ARTICLE

Basic Science

Hyperglycemia and microRNAs in prostate cancer

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BACKGROUND: Hyperglycemia can promote the development of prostate cancer (PCa). Differential expression levels of miRNAs between PCa patients and controls were also reported. Therefore, we examined the relationship between hyperglycemia and miRNA levels in PCa.
METHODS: Relative expression of urinary miR-574-3p, miR-375, miR-205-5p, miR-200b-3p, miR-187-3p, miR-182-5p, and miR-100-5p were investigated in 105 PCa patients and 138 noncancer controls by Real-Time quantitative PCR. Fasting plasma glucose measurements were retrieved from clinical records. The differential miRNA expressions among groups were compared using non-parametric tests. Correlations with glucose and prostate-specific antigen (PSA) were tested using Pearson correlation coefficient.
RESULTS: When we analyzed miRNA expression according to glycemic state, significant down-regulations were found for miR-200b-3p, miR-187-3p, miR-182-5p, and miR-100-5p in noncancer controls with high glucose. The lowest down-regulations were observed for miR-187-3p, miR-182-5p, and miR-100-5p. Subsequently, when hyperglycemia was considered in PCa, significant dysregulations of selected miRNAs were found in hyperglycemic PCa patients than in controls with high glucose. In particular, miR-375 and miR-182-5p showed a 3-FC in hyperglycemic PCa patients than controls who left hyperglycemia untreated. Conversely, only a down-regulation of miR-574-3p was observed in PCa patients regardless of glycemic status and only modest down-regulation of miR-574-3p, miR-200b-3p, miR-187-3p and miR-182-5p were found in normoglycemic PCa patients. Next, significant correlations between miRNAs and glucose (miR-200b-3p, miR-100-5p) and PSA (miR-205-5p and miR-187-3p) were detected in controls. Similarly, miR-205-5p and miR-187-3p were correlated with glucose in PCa patients, while miR-574-3p and miR-375 showed inverse relationships.
CONCLUSIONS: miRNA dysregulations can occur in hyperglycemic PCa patients as compared to noncancer controls who left hyperglycemia untreated. Hyperglycemia can consistently promote the expression of miR-375 and miR-182-5p. Uncontrolled hyperglycemic state could contribute to the creation of a suitable microenvironment for later PCa development by promoting gene expression.

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INTRODUCTION

Prostate cancer (PCa) is the most common male cancer in Western countries and the second cause of death due to malignancy among men [1]. The introduction of serum prostate specific antigen (PSA) testing for early detection and screening resulted in lower PCa mortality but also in significantly increased cancer incidence [2]. PSA screening has even caused an increment of over-diagnosis, and over-treatments [2]. Transrectal ultrasound and, recently, multiparametric Magnetic Resonance Imaging (MRI) guided tumor biopsies remain the gold standard methods for PCa prostate diagnosis [3], but cause important stress and health risk for urological patients.

Over the last years, much emphasis has been given to microRNAs (miRNAs), a large family of evolutionarily conserved small noncoding RNAs with a main role in gene regulation by acting at the posttranscriptional level [4]. At functional level, miRNAs modulate the expression of their target genes by imperfect base pairing to the 3′-untranslated regions of mRNAs inducing silencing of target mRNAs [4]. miRNA family plays a role in various biological and pathological processes, including cellular

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proliferation, differentiation, metastasis and cell death [5]. Early works showed that dysregulated miRNA expression contribute to human cancer through diverse mechanisms by acting as oncogene or tumor suppressor [6]. Specific signatures of miRNAs were identified in solid tumors [7], including PCa [8]. Moreover, a number of studies demonstrated differential expression levels of urinary miRNAs between PCa patients and controls [9].

In the last years, a growing body of evidence has shown a main role of glucose in PCa [10–12]. A 52% higher risk of PCa was found in hyperglycemic men as compared to normoglycemic subjects [11]. Later, an elevated risk of cancer death in men with high glucose was reported by the same Authors [12]. Increments of 150% and 220% risks of lethal and fatal PCa, respectively, were demonstrated in a communities cohort study [10]. Besides, high sugar and triglycerides have been linked to PCa aggressiveness and severity [13].

In the current study, we analyzed the expression of urinary miR-574-3p, miR-375, miR-205-5p, miR-200b-3p, miR-187-3p, miR-182-5p and miR-100-5p, as target miRNAs for PCa [14] in a case-cohort study considering the concentrations of glucose, a determinant of miRNA expression [15]. Relative expression levels of miRNAs were analyzed in urinary cell-free fraction by Real-Time quantitative PCR. Our aim was to examine the relationship between miRNAs and PCa in men with low and high sugar levels.

METHODS

Study population

The case-cohort study included 243 patients undergoing clinical prostatic examination and standard biopsy template (at least 16 cores) at Careggi University Hospital, Florence, Italy. The inclusion criteria were to be 50–70 years aged and have clinical suspect of PCa by PSA ≥ 3.0 ng/ml. The exclusion criteria were previous positive biopsy; presence of specific PCa symptoms; treatments with 5-alpha reductase and other tumors. Data, including Gleason score (GS), pre-biopsy total serum PSA and fasting plasma glucose measurements were retrieved from clinical records. Study procedures were done in accordance with the Declaration of Helsinki for human studies. The study protocol was approved by the Institutional Review Boards and registered in the Regional Trial Register (N. bio16001).

