

Clinical Utility of miRNA-1, miRNA-29g and miRNA-133s Plasma Levels in Prostate Cancer Patients With High-Intensity Training After Androgen-Deprivation Therapy

A. GAZOVA¹, A. SAMAKOVA³, E. LACZO⁴, D. HAMAR⁴, M. POLAKOVICOVÁ^{4,5}, M. JURIKOVA⁶, J. KYSELOVIC²

¹Institute of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Comenius University Bratislava, Slovak Republic, ²5th Department of Internal Medicine, Faculty of Medicine, Comenius University Bratislava, University Hospital Bratislava, Slovak Republic, ³Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University Bratislava, Slovak Republic, ⁴Faculty of Physical Education and Sports, Comenius University Bratislava, Slovak Republic, ⁵Faculty of Pharmacy, Comenius University Bratislava, Slovak Republic, ⁶Institute of Histology and Embryology, Faculty of Medicine, Comenius University Bratislava, Slovak Republic

Received June 8, 2019

Accepted September 20, 2019

Summary

The randomized trials showed that the addition of training resistance program to androgen-deprivation therapy (ADT) had many beneficial effects for prostate cancer (PC) patients (significant protective effect on the volume of muscle mass) and the studies have revealed a panel of miRNAs, which are deregulate in PC and may serve as promising biomarkers of PC risk. The primary aim of our present study was to investigate the effect of exercise training to changes in body composition (muscle strength) and the secondary endpoint was to investigate the impact of an exercise training program on plasma levels of selected myogenic microRNAs (miRNAs) (miRNA-1, miRNA-29b, and miRNA-133) in PC patients undergoing the ADT. Effect of ADT and exercise intervention showed significant increase (experimental group vs. control group) the changes in body composition, free testosterone levels, IL-6 and plasma levels of myogenic miRNAs and significant reduced insulin serum levels. In conclusion, resistance training with ADT in the treatment of PC significantly changed the physical and metabolic function and the plasma levels of specific myogenic miRNAs. Our data support with the other publicized results.

Key words

Prostate cancer • Androgen-deprivation therapy • miRNAs • Training

Corresponding author

A. Gazova, Faculty of Medicine, Comenius University, Špitálska 24,813 72 Bratislava, Slovak Republic. E-mail: andrea.gazova@fmed.uniba.sk

Introduction

Prostate cancer (PC) is one of the most commonly diagnosed malignancies in men. Early diagnosis of cancer and timely detection of disease progression are crucial for the effective treatment and beneficial clinical outcome. Androgen-deprivation therapy (ADT) is commonly used in the treatment of PC (Keating *et al.* 2006). Despite some controversies surrounding the appropriate scope of use, there is evidence that ADT is effective at slowing tumor progression, reducing symptoms, and improving survival in PC patients. ADT is accompanied by a range of adverse effects such as acceleration of the age-related decline in bone mineral density, increasing adiposity and decreasing lean body mass within 3 to 12 months of initiation of treatment. The loss of muscle mass is associated with reduced muscular strength and increasing falls, fracture risk and ADT-induced fatigue. Exercise training has been proposed as natural and non-invasive strategy for ameliorating these adverse effects (Taafle

et al. 2017, Ostergren *et al.* 2016, Basaria *et al.* 2006).

Randomized trials have demonstrated that the addition of a resistance training program to ADT therapy had many beneficial effects for PC patients and resulted in the mitigation of selected adverse effects. Studies comparing the impact of routine treatment on oncological patients with a specialized exercise program have shown a significant protective effect on the volume of muscle mass with the inclusion of a specialized exercise program (Battaglini *et al.* 2007, Coleman *et al.* 2003).

During the last decade, a major advancement in molecular biology was the discovery of non-coding RNAs, including microRNA (miRNA) which serve important biological functions. Since their discovery, miRNAs have shown many promising perspectives in a wide range of clinical applications, not only for diagnostic purposes, but in therapy as well. Results from numerous clinical studies have revealed a panel of miRNAs, which are deregulated in PC and may serve as promising biomarkers of PC risk, progression and potential therapeutic targeting. Similarly, miRNA expression levels also vary considerably depending on the mode of exercise and muscular pathology (Eisenberg *et al.* 2007, Polakovicova *et al.* 2016, Gerdes *et al.* 2014, Calin *et al.* 2012).

