someya *et al*, 2015	48	Localised PCa	RT	miRNA array and RT-qPCR	miRNA-99a	In the RT-induced grade 2-3 rectal bleeding group, miRNA-99a expression was significantly higher (P=0.013) after RT. Thus, high miRNA-99a expression could be used as a promising marker for predicting rectal bleeding after RT.	(30)
Rana *et al*, 2019	12	Localised PCa	RT	RT-qPCR	Low-toxicity patients: miRNA-132-5p, miRNA-23a-3p and miRNA-1-3p	In the low-toxicity group, three miRNAs exhibited differential expression at post-RT compared with pre-RT: miRNA-132-5p (upregulated; P=0.001), miRNA-23a-3p (downregulated; P=0.020) and miRNA-1-3p (upregulated; P=0.047).	(31)
					High-toxicity patients: miRNA-132-5p, miRNA-197-3p, miRNA-151a-5p and miRNA-18b-5p	In the high-toxicity group, four miRNAs exhibited differential expression at post-RT compared with pre-RT: miRNA-132-5p (downregulated; P=0.003), miRNA-197-3p (upregulated; P=0.017), miRNA-151a-5p (upregulated; P=0.031) and miRNA-18b-5p (upregulated; P=0.020).	

GU, genitourinary; Ku80, Ku autoantigen, 80 kDa; miRNA, microRNA; PCa, prostate cancer; RT, radiotherapy; RT-qPCR, reverse transcription-quantitative PCR.

Table III. miRNAs dysregulated following RT in prostate cancer based on RT-induced toxicity severity.

		miRNA expression post-RT		
Author/s, year	RT-induced toxicity	Increased miRNAs	Decreased miRNAs	(Refs.)
Someya *et al*, 2018; Rana *et al*, 2019	Low toxicity	miRNA-132-5p (P=0.001), miRNA-1-3p (P=0.047), miRNA-410 (P=0.020) and miRNA-221 (P=0.013)	miRNA-23a-3p (P=0.020)	(29,31)
Kopcalic *et al*, 2019; Someya *et al*, 2015; Rana *et al*, 2019	High toxicity	miRNA-197-3p (P=0.017), miRNA-151a-5p (P=0.031), miRNA-18b-5p (P=0.020), miRNA-99a (P=0.013) and miRNA-21 (P=0.068)	miRNA-132-5p (P=0.003)	(26,30,31)

miRNA, microRNA; RT, radiotherapy.

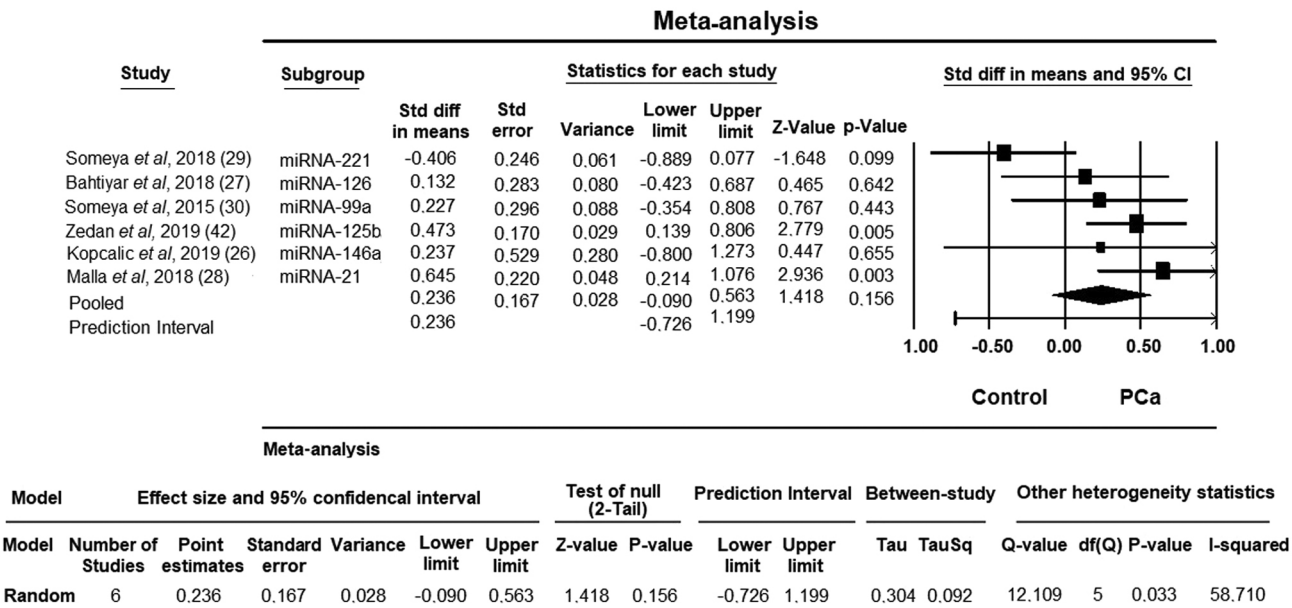
found to be 1693 when trivial value was taken as 0.001, that is, in order to make the relevant Fisher's Z value insignificant. Kendall's tau z value was found to be 0.001 and one-way P-value was found to be 0.500. This is an indication that there is no publication bias. According to the Egger regression intercept results, the intercept value is (β0)=-1.59, t=0.707 and the P-value is 0.259. As demonstrated in Fig. 3, the asymmetry of the funnel plot and Deeks' funnel plot was tested using linear regression in order to assess publication bias. The funnel plot and Deeks' funnel plot did not exhibit any noticeable asymmetry, as shown by a P-value of >0.05 in Fig. 3. The likelihood of a bias is reduced, the closer the regression line is to 90˚, and the smaller the angle between it and the DOR axis. The angle in this figure is extremely near to 90˚, which suggests that there is no discernible publication bias and that the meta-analysis findings are trustworthy. These three tests for publication bias, along with the funnel plot and Deeks's funnel plot results, demonstrated that the results were trustworthy and devoid of publication bias (P>0.05; Fig. 3).