MicroRNA analysis

After informed consent, urines were collected on the day of biopsy. Total RNA was isolated from urine supernatant using miRNeasy Mini Kit (Qiagen, Venlo, The Netherlands) according to protocol's instructions and stored at −80°C. miR-specific cDNA synthesis was performed using miScript II Reverse Transcription Kit (Qiagen, Venlo, The Netherlands) in PTC-100™ Thermal Cycler (MJ Research, inc., Québec, Canada). cDNAs were preamplified using miScript PreAMP PCR Kit (Qiagen) and miScript PreAMP Custom Primer Mix (Qiagen). Mature miRNA expression profiling was determined by RT-qPCR using custom miScript miRNA PCR Arrays (Qiagen, Venlo, The Netherlands) and miScript SYBR Green PCR Kit (Qiagen, Venlo, The Netherlands) using 7900 RT-PCR System (Applied Biosystems™, Thermo Fisher Scientific, Massachusetts, USA). Samples were blindly analyzed in duplicated. microRNA expression was normalized against an endogenous normalizer [16]. The ΔΔCt method was used for the calculation of the relative expression of miRNAs and fold-change (FC).

Statistical methods

miRNAs levels were reported as median and range. Study participants were grouped according to clinicopathological parameters. Glycemic state was based on WHO criteria [17]. Levene's statistic was used to assess if there was a significant difference in variance between groups. Distribution of data was determined using Shapiro-Wilk Test and non-parametric tests were used as appropriate. Continuous variables were compared using Mann-Whitney *U* (two-sided) and Kruskal-Wallis rank tests. The levels of miRNA expression were tested for correlation with glucose levels and PSA using the Pearson correlation coefficient. Statistical significance was taken as $p < 0.05$. Data were analyzed using IBM SPSS Statistics® software (version 20.0, Chicago. IL, USA).

RESULTS

Patient characteristics

After inclusion, there were 243 men with PSA ≥ 3 ng/ml in the final cohort. Based on biopsy outcome, the cohort consisted of 105 PCa patients of whom 46% patients with GS ≥ 4 + 3, and 138 patients, who tested negative to prostate biopsy, who were included as noncancer controls. In PCa patients, 85 men were normoglycemic and 20 had high glucose. Among noncancer controls, 107 men were normoglycemic and 31 were hyperglycemic. There was no significant difference in variance between groups. Clinicopathologic characteristics are summarized in Table 1.

Glucose and microRNAs

We first decided to analyze the expression of urinary miRNAs (miR-574-3p, miR-375, miR-205-5p, miR-200b-3p, miR-187-3p, miR-182-5p, and miR-100-5p) in controls according to glycemic state. Median ΔC_T and range of miRNAs are shown in Table 2 and Fig. 1. Results show a general tendency of urinary miRNAs to be down-regulated at high glucose levels. As reported in Table 2, the expression of miR-200b-3p, miR-187-3p, miR-182-5p, and miR-100-5p were significantly reduced in hyperglycemic controls with respect to those with normal glucose (*p* values of 0.048, 0.001, 0.010 and 0.022, respectively). The lowest down-regulations were observed for miR-187-3p, miR-182-5p, and miR-100-5p with FC value of 0.5.

Glucose, prostate cancer, and microRNAs

The expression of miRNAs was initially examined regardless of glycemic status (Table 3). Findings revealed that only a modest statistically significant down-regulation of miR-574-3p was present in PCa patient group than in controls. Subsequently, we explored the relationship between miRNAs and PCa at the level of each layer of glucose, hyperglycemic and normoglycemic one. Table 3 and Fig. 1 report that significant dysregulations of miR-574-3p, miR-375, miR-205-5p, miR-187-3p and miR-182-5p occurred in hyperglycemic PCa patients than in controls with high glucose. In particular, the relative expression of miR-574-3p, miR-375 and miR-182-5p were significantly over-expressed, *p* values of 0.014, 0.041 and 0.043, respectively. As shown in Fig. 1, data suggest that hyperglycemia over induce the expression of miR-375 and miR-182-5p in PCa patients with an FC expression value of 3.0. Specifically, the median ΔC_T and range values of miR-375 and miR-182-5p were −3.1 (−6.5, 0.4) and 2.9 (−0.5, 5.9) in hyperglycemic controls and −4.6 (−7.2, −0.8) and 1.3 (0.4, 5.1) in PCa patients with high glucose, p-values of 0.041 and 0.043, respectively. Also, modest but significant decrements were determined for miR-205-5p and miR-187-3p (*p* values of 0.042 and <0.0001) in the

Table 1. Clinicopathological characteristics of the study population, according to cancer status, prostate-specific antigen (PSA), Gleason score (GS) and glycemic state.

Characteristics	*N*	
Study population	243	61.7 years ± 5.4 (SD)
Prostate cancer patients	105	62.9 years ± 4.5 (SD)
PSA	105	10.9 ng/ml ± 11.4 (SD)
Gleason score	57	GS ≤ 3 + 4
	48	GS ≥ 4 + 3
Normoglycemic patients	85	<110 mg/dl plasma glucose
Hyperglycemic patients	20	≥110 mg/dl plasma glucose
Noncancer controls	138	60.8 years ± 5.9 (SD)
PSA	136	7.6 ng/ml ± 3.5 (SD)
Normoglycemic controls	107	<110 mg/dl plasma glucose
Hyperglycemic controls	31	≥110 mg/dl plasma glucose

Table 2. Relative expression levels and range of miR-574-3p, miR-375, miR-205-5p, miR-200b-3p, miR-187-3p, miR-182-5p and miR-100-5p 5p in noncancer controls according to glycemic state.