In serum and plasma, expression patterns of miRNAs in miRNA-1, miRNA -29b, miRNA-133a, miRNA-206, miRNA-208a, miRNA-208b miRNA-486 and miRNA-499 have shown differences in response to various endurance and strength training modalities (Zen *et al.* 2012, De Planell-Saguer *et al.* 2013, Chen *et al.* 2008, Baggish *et al.* 2011, Baggish *et al.* 2014, Mooren *et al.* 2013, Clauss *et al.* 2016, Aoi *et al.* 2013). These miRNAs could play a role also in myogenesis, muscle regeneration (Horak *et al.* 2016, Moresi *et al.* 2015, Kovanda *et al.* 2014), and in different models of muscle atrophy (Li *et al.* 2017). On the other hand, different studies have shown a specific expression profile of miRNAs in several types of cancer including PC (Hasáková *et al.* 2017). Cumulative results show that these miRNAs could be involved in all steps of PC development, such as cell proliferation, differentiation, and progression (Filella *et al.* 2018).

To determine the relationship between the role of physical activity on PC patients and miRNAs, miRNA profiling needs to be performed. Thus, the aim of our present study was to investigate the impact of a specialized strength-training program on physical and metabolic function and plasma levels of

selected myogenic miRNAs (miRNA-1, miRNA-29b, miRNA-133a) in PC patients undergoing ADT.

Methods

Patients and trial design

The randomized open prospective study was conducted from August 2014 to October 2014. The protocol was approved by the Ethics committee for research projects established at the Faculty of Physical Education and Sport, Comenius University in Bratislava (Decision No. 10-1-13/FTVSUK).

Potential participants were primarily identified by their treating urologist/oncologist and referred to the study coordinator to confirm eligibility, describe the study, and obtain informed consent. In total, 32 prostate cancer patients were recruited and treated in two specialized health establishments in Bratislava, Slovak Republic. Every eligible patient had histologically confirmed localized prostate cancer, without metastatic disease or other active oncological illness except for skin conditions or anaemia. Patients were required to be treated with long-term ADT for 24-36 weeks. Inclusion criteria also included patients aged 60-78 years, a sedentary lifestyle without any exercise, structured resistance or aerobic training lasting more than 45 minutes per week in the previous 12 months. Exclusion criteria included evidence of heart disease, advanced hypertension, insulin dependent diabetes mellitus, chronic obstructive pulmonary disease, hepatic or liver ailments or presence of psychiatric illnesses. After the initiation phase of the trial, nine patients withdrew consent, leaving 23 patients for evaluation.

Participants were randomized to one of the two study arms: the control group (ADT + normal daily activities) or experimental group (ADT + supervised specialized strength training program). Study endpoints were assessed at baseline and after 16 weeks. The primary study endpoint was to investigate the effect of exercise training to changes in body composition, physical function, and muscle strength and cardio-metabolic alterations in PC patients undergoing ADT. The prespecified secondary endpoint was to investigate the impact of the strength training program on plasma levels of selected myogenic miRNAs (miRNA-1, miRNA-29b and miRNA-133a) in these patients.

Exercise training program

Participants in the experimental group undertook

progressive resistance training thrice a week for 16 weeks. Exercise training was carried out at the Faculty of Physical Education and Sport, Comenius University in Bratislava. Fifteen participants were placed in the

experimental group and the training program was designed by recommendations for movement and activity of oncology patients, published by Schmitz *et al.* (2010). See the characterization of training program in Table 1.

Table 1. Characterization of training program.