Discussion

In clinical practice, when RT is performed to treat localized PCa, a high dose needs to be delivered to the prostate, while reducing the damage to the surrounding normal tissues. The advancement of therapeutic procedures such as intensity-modulated RT has permitted the escalation of the dose delivered to the prostate, improving local tumour control without markedly increasing RT-induced toxicity (43). At present, researchers are trying to understand the important mechanisms involved in RT-induced toxicity and identify possible molecular biomarkers that could predict RT-induced toxicity (44). Some previous studies have stated that mechanisms such as inflammation and chronic oxidative stress, reduction of tissue stem and progenitor cells, and damage to the microenvironment are involved in RT-induced toxicity (45,46).

In PCa, miRNA expression is dysregulated, and this can modulate the expression of oncogenes and tumour suppressor genes (47-50). Treatment resistance is still a great challenge; in this case, miRNAs could be a novel therapeutic target and predict response to treatments, such as chemotherapy and RT in patients with cancer (47-49,51). Therefore, miRNA expression provides novel perceptions of what treatment is the most appropriate, and if treatment must be changed or adjusted. Furthermore, regarding side effects, changes in miRNA expression can be used to overcome these toxicities or to understand their signs before the need to interrupt the therapy with possible impairment in therapeutic results (26,33,52). For the treatment of patients with PCa with RT, dose-escalation has been established to improve biochemical recurrence control; however, an increased RT dose increases the risk of late GU and GI toxicity (53,54). When considering doses of ≥60 Gy, the majority of dose-volume parameters are linked to late rectal toxicity (55). In addition, grade ≥2 rectal toxicity rates are considerably higher for dose-volume histograms passing above these thresholds than those passing below (55).

miRNA-155 expression can increase or decrease depending on the type of RT, the dose of RT and the rates of RT (32,56). These essential factors contribute to the cellular response to RT as reflected by miRNA expression levels (56). For example,



Meta-analysis

Model	Effect size and 95% confidencal interval						Test of null (2-Tail)		Prediction Interval		Between-study		Other heterogeneity statistics			
Model	Number of Studies	Point estimates	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Lower limit	Upper limit	Tau	TauSq	Q-value	df(Q)	P-value	I-squared
Random	6	0.236	0.167	0.028	-0.090	0.563	1.418	0.156	-0.726	1.199	0.304	0.092	12.109	5	0.033	58.710

Figure 2. Forest plot of effect size, 95% confidence interval, prediction interval and Q-value with corresponding heterogeneity statistics. The points show the estimated and effect size of miRNA expression in PCa patients with RT-induced toxicity. df, degrees of freedom; miRNA, microRNA; PCa, prostate cancer; Std diff, standardised mean difference.