	N	miR-574-3p Median ΔC_T (range)	*p* value	miR-375 Median ΔC_T (range)	*p* value	miR-205-5p Median ΔC_T (range)	*p* value	miR-200b-3p Median ΔC_T (range)	*p* value	miR-187-3p Median ΔC_T (range)	*p* value	miR-182-5p Median ΔC_T (range)	*p* value	miR-100-5p Median ΔC_T (range)	*p* value
Normoglycemic controls	107	−0.3 (−2.3, 7.0)	Baseline	−3.5 (−7.2, 0.5)	Baseline	−0.5 (−4.8, 2.7)	Baseline	−3.1 (−5.9, 0.1)	Baseline	2.8 (0.0, 4.9)	Baseline	2.0 (−0.8, 5.3)	Baseline	−2.3 (−5.9, 3.1)	Baseline
Hyperglycemic controls	31	−0.2 (−2.3, 3.5)	0.746	−3.1 (−6.5, 0.4)	0.572	0.1 (−4.1, 4.4)	0.275	−2.6 (−5.1, 1.1)	0.048	3.6 (0.7, 5.7)	0.001	2.9 (−0.5, 5.9)	0.010	−1.4 (−4.8, 3.5)	0.022

hyperglycemic group. On the other hand, when we investigated the patterns of expression of miRNAs in normoglycemic PCa patients and controls (Table 3 and Fig. 1), we observed modest significant downregulations of the relative expression levels of miR-574-3p, miR-200b-3p, miR-187-3p, and miR-182-5p in normoglycemic PCa patient group, *p* values of 0.004, 0.029, 0.002, and 0.040, respectively.

Glucose, prostate-specific antigen and microRNAs

Then, we investigated the occurrence of correlations between the expression levels of miRNAs, glucose and PSA in controls and PCa patients (Table 4). In controls, significant relationships were determined between miR-200b-3p, miR-100-5p and glucose, *p* values of 0.047 and 0.018, respectively. Findings show that the expression of 2 miRNAs varied with increasing PSA in controls. Specifically, the correlations of miR-205-5p and miR-187-3p with PSA were significant, *p* values of 0.021 and 0.017, respectively. Conversely, in PCa group, miRNAs were significantly correlated only with glucose. In more detail, miR-205-5p and miR-187-3p were positively correlated with glucose, *p* values of 0.039 and 0.020, respectively. Whereas 574-3p and miR-375 showed inverse relationships, p-values of 0.011 and 0.005, respectively. A heatmap illustrating the correlations between miRNA expression levels, fasting plasma glucose, and serum PSA in both noncancer controls and PCa patients is presented to easily identify patterns of associations (Fig. 2).

DISCUSSION

Despite the relevance of high glucose in PCa [11], little is known about how sugar influences miRNA expression in this malignant pathology. Therefore, we have initially compared the expression levels of seven target miRNAs (miR-574-3p, miR-375, miR-205-5p, miR-200b-3p, miR-187-3p, miR-182-5p and miR-100-5p) [14] in urinary cell-free fraction of controls with and without high glucose. As a result, we observed that significant dysregulations of miRNA expression occur in response to hyperglycemic state. In noncancer controls, the expression of miR-200b-3p, miR-187-3p, miR-182-5p and miR-100-5p was significantly down-regulated in those with high glucose compared to normoglycemic one. The most consistent alterations were observed for miR-187-3p, miR-182-5p and miR-100-5p in controls with high glucose. Our findings are in line with early reports showing that dysregulated miRNA levels occur in response to high glucose. Formerly, the study of Oger et al. [18] reported that down-regulated expression of miR-200b was present in obese diabetic patients with high glucose. A down-regulation of miR-182-5p was found in individuals with prediabetes and type 2 diabetes mellitus [19]. Similarly, Pek et al. [20] reported lower expression levels of miR-100 in diabetic patients.

Our main result provides information within the pathology of PCa, highlighting the link between hyperglycemia and cancer development. In fact, when we examined if hyperglycemia was a confounder of the relationship between miRNAs and PCa, we observed that the relationship was different at the level of each layer of glucose. An effective influence of high glucose on the expression of selected miRNAs was found after stratification for glycemic state. Consistent up-regulations were observed for miR-574-3p, miR-375 and miR-182-5p, whereas low decrements were determined for miR-205-5p and miR-187-3p. As mentioned above, miR-375 and miR-182-5p had a 3-fold expression change in hyperglycemic PCa patients as compared to noncancer controls who left hyperglycemia untreated. On the other hand, only the expression of miR-574-3p was significantly dysregulated in PCa patients when compared to controls regardless of glycemic status, and modest down-