		Experimental group				
<i>Duration (month)</i>	<i>Training (a week)</i>	Characterization of training unit:				
		Resistance	No. of series:	No. of exercise in series	Repetition of exercises	Rest periods (min)
1st period: developing power capabilities						
2 units per week: practice exercise technique						
<i>1 month</i>	<i>3 times</i>	30 %	2	5	10-15	2 after exercise 5 after series
1 unit per week: compensating increase load, developing mobility						
2nd and 3th periods: progressively increasing resistance						
2 units per week: suppressing muscle mass loss and strength development						
<i>2 months</i>	<i>3 times</i>	90-100 %	2	5	10-12	2 after exercise 5 after series
1 unit per week: compensate the weight						
4th period: progressively increasing resistance						
2 units per week: suppressing muscle mass loss and strength development						
<i>1 month</i>	<i>3 times</i>	90-100 %	3	5	10-15	2 after exercise 5 after series
1 unit per week: compensate the weight						

Control group was without training

Assessments

At baseline and after 16 weeks of trial, patients were assessed by means of physical examination (body composition, functional ability, bone mineral density, and venous blood samples). Strength abilities were assessed on the third day.

Total RNA was extracted from 100 µl of plasma using TRI Reagent™ Solution (Invitrogen™) according to the manufacturer's instructions and with use of phenol-chloroform extraction. For quantitative real-time reverse-transcription PCR analysis, a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) was

used. Quantitative PCR was performed with the TaqMan Universal PCR Master Mix II (Applied Biosystems). Each sample was analyzed in triplicate. Every batch of amplifications included 3 water blanks as negative controls for each of the reverse-transcription and PCR steps. All data were collected and analyzed with LinReg PCR used the methods $\Delta\Delta C_t$, as result we show expression fold change. We conducted TaqMan MicroRNA assays for hsa-miR-1, hsa-miR-29b-3p, hsa-miR-133a-3p (Invitrogen). The small RNAs (SNU6) frequently used for normalization of miRNA expression, was included as well.

Endpoints

Study endpoints were assessed at baseline and 16 weeks. The primary study endpoint was to investigate the effect of exercise training to changes in body composition, physical function, and muscle strength and cardio-metabolic alterations in PC patients undergoing ADT. The prespecified secondary endpoint was to investigate the impact of the strength training program on plasma levels of selected myogenic miRNAs (miRNA-1, miRNA-29b, miRNA-133a) in these patients.

Statistical analysis

Data were analyzed using Minitab 17 (Minitab, Pennsylvania State University, USA). Statistical analyses were performed with independent and paired sample T-Tests.

Results

Characteristics of the patients

The randomization included 32 patients, 9 of whom withdrew consent, leaving 23 patients for evaluation, from August 2014 to October 2014. The average age of the patients was 70.69 ± 7.5 years in the experimental group, and 69.21 ± 5.8 years in the control group. The average weight was 90.5 ± 14.5 kg, height 173.3 ± 21.7 cm in the experimental group, and 94.0 ± 12.2 kg with 174.3 ± 16.7 cm in the control group. The median BMI was 30.14 ± 6.4 kg/m² in the experimental and 30.85 ± 5.2 kg/m² in the control group (Table 2).

Endpoints

The primary study endpoint was to investigate the effect of exercise training to changes in body composition, physical function, and muscle strength and cardio-metabolic alterations in PC patients undergoing ADT. The primary endpoint difference of results at 16th week are shown in Fig. 1A, 1B. There were no significant between-group differences in the changes of weight, BMI and bone mineral density.