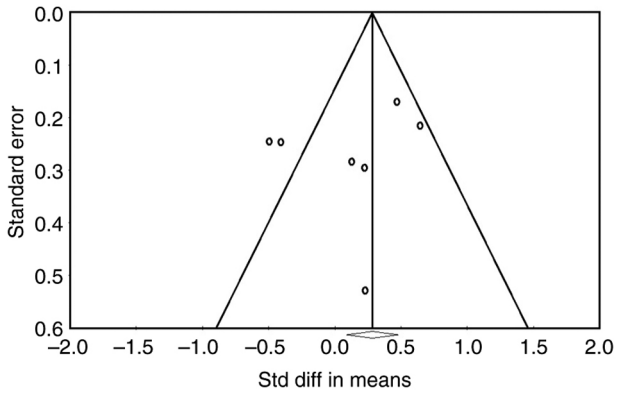


Figure 3. Funnel plot of research included in the meta-analysis demonstrating the relationship between microRNA expression and radiotherapy-induced toxicity in prostate cancer. The funnel plot shows the study size, standard error and precision on the vertical axis, and presents the effect size function on the horizontal axis. The dots show the individual studies, and although the asymmetry observed defines publication bias, the highest size of this area contains regions of high significance. Std diff, standardised mean difference.

Korpela *et al* (57) reported that miRNA-21, miRNA-146a and miRNA-155 expression levels were increased post-RT compared with pre-RT. In addition, another study demonstrated that miRNA-21 levels varied during and after RT (58). Stepanović *et al* (59) also reported that miR-34a expression was elevated at the 15 and 30th fraction of RT compared with pre-RT samples. However, none of these biomarkers have shown encouraging results that could be applied clinically (44).

Several characteristics of miRNAs make them appropriate candidates for molecular biomarker development (60), including the high stability of miRNAs in the blood and urine (60,61). Furthermore, miRNAs can also remain stable after incubation at room temperature and after undergoing repeat freeze-thaw cycles (61). miRNAs can easily be detected with a standard RT-qPCR (60). However, there are still some drawbacks to using miRNAs reliably as predictive biomarkers of RT-induced toxicity. First, there is a shortage of miRNA studies investigating RT-induced toxicity in PCa; therefore, determining miRNA as a potential biomarker in RT oncology is necessary for additional clinical investigations. Second, more research on blood samples is necessary for consistent prospective study protocols. This research should include controlled sample sizes, the interpretation of statistical results, and the use of plasma or serum. Third, in the future, prospective studies should consider blood sampling before, during and after RT to evaluate miRNA expression levels and follow-up to quantify acute and late RT-induced toxicity.

The present study included published data from previous clinical studies regarding the influence of RT on miRNA expression levels and their association with the severity of RT-induced toxicity in patients with PCa. After considering the evidence indicating excellent stability and less difficulty in quantifying miRNAs in liquid biopsies, miRNA could be used as RT-induced toxicity biomarkers. Transcription of miRNA in lymphocytes is active and responsive to various environmental signals and irradiation (62). Therefore, to study biomarkers in RT oncology, a systematic review and meta-analysis of miRNA expression levels and their association with the severity of RT-induced toxicity is an appropriate and acceptable method.

One of the aims of the present study was to perform a meta-analysis of miRNA expression profiling studies investigating RT-induced toxicity in PCa to identify novel candidate biomarkers and/or therapeutic targets. To the best of our knowledge, the present study was the first meta-analysis to focus on the role of miRNAs in RT-induced PCa toxicity, with a systematically quantified evaluation of the diagnostic value. A total of 10 candidate miRNAs (hsa-let-7a-5p, miRNA-21, miRNA-93,

miRNA-99a, miRNA-125b, miRNA-146a, miRNA-155, miRNA-210, miRNA-221 and miRNA-410) from six articles were identified using electronic databases (26-30,42). These findings suggested that identifying miRNAs with altered expression in PCa may help identify novel biomarkers for PCa that can be used to track and influence disease progression. A shortcoming of the interpretation of the miRNA expression profile is the lack of consistency between study results. The diversity of the study population may result from various study designs, variations in expression profiling platforms, and genetic, environmental and clinicopathological variations among organ and/or tissue donors. Further validation in large patient cohorts is required to confirm the significance of these miRNAs as PCa biomarkers and therapeutic targets.

Meta-analysis of the European ancestry cohorts identified three genomic signals: Single nucleotide polymorphism rs17055178 with rectal bleeding ($Pmeta=6.2x10^{-10}$), rs10969913 with decreased urinary stream ($Pmeta=2.9x10^{-10}$) and rs11122573 with hematuria ($Pmeta=1.8x10^{-8}$), and association with RT-induced toxicity events such as rectal bleeding, lower urinary stream and higher urinary frequency (63). Whole transcriptome and pathway analysis of liquid biopsies might reveal mechanisms underlying the pathogenesis of acute or late radiotoxicity. There is a lack of meta-analyses investigating associations between miRNAs and side effects in patients with PCa who have undergone RT, and the present study may be among the first ones.