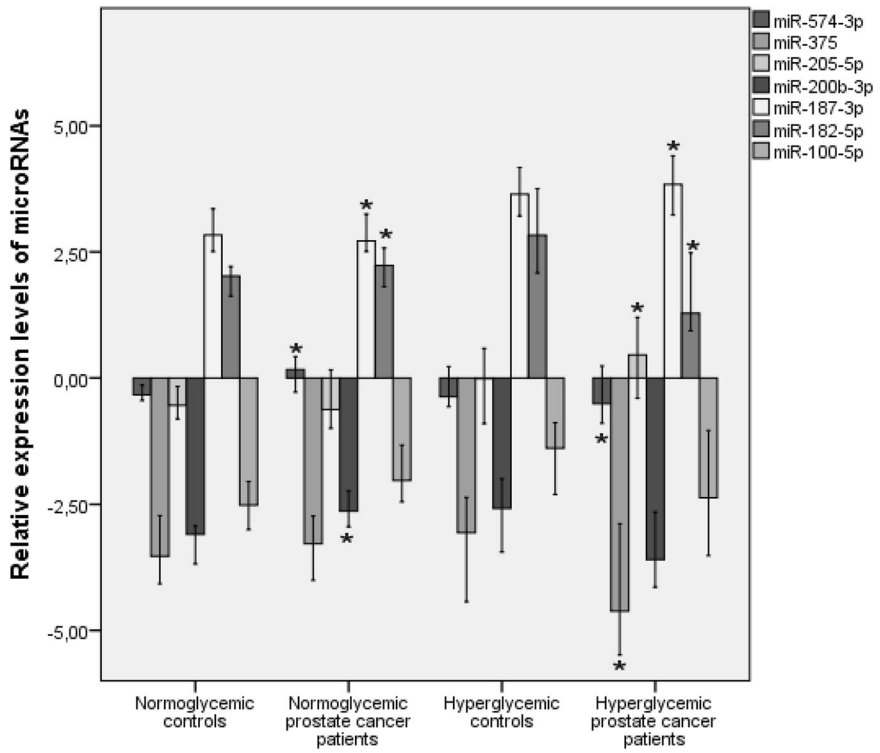

Fig. 1 Relative expression levels of miR-574-3p, miR-375, miR-205-5p, miR-200b-3p, miR-187-3p, miR-182-5p, and miR-100-5p according to prostate cancer and glycemic state. Interval confidences and significances (*) are shown.

regulations of miR-574-3p, miR-200b-3p and miR-182-5p were found in PCa patients with normal glycemic state.

Overall, findings suggest that miR-375 and miR-182-5p could be up-regulated in hyperglycemic PCa patients and be involved in PCa development. Our results are in agreement with the study of Xiaojuan et al. [21], who showed that hyperglycemia induces the expression of miR-186, miR-301a, miR-365 and miR-193 in rodent models. Gajeton et al. [22] showed that a miR-467-dependent pathway, capable of promoting tumor growth, was activated by hyperglycemia in experimental rodents. Furthermore, Hudson [23] demonstrated that miR-574-3p was up-regulated in primary prostate tumors. Similarly, increased levels of miR-375 were reported in patients with PCa as compared to controls [24, 25]. Abramovic et al. [24] reported a pattern of miR-182 up-regulation in PCa patients relative to controls. In another investigation, Konoshenko et al. [26] showed that the levels of miR-182 were effective in discriminating PCa patients from controls but in combination with miR-125 or miR-30. Wang et al. [27] reported that miR-205 was frequently downregulated in PCa. Also, the expression of miRNA-187 was found to be decreased in PCa tissue specimens [28]. In the opposite direction, it is possible that hyperglycemic state could not be associated to PCa development by gene expression promotion. Dysregulations of miRNAs could simply be a reflection of the existence of PCa, a condition that could lead to a sensitivity to hyperglycemia.

Next, the relationships of miRNAs with glucose and PSA showed differences among PCa patients and controls. The levels of miR-574-3p and miR-375 showed inverse relationships with glucose in PCa patients, in keeping with previous studies [29, 30], and reflecting their different potential involvements in prostate carcinogenesis, possibly by androgen receptor regulation [31]. Whereas, miR-205-5p and miR-187-3p levels were correlated with PSA in controls, perhaps predicting increased cancer risk [32].

Knowing the gene regulatory action of miRNAs [5], glycemic derangements could contribute to the creation of a favorable microenvironment for later PCa development. Dysregulated expression can be caused by hyperglycemic state through different mechanisms including transcriptional modifications affecting transcriptional factors and miRNA expression [33] and oxidative stress [34]. Xiaojuan et al. [21] reported that high glucose induces miR-301a expression in prostate tissues and PCa cell lines. The oncogenic properties of miR-375 can be due to its ability of regulating *homolog A, COPII coat complex component A, fibroblast growth factor 2*, and *pyruvate dehydrogenase kinase 1* [35] and the Wnt signaling pathway promoting cell differentiation [36, 37]. Besides, up-regulation of miR-182 expression promotes cancer cell proliferation and invasion role in PCa by targeting multiple genes in experimental PCa cells [38]. The up-regulation of miR-182 in PCa contributes to Epithelial-Mesenchymal Transition (EMT) by targeting the expression of microphthalmia-associated transcription factor [39]. Overexpression of miR-182 in prostate experimental cells even decreases the transcript levels of *snail family transcriptional repressor 2* (*SNAI2*), an important regulator of EMT pathway [40]. On the other hand, hyperglycemia causes cell distress by glucose auto-oxidation and the production of reactive oxygen species [41], compounds capable of inducing aberrant DNA methylation, a mechanism able to dysregulate miRNA expression [42], by oxidative damage [43]. Early, we showed significant inverse relationships between oxidative damage and DNA methylation aberrations at *long interspersed nuclear element-1* and at the promoter domain of *interleukin-6* among the participants to the Map Ta Phut study, who were exposed to complex mixtures of environmental and industrial toxicants [44]. As result of hyperglycemic state, increased oxidative stress could cause PCa through DNA damage and subsequent DNA methylation alterations in PCa-related genes.