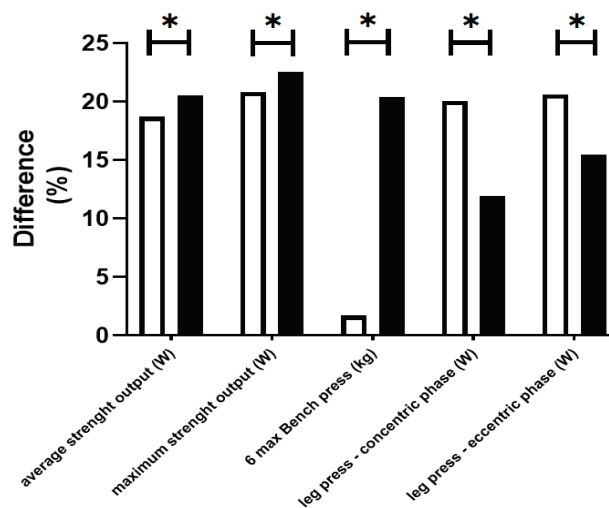


Fig. 1A Mean Percent Change from Baseline in Average and Maximal Strength Output, 6 max Bench Press, Concentric and Eccentric Phase of Leg Press. Within each group * indicate significant difference between baseline and the end of intervention (* $p < 0.05$). □ - control group (ADT only), ■ - experimental group (ADT + Training Program).

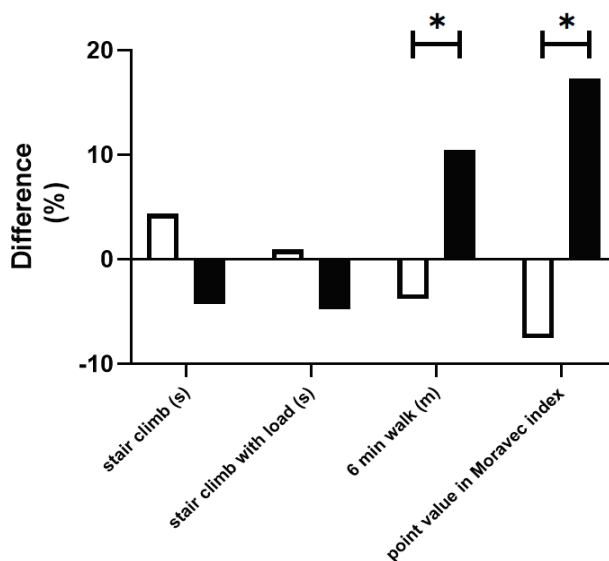


Fig. 1B Mean Percent Change from Baseline in Stair Climb, Stair Climb with Load, 6 min Walk, Point Value in Moravec index. Within each group * indicate significant difference between baseline and the end of intervention (* $p < 0.05$). □ - control group (ADT only), ■ - experimental group (ADT + Training Program).

Table 2. Baseline Characteristics of the Study Participants. Data expressed as mean \pm standard deviation.

Characteristic	Control Group (n = 8) ADT only	Experimental Group (n = 15) ADT and exercise intervention
Age (years)	70.69 ± 7.5	69.21 ± 5.8
Weight (kg)	90.5 ± 14.5	94.0 ± 12.2
Height (cm)	173.3 ± 21.7	174.3 ± 16.7
BMI (kg/m ²)	30.14 ± 6.4	30.85 ± 5.2

Table 3. Changes in body composition, physical function and metabolic function.