According to the studies reported, miRNAs have been linked to significant events such as DNA damage repair, oxidative stress, cell cycle regulation, inflammation, cell death and apoptosis, and hypoxia (64-71). For example, miRNA-21 is associated with apoptosis, targeting PTEN, programmed cell death protein 4 and BCL2 (64). miR-99a is associated with cell cycle regulation (65). miR-221 is also associated with apoptosis via targeting of PTEN (66). miRNA-18b is downregulated in LnCaP cells after RT (67). miR-132-5p is associated with fibrosis, so it may be closely related to toxicity (68). miR-197-3p and miR-23a-3p are associated with inflammation and liver fibrosis, and apoptosis in diabetic kidney disease (69,70). miRNA-410 can inhibit cytokine release, indicating its involvement in the inflammatory response by targeting NF-κB (71).

Lymphocyte models for the investigation of responses to radiation in terms of genetics and epigenetics are especially informative and important. When exposed to radiation, quickly dividing cells such as hematopoietic cells react first (72). Lymphocytes from circulation are radiosensitive (73-75). It has also been demonstrated that the transcriptome of lymphocytes changes after irradiation (3 h after *ex vivo* irradiation with 2-Gy ɣ-rays) (76). Furthermore, genetic/epigenetic information can be transferred to distant cells and organs by circulation and miRNA trafficking (via exosomes which enter and exit lymphocytes) (77). Therefore, miRNA changes in response to RT are noteworthy and should be utilized as adjunctive factors for the prediction of therapy response, aside from information obtained from serum or plasma samples. The studies described indicate that miRNA changes in plasma/serum and PBMCs/peripheral blood mononuclear lymphocytes may have the potential for use in clinical practice (26-31,42). Additionally, it should be noted that events in the cells, which are the repercussion of radiation exposure, may increase or decrease miRNA levels (67). These miRNA level changes should also be considered as predictive parameters of response to therapy, regardless of their absolute values or targets and genes/pathways they are silencing.

The present study highlighted the importance of transcriptome and non-coding transcript changes. The changes in the transcriptome (coding and non-coding) may be used for prediction of not only response to RT but also chemotherapy, as well as for other types of malignancies. Furthermore, changes in miRNA levels during therapy may be used in the future to modulate therapy, providing information ranging from how to alter the course of treatment and avoiding surrounding tissue damage, to lowering the incidence of RT side effects (78). The differential expression of the miRNA transcriptome between normal and malignant tissues may be the key feature for miRNA utilization as radioprotectors. In the present systematic review and meta-analysis, miRNAs (miRNA-132-5p, miRNA-1-3p, miRNA-410, miRNA-221, miRNA-23a-3p, miRNA-197-3p, miRNA-151a-5p, miRNA-18b-5p, miRNA-99a and miRNA-21) are listed, which are potential candidates for panels of radiotoxicity prediction. It is important to determine which miRNA molecule is the best candidate to be evaluated from a particular sample type (liquid biopsy, blood, serum, plasma, lymphocytes, exosomes or tissue specimens), and if a miRNA is associated with RT-induced toxicities. The next step to verify specificity and sensitivity of these miRNAs as biomarkers is to conduct extensive validation studies.

According to the present systematic review, miR-21, miR-99a, miR-221, miR-18b, miR-132-5p, miR-197-3p, miR-23a-3p and miR-410, miRNA 1-3p and miRNA-151a-5p are radiosensitive, and directly involved in inflammation, fibrosis and apoptosis of the GI and GU tract. Therefore, they might be utilised in the future for prediction and modulation of the radiation response of individual patients to increase the quality of life of patients with PCa. The meta-analysis identified that miRNA-21 and miRNA-125b were significant PCa-associated miRNAs differentially expressed in RT-induced toxicity in PCa. However, further extensive validation is required to determine the association between miRNA expression levels and RT-induced toxicity in PCa and to prove their predictive value.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JS, NP, TT, GS and SSS contributed to the conception, design of the study and critically revised the manuscript. JS, NP, DUA,

SOY, KDM, MSE and SM prepared the materials, collected the data and performed the analysis. JS, TT, DUA, SOY, GS, MSE and KDM critically revised the the manuscript. SSS, NP, TT, and SM confirm the authenticity of all the raw data. All authors revised the manuscript. SSS supervised the over all study. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

JS ORCID, 0000-0002-0457-2650.

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