This study has several strengths such as a prospective case-cohort study design and reliable glucose measurements; profiling miRNAs from urine is an accepted surrogate tissue for PCa [14], which offers key advantages for the urological patient since it is an easier and non-invasive sampling method compared to biopsy and peripheral blood [45]. In addition, the urinary cell-free fraction

Table 3. Relative expression levels and range of miR-574-3p, miR-375, miR-205-5p, miR-200b-3p, miR-187-3p, miR-182-5p, and miR-100-5p in the case-cohort with and without glycemic and cancer state stratification.

		miR-574-3p		miR-375		miR-205-5p		miR-200b-3p		miR-187-3p		miR-182-5p		miR-100-5p	
	N	Median ΔC_T (range)	*p*-value	Median ΔC_T (range)	*p* value	Median ΔC_T (range)	*p* value	Median ΔC_T (range)	*p* value	Median ΔC_T (range)	*p* value	Median ΔC_T (range)	*p* value	Median ΔC_T (range)	*p* value
Controls	138	−0.3 (−2.3, 7.0)	Baseline	−3.4 (−7.1, 0.5)	Baseline	−0.4 (−4.8, 4.4)	Baseline	−3.0 (−5.9, 1.1)	Baseline	3.2 (0.0, 5.7)	Baseline	2.2 (−0.8, 5.9)	Baseline	−2.2 (−5.9, 3.5)	Baseline
Prostate cancer patients	105	0.0 (−2.1, 5.5)	0.015	−3.6 (−7.2, 0.6)	0.948	−0.4 (−6.0, 3.2)	0.862	−2.8 (−6.2, 2.5)	0.340	3.1 (−0.8, 6.3)	0.973	2.0 (0.0, 6.6)	0.539	−2.0 (−6.3, 2.0)	0.676
Normoglycemic controls	107	−0.3 (−2.3, 7.0)	Baseline	−3.5 (−7.2, 0.5)	Baseline	−0.5 (−4.8, 2.7)	Baseline	−3.1 (−5.9, 0.1)	Baseline	2.8 (0.0, 4.9)	Baseline	2.0 (−0.8, 5.3)	Baseline	−2.3 (−5.9, 3.1)	Baseline
Normoglycemic prostate cancer patients	85	0.1 (−2.1, 5.5)	0.004	−3.3 (−6.6, 0.6)	0.420	−0.6 (−6.0, 3.2)	0.438	−2.7 (−6.2, 2.5)	0.029	2.6 (−0.8, 5.6)	0.002	2.1 (0.0, 6.6)	0.040	−2.0 (−6.3, 2.0)	0.077
Hyperglycemic controls	31	−0.2 (−2.3, 3.5)	Baseline	−3.1 (−6.5, 0.4)	Baseline	0.1 (−4.1, 4.4)	Baseline	−2.6 (−5.1, 1.1)	Baseline	3.6 (0.7, 5.7)	Baseline	2.9 (−0.5, 5.9)	Baseline	−1.4 (−4.8, 3.5)	Baseline
Hyperglycemic prostate cancer patients	20	−0.5 (−1.6, 1.1)	0.014	−4.6 (−7.2, −0.8)	0.041	0.5 (−2.8, 2.3)	0.042	−3.6 (−5.1, −1.9)	0.069	3.8 (0.6, 6.3)	<0.0001	1.3 (0.4, 5.1)	0.043	−2.5 (−5.7, 1.4)	0.291

Table 4. Correlation analysis between the relative expression levels of miR-574-3p, miR-375, miR-205-5p, miR-200b-3p, miR-187-3p, miR-182-5p and miR-100-5p, fasting plasma glucose and prostate-specific antigen.

	miR-574-3p		miR-375		miR-205-5p		miR-200b-3p		miR-187-3p		miR-182-5p		miR-100-5p	
	Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value
Noncancer controls														
Fasting plasma glucose	0.021	0.406	0.017	0.423	0.095	0.170	0.164	0.047	0.107	0.118	0.107	0.141	0.177	0.018
Prostate-specific antigen	−0.093	0.139	−0.076	0.189	0.197	0.021	−0.049	0.308	0.187	0.017	−0.038	0.349	0.088	0.152
Prostate cancer patients														
Fasting plasma glucose	−0.175	0.039	−0.200	0.020	0.244	0.011	−0.139	0.099	0.253	0.005	−0.147	0.087	0.064	0.259
Prostate-specific antigen	0.031	0.378	0.093	0.171	−0.104	0.168	0.066	0.271	−0.074	0.228	0.005	0.482	0.020	0.419