Parameter	Control Group ADT only			Experimental Group ADT and Training Program		
	PRE	POST	Difference (%)	PRE	POST	Difference (%)
Physical function						
<i>Average strength output (W)</i>	195.62±44.54	232.24±40.92	+18.72	199.41±45.66	240.31±22.24	+20.51 *
<i>Maximum strength output (W)</i>	546.55±109.54	660.35±111.1	+20.82	576.62±158.3	706.67±129.7	+22.55 *
<i>6 max Bench press (kg)</i>	32.78±7.11	33.33±9.12	+1.69	39.62±14.73	47.69±13.92	+20.39 *
<i>Leg press - concentric phase (W)</i>	111.25±28.90	133.58±27.44	+20.07	135.30±34.34	151.46±38.74	+11.95 *
<i>Leg press - eccentric phase (W)</i>	151.07±59.08	182.22±55.02	+20.62	168.54±60.29	194.60±65.49	+15.44 *
<i>Stair climb (s)</i>	10.77±1.55	11.24±1.46	+4.36	11.07±2.34	10.62±2.59	-4.25
<i>Stair climb with load (s)</i>	11.45±1.48	11.56±0.86	+0.96	11.45±2.40	10.93±2.42	-4.76
<i>6 min walk (m)</i>	564.13±56.23	542.88±60.81	-3.77	575.64±71.31	635.86±58.32	+10.46 *
<i>Point value in Moravec index</i>	101.50±21.55	94.38±23.18	-7.55	103.07±21.68	120.93±18.51	+17.33 *
Metabolic function						
<i>Total testosterone (nmol/l)</i>	0.45±0.12	0.449±0.13	-0.22	0.35±0.18	0.69±0.21	+95.74 *
<i>Free testosterone (nmol/l)</i>	5.18±2.33	5.80±1.89	+12.07	3.62±1.12	5.37±1.48	+48.34
<i>Insulin (mIU/l)</i>	26.28±7.08	47.32±7.72	+80.06	26.34±8.35	21.42±7.93	-22.94 *
<i>Interleukin-6 (pg/ml)</i>	2.80±1.05	4.0±1.62	+42.87*	3.26±1.22	4.51±1.34	+38.36
<i>PSA (µg/l)</i>	0.22±0.08	0.78±0.21	+255.62	0.51±0.28	0.61±0.21	+18.91
<i>Cortisol (nmol/l)</i>	538.68±121.88	441.23±118.7	-22.08	435.68±84.46	399.57±94.9	-9.03

*p<0.05 with the use of paired t-test for the comparison between the trial entry and the end of study. Data expressed as mean ± standard deviation.

Impact of ADT and exercise intervention showed significant increase (experimental group vs control group) in lean body mass (+4.65 %, p=0.02 vs -1.16 %, p=0.798), average strength output (+20.51 %, p=0.006 vs +18.72 %, p=0.318) and maximum strength output (+22.51 %, p=0.04 vs +20.82 %, p=0.318) (Fig. 1A). There were significantly improved physical functions (experimental group vs control group) in the 6 max bench press (+20.39 %, p=0.041 vs +1.69, p=0.176), concentric (+11.95 %, p=0.005 vs +20.07 %, p=0.051) and eccentric (+15.44 %, p=0.049 vs +20.62 %, p=0.081) phase of leg press (Fig. 1A), 6 min walk (+10.46 %, p=0.045 vs -3.77 %, p=0.528) and in point value in Moravec index (+17.33 %, p=0.006 vs -7.55 %, p=0.855) (Fig. 1B). ADT with or without exercise intervention indicated no significant changes in stair

climbing (Table 3 – Physical function).

The rates of metabolic disease progression including selected biochemical levels were lower in the experimental group (ADT + training program) than in the control group (ADT only). In the experimental group were significantly reduced insulin serum levels (PRE: 26.336 mIU/l, POST: 21.422 mIU/l, -22.94 %, p=0.039) and were significantly increased the levels of free testosterone (PRE: 0.352 nmol/l, POST: 0.689 nmol/l, +95.74 %, p=0.021) compared to control group. Interleukin-6 was significantly increased in the control group (PRE: 2.799 pg/ml, POST: 3.999 pg/ml, +42.87 %, p=0.035) compared to the experimental group (PRE: 3.256 pg/ml, POST: 4.505 pg/ml, 38.36 %, p=0.145) (Table 3 – Metabolic Changes).

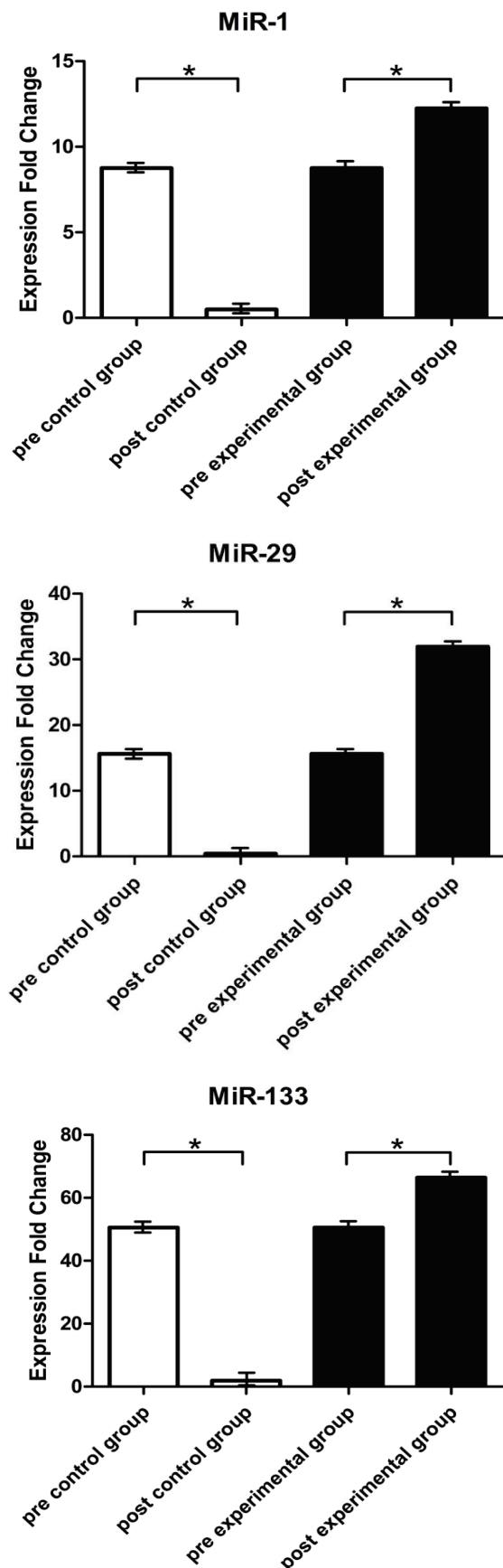


Fig. 2. Fold change of expression levels of miR-1, miR-29b, miR-133a at baseline and after 16 weeks. (* $p < 0.05$ - within each group * indicate significant difference between baseline and the end of intervention and the control).

MicroRNA analyses

In an effort to identify miRNAs involved in post-ADT cardiovascular changes, we compared miRNA expression profiles in the control and experimental group at baseline and after 16 weeks of intervention. The expression fold-change of miRNA-1, miRNA-29 and miR-133 significantly decreased in control group after 16 weeks of ADT. Real-time PCR analysis indicated that the expression fold-change of miRNA-1, miRNA-29 and miR-133 in experimental group were significantly increased in response to 16 weeks of ADT and strength training program (Fig. 2).

Discussion

In this randomized trial, we have demonstrated that the addition of a strength training (ST) program to ADT had many positive effects for patients and resulted in the mitigation of selected adverse effects. Previously it has been shown, that a regular exercise program was effective in preventing muscle mass loss (Segal *et al.* 2009). Studies comparing the impact of routine treatment on oncological patients with a specialized exercise program have resulted in a significant protective effect on the volume of muscle mass with the inclusion of a specialized exercise program (Battaglini *et al.* 2007, Coleman *et al.* 2003, Collins *et al.* 2012).

We hypothesized that in patients on ADT, strength training would increase muscle power, thereby improving body composition and physical function in older men with PC. We demonstrated a significant increase in average (+20.51 %, $p = 0.006$) and maximum (+22.55 %, $p = 0.040$) strength output in the experimental group compared to the control group. Hanson and colleagues reported the results of a 12 week ST regimen on 17 men with PC on ADT. They found that ST significantly increased average strength output (+17 %) in ADT-treated prostate cancer patients (Hanson *et al.* 2013). LeStayo *et al.* (2011) found an increase in average strength output (+28.91 %) in an oncological group without ADT.