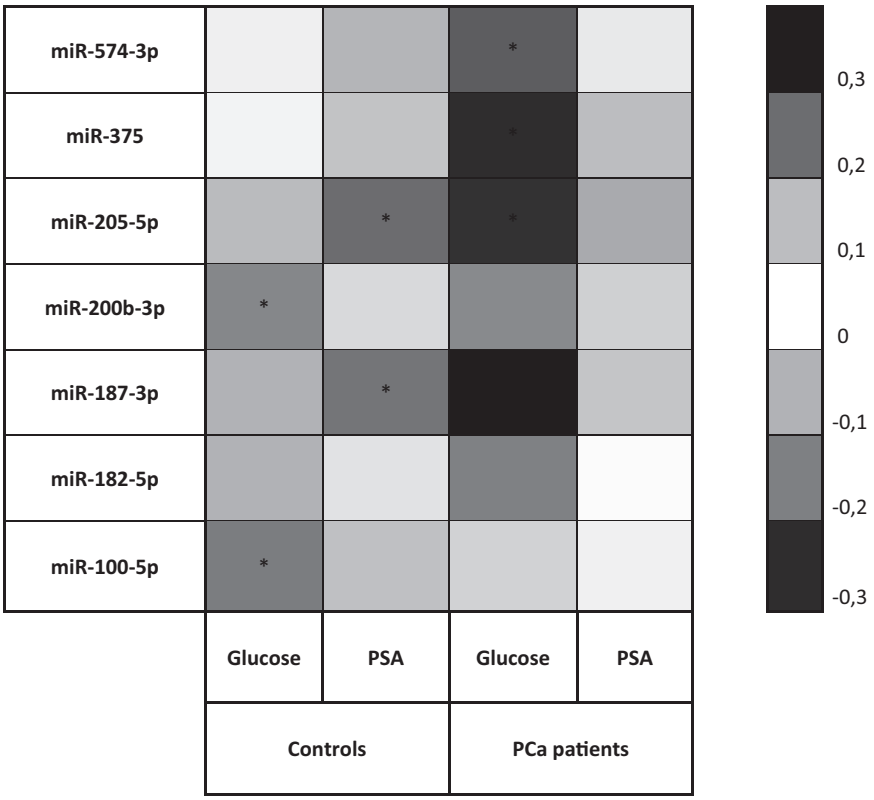

Fig. 2 Heatmap showing correlations between the expression levels of miR-574-3p, miR-375, miR-205-5p, miR-200b-3p, miR-187-3p, miR-182-5p, miR-100-5p, and the concentrations of fasting plasma glucose and serum prostate-specific antigen (PSA) among noncancer controls and prostate cancer (PCa) patients. Color key represents Pearson correlation coefficients in a color gradient (red: positive and green: negative values). Significances (*) are reported.

is free of contaminants whereas urine sediment contains renal tubular cells, urothelial cells, erythrocytes and tumor cells that could be a source of bias [46].

Hyperglycemia-related dysregulations with over and under expression of miRNAs are yet to be fully understood. Current knowledge indicates that miRNAs play a role in glucose metabolism and hyperglycemia-associated cancer grow [21]. For instance, mir-375 down-regulation enhances insulin secretion whereas mir-375 over-expression decreases insulin secretion and transcription altering glucose metabolism, leading to chronic hyperglycemia and promoting proliferation, invasion and migration of cancer cells [47, 48]. Currently, PSA is the most employed biomarker for PCa, however, PSA has limited specificity and leads to overdiagnosis which in turn results in overtreatment. To increase specificity and reduce the number of unnecessary biopsies, multiparametric MRI and several biomarkers, such the 4 K score, the prostate cancer 3 (PCA3), a long non-coding RNA, the Mi-prostate score, a predictive algorithm including PSA, PCA3 and TMPRSS2:ERG [49], have become available. Interestingly, the differential expression of miR-375 and miR-182-5p between hyperglycemic PCa patients and controls (3-fold expression change) was higher than that of miR- PCA3 (2-fold expression change) [50]. The expression levels of urinary miR-375 and miR-182-5p as possible biomarkers of PCa will have a role as a future clinically relevant test alone or in combination with other biomarkers in reducing overdiagnoses in indolent disease and identifying at higher-risk individuals. Next steps for this research will involve potential validation studies in independent cohorts. These approaches would enhance the reproducibility and reliability of the reported results. Other specific avenues for further investigations will be based on experiments to elucidate the mechanisms through which hyperglycemia influences miRNA expression and on clinical trials aimed to validate the diagnostic and therapeutic potential of miR-375 and miR-182-5p. Such investigations will have the potential to achieve an accurate molecular risk stratification. PCa patients under high glucose and high levels of miR-375 and miR-182-5p could be monitored and targeted with inhibitors to block cell proliferation and differentiation.

CONCLUSIONS

miRNA dysregulations occur in hyperglycemic PCa patients as compared to noncancer controls who left hyperglycemia untreated. Hyperglycemia can promote miR-375 and miR-182 expression. Given the known gene regulatory action of miRNAs, uncontrolled hyperglycemic state could contribute to the creation of a suitable microenvironment for later PCa development by gene expression promotion. Future studies should be performed to examine if miR-375 and miR-182-5p represent useful biomarkers for early diagnosis and promising therapeutic targets.