Lean body mass is a frequent primary endpoint in randomized trials that follow the efficacy of an exercise program on the body composition of oncology patients using ADT (Galvao *et al.* 2006). We assume that there are two opposing changes occurring, namely a decrease in visceral reserves and an increase in the volume of muscle mass. The results of our study found a statistically significant increase in lean body mass by

4.65 %. Studies by Cormie and colleagues showed an almost identical, statistically significant alteration in the proportion of lean body mass, at a level of 3-4 %, after a three-month power program (Cormie *et al.* 2014). We did not either see a significant change in the experimental or control group when we evaluated changes in body mass index or body weight. Body mass and weight are highly dependent on the systemic regulation of storage and consumption of energy supplies and on the management of metabolism. Different ranges and prevalence of metabolic disorders in subject groups could be the main reasons for the lack of statistically significant changes in the body mass index or body weight.

Small endogenous non-coding RNAs (18-25 nt) known as miRNAs can prevent protein expression through cleavage of specific target mRNAs or through inhibition of their translation (Bartel 2004). miRNAs represent an interesting target for biomarker discovery. Every miRNA can interact with various cellular pathways, and therefore alterations in the expression of few miRNAs may impact the deregulation of several cellular processes contributing to cancer complexity. The issue of early cancer detection represents a promising field for miRNA-based diagnostics, as currently there is a lack of early detection methods and screening tests in many types of cancer. Recent clinical and experimental studies have demonstrated that microRNAs have distinct expression profiles in normal and cancer cells, which are tissue specific (Franco *et al.* 2011), microRNAs can also influence all hallmarks of cancer either by promoting or suppressing tumour development and progression (Vanacore *et al.* 2017).

Kojima *et al.* (2012) observed significantly lower expression levels of miRNA-1 and miRNA-133a in PC tissues compared with non-prostatic cancer tissue PC and suggested that these changes are key steps in oncogenesis or progression in PC. They also demonstrated that restoration of miRNA-1 or miRNA-133a expression suppressed cancer cell proliferation, migration, and invasion in PC. In addition, several studies have shown that miRNA-133a is under-expressed in pancreatic ductal adenocarcinoma, colon cancer, and tongue squamous cell carcinoma (Basaria *et al.* 2006). In tongue squamous cell carcinoma miRNA-133a inhibited cell proliferation and induced apoptosis (Wong *et al.* 2008). In our study, we detected significantly lower expression levels of miRNA-1 in the control group while a significantly higher expression level was noted in the experimental group. A similar

trend was noted in the level of expression of miRNA-133a, levels were significantly increased in the experimental group compared to control. Kojima *et al.* (2012) have shown that the restoration of miRNA-1 or miRNA-133a expression suppressed cancer cell proliferation, migration, and invasion in PC. Several reports demonstrated that miRNA-133a was under-expressed in pancreatic ductal adenocarcinoma, colon cancer and tongue squamous cell carcinoma (Basaria *et al.* 2006), and in tongue squamous cell carcinoma, miRNA-133a inhibited cell proliferation and induced apoptosis (Wong *et al.* 2008). Taken together, miRNA-1 and miRNA-133a appear to be important miRNAs, acting as tumor suppressors in several human cancers including PC.

Thus, miRNAs have been reported to play fundamental roles in diverse biological and pathological processes, including muscle development and regeneration. Different miRNAs, including miRNA-1, miRNA-133 and miRNA-206 have been shown to participate in myogenesis and muscle regeneration (Horak *et al.* 2016, Moresi *et al.* 2015, Kovanda *et al.* 2014). In contrast, miRNAs have also been shown to play a role in different models of muscle atrophy, including miRNA-1, miRNA-133, miRNA-23a, miRNA-21, miRNA-27, miRNA-628, miRNA-431 and miRNA-206 (Li *et al.* 2017). Li *et al.* identified miRNA-29b induction and activity as a novel pathway contributing to muscle atrophy. The expression of miRNA-29b is significantly increased in *in vivo* and *in vitro* models of muscle atrophy. In our study, levels of miRNA-29b in the control group were lower than in the experimental group.

Conclusions

In conclusion, strength training in combination with ADT in the treatment of PC, alters the levels of specific microRNAs, suggesting the possibility that these may serve as relevant biomarkers of cell proliferation and prostate cancer.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This publication is the result of the project implementation: VEGA 1/0949/15, APVV-14-0416 and MZ SR 2016/13-FAUK-1.

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