DATA AVAILABILITY

The dataset used during the current study is available from the corresponding author upon reasonable request.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
2. Auvinen A, Moss SM, Tammela TLJ, Taari K, Roobol MJ, Schrader FH, et al. Absolute effect of prostate cancer screening: balance of benefits and harms by

center within the European randomized study of prostate cancer screening. Clin Cancer Res. 2015;22:243–9.
3. Ahdoot M, Wilbur AR, Reese SE, Lebastchi AH, Mehralivand S, Gomella PT, et al. MRI-targeted, systematic, and combined biopsy for prostate cancer diagnosis. N. Engl J Med. 2020;382:917–28.
4. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136:215–33.
5. Nair VS, Pritchard CC, Tewari M, Ioannidis JPA. Design and analysis for studying microRNAs in human disease: a primer on -omic technologies. Am J Epidemiol. 2014;180:140–52.
6. Volinia S, Calin GA, Liu C-G, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA. 2006;103:2257–61.
7. Ardekani AM, Naeini MM. The role of MicroRNAs in human diseases. Avicenna J Med Biotechnol. 2010;2:161–79.
8. Fabris L, Ceder Y, Chinnaiyan AM, Jenster GW, Sorensen KD, Tomlins S, et al. The potential of MicroRNAs as prostate cancer biomarkers. Eur Urol. 2016;70:312–22.
9. Aveta A, Cilio S, Contieri R, Spena G, Napolitano L, Manfredi C, et al. Urinary MicroRNAs as biomarkers of urological cancers: a systematic review. Int J Mol Sci. 2023;24:10846.
10. Marrone MT, Selvin E, Barber JR, Platz EA, Joshu CE. Hyperglycemia, classified with multiple biomarkers simultaneously in men without diabetes, and risk of fatal prostate cancer. Cancer Prev Res. 2019;12:103.
11. Murtola TJ, Vihervuori VJY, Lahtela J, Talala K, Taari K, Tammela TLJ, et al. Fasting blood glucose, glycaemic control and prostate cancer risk in the Finnish Randomized Study of Screening for Prostate Cancer. Br J cancer. 2018;118:1248–54.
12. Murtola TJ, Salli SM, Talala K, Taari K, Tammela TLJ, Auvinen A. Blood glucose, glucose balance, and disease-specific survival after prostate cancer diagnosis in the Finnish Randomized Study of Screening for Prostate Cancer. Prostate Cancer Prostatic Dis. 2019;22:453–60.
13. Arthur R, Muller H, Garmo H, Holmberg L, Stattin P, Malmstrom H, et al. Association between baseline serum glucose, triglycerides and total cholesterol, and prostate cancer risk categories. Cancer Med. 2016;5:1307–18.
14. Paiva RM, Zauli DAG, Neto BS, Brum IS. Urinary microRNAs expression in prostate cancer diagnosis: a systematic review. Clin Transl Oncol. 2020;22:2061–73.
15. Al-Mahayni S, Ali M, Khan M, Jamsheer F, Moin ASM, Butler AE. Glycemia-induced miRNA changes: a review. Int J Mol Sci. 2023;24:7488.
16. Hu Z, Dong J, Wang L-E, Ma H, Liu J, Zhao Y, et al. Serum microRNA profiling and breast cancer risk: the use of miR-484/191 as endogenous controls. Carcinogenesis. 2012;33:828–34.
17. Echouffo-Tcheugui JB, Selvin E. Prediabetes and what it means: the epidemiological evidence. Annu Rev Public Health. 2021;42:59–77.
18. Oger F, Gheeraert C, Mogilenko D, Benomar Y, Molendi-Coste O, Bouchaert E, et al. Cell-specific dysregulation of microrna expression in obese white adipose tissue. J Clin Endocrinol Metab. 2014;99:2821–33.
19. Weale CJ, Matshazi DM, Davids SFG, Raghubeer S, Erasmus RT, Kengne AP, et al. Circulating miR-30a-5p and miR-182-5p in prediabetes and screen-detected diabetes mellitus. Diabetes Metab Syndr Obes. 2020;13:5037–47.
20. Pek SLT, Sum CF, Lin MX, Cheng AKS, Wong MTK, Lim SC, et al. Circulating and visceral adipose miR-100 is down-regulated in patients with obesity and Type 2 diabetes. Mol Cell Endocrinol. 2016;427:112–23.
21. Li X, Li J, Cai Y, Peng S, Wang J, Xiao Z, et al. Hyperglycaemia-induced miR-301a promotes cell proliferation by repressing p21 and Smad4 in prostate cancer. Cancer Lett. 2018;418:211–20.
22. Gajeton J, Krukovets I, Muppala S, Verbovetskiy D, Zhang J, Stenina-Adognravi O. Hyperglycemia-induced miR-467 drives tumor inflammation and growth in breast cancer. Cancers. 2021;13:1346.
23. Hudson RS, Yi M, Esposito D, Watkins SK, Hurwitz AA, Yfantis HG, et al. MicroRNA-1 is a candidate tumor suppressor and prognostic marker in human prostate cancer. Nucleic Acids Res. 2012;40:3689–703.
24. Abramovic I, Vrhovec B, Skara L, Vrtaric A, Nikolac Gabaj N, Kulis T, et al. MiR-182-5p and miR-375-3p have higher performance than PSA in discriminating prostate cancer from Benign prostate hyperplasia. Cancers. 2021;13:2068.
25. Szczyrba J, Loprich E, Wach S, Jung V, Unteregger G, Barth S, et al. The MicroRNA profile of prostate carcinoma obtained by deep sequencing. Mol Cancer Res. 2010;8:529–38.
26. Konoshenko MY, Lekchnov EA, Bryzgunova OE, Zaporozhchenko IA, Yarmoschuk SV, Pashkovskaya OA, et al. The panel of 12 cell-free microRNAs as potential biomarkers in prostate neoplasms. Diagnostics. 2020;10:38.
27. Wang NL, Li Q, Cheng NH, Guan G, Wang ZL, Qin Y, et al. miR-205 is frequently downregulated in prostate cancer and acts as a tumor suppressor by inhibiting tumor growth. Asian J Androl. 2013;15:735–41.
28. Nayak B, Khan N, Garg H, Rustagi Y, Singh P, Seth A, et al. Role of miRNA-182 and miRNA-187 as potential biomarkers in prostate cancer and its correlation with the staging of prostate cancer. Int Braz J Urol. 2020;46:614–23.
29. Li Y, Li J, Yu H, Liu Y, Song H, Tian X, et al. HOXA5-miR-574-5p axis promotes adipogenesis and alleviates insulin resistance. Mol Ther Nucleic Acids. 2022;27:200–10.
30. Raza ST, Rizvi S, Afreen S, Srivastava S, Siddiqui Z, Fatima N, et al. Association of the circulating micro-RNAs with susceptible and newly diagnosed type 2 diabetes mellitus cases. Adv Biomark Sci Technol. 2022;5:57–67.
31. Lieb V, Weigelt K, Scheinost L, Fischer K, Greither T, Marcou M, et al. Serum levels of miR-320 family members are associated with clinical parameters and diagnosis in prostate cancer patients. Oncotarget. 2018;9:10402–10416.
32. Singh PK, Preus L, Hu Q, Yan L, Long MD, Morrison CD, et al. Serum microRNA expression patterns that predict early treatment failure in prostate cancer patients. Oncotarget. 2014;5:824–40.
33. Ramteke P, Deb A, Shepal V, Bhat MK. Hyperglycemia associated metabolic and molecular alterations in cancer risk, progression, treatment, and mortality. Cancers. 2019;11:1402.
34. Yaribeygi H, Atkin SL, Sahebkar A. A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress. J Cell Physiol. 2018;234:1300–12.
35. He S, Shi J, Mao J, Luo X, Liu W, Liu R, et al. The expression of miR-375 in prostate cancer: a study based on GEO, TCGA data and bioinformatics analysis. Pathol Res Pract. 2019;215:152375.
36. Pickl JMA, Tichy D, Kuryshev VY, Tolstov Y, Falkenstein M, Schuler J, et al. Ago-RIP-Seq identifies Polycomb repressive complex I member CBX7 as a major target of miR-375 in prostate cancer progression. Oncotarget. 2016;7:59589–603.
37. Liu Y, Yang C, Chen S, Liu W, Liang J, He S, et al. Cancer-derived exosomal miR-375 targets DIP2C and promotes osteoblastic metastasis and prostate cancer progression by regulating the Wnt signaling pathway. Cancer Gene Ther. 2022;30:437–49.
38. Wang D, Lu G, Shao Y, Xu D. MiR-182 promotes prostate cancer progression through activating Wnt/β^2-catenin signal pathway. Biomed Pharmacother. 2018;99:334–9.
39. Stafford MYC, McKenna DJ. MiR-182 is upregulated in prostate cancer and contributes to tumor progression by targeting MITF. Int J Mol Sci. 2023;24:1824.
40. Ye X, Tam WL, Shibue T, Kaygusuz Y, Reinhardt F, Ng Eaton E, et al. Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. Nature. 2015;525:256–60.
41. Gonzalez P, Lozano P, Ros G, Solano F. Hyperglycemia and oxidative stress: an integral, updated and critical overview of their metabolic interconnections. Int J Mol Sci. 2023;24:9352.
42. Pellacani D, Droop AP, Frame FM, Simms MS, Mann VM, Collins AT, et al. Phenotype-independent DNA methylation changes in prostate cancer. Br J Cancer. 2018;119:1133–43.
43. O'Hagan HeatherM, Wang W, Sen S, DeStefano Shields C, Lee Stella S, Zhang Yang W, et al. Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG islands. Cancer Cell. 2011;20:606–19.
44. Peluso M, Srivatanakul P, Jedpiyawongse A, Sangrajrang S, Munnia A, Piro S, et al. Aromatic DNA adducts and number of lung cancer risk alleles in Map-Ta-Phut Industrial Estate workers and nearby residents. Mutagenesis. 2013;28:57–63.
45. Kutwin P, Konecki T, Borkowska EM, Magdalena T-B, Jablonowski Z. Urine miRNA as a potential biomarker for bladder cancer detection - a meta-analysis. Cent Eur J Urol. 2018;71:177–85.
46. Abramovic I, Ulamec M, Katusic Bojanac A, Bulic-Jakus F, Jezek D, Sincic N. miRNA in prostate cancer: challenges toward translation. Epigenomics. 2020;12:543–58.
47. Li W, Zhang X, Sang H, Zhou Y, Shang C, Wang Y, et al. Effects of hyperglycemia on the progression of tumor diseases. J Exp Clin Cancer Res. 2019;38:327.
48. Brennan E, McClelland A, Hagiwara S, Godson C, Kantharidis P. miRNAs in the pathophysiology of diabetes and their value as biomarkers. In: *Epigenetic biomarkers and diagnostics*. Boston: Academic Press; 2016, p. 643–61.
49. Chen J-Y, Wang P-Y, Liu M-Z, Lyu F, Ma M-W, Ren X-Y, et al. Biomarkers for prostate cancer: from diagnosis to treatment. Diagnostics. 2023;13:3350.
50. Pang KH, Rosario DJ, Morgan SL, Catto JWF. Evaluation of a short RNA within prostate cancer gene 3 in the predictive role for future cancer using non-malignant prostate biopsies. PloS One. 2017;12:e0175070.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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