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ORIGINAL PAPER

MiRNAs and tempol therapeutic potential in prostate cancer: a preclinical approach

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Abstract

This study investigated tempol action on genes and miRNAs related to NFκB pathway in androgen dependent or independent cell lines and in TRAMP model in the early and late-stages of cancer progression. A bioinformatic search was conducted to select the miRNAs to be measured based on the genes of interest from NFκB pathway. The *miR-let-7c-5p*, *miR-26a-5p* and *miR-155-5p* and five target genes (BCL2, BCL2L1, RELA, TNF, PTGS2) were chosen for RT-PCR and gene enrichment analyses. In vitro, PC-3 and LNCaP cells were exposed, respectively, to 1.0 or 2.0 mM of tempol during 48 h. In vivo, five experimental groups were evaluated regarding tempol effects in the early (CT12 and TPL12 groups) and late-stages (CT20, TPL20-I and TLP20-II) of PCa development. TPL groups were treated with 50 mg/kg or 100 mg/kg of tempol. The ventral lobe of the prostate and the plasma was collected. Tempol treatment increased miRs expression in PC-3 and LNCaP. For both cell lines, tempol decreased RELA expression. In TRAMP model, tempol increased miRNA expression in prostate for all treated groups. Tempol upregulated the miRNA expressions related to the NFκB pathway in the prostate tissue and human tumor cell lines. Their increase is mainly linked to increased cell death and delayed CaP aggressivenes. Thus, tempol's capacity for miRNA-mediated gene silencing to decrease tissue proliferation and cell survival processes is part of its tissue mechanics.

Keywords Prostate cancer · Tempol · miRNAs · NFκB

Introduction

Prostate cancer (PCa) is one of the leading causes of morbidity and mortality in men worldwide. It is estimated that more than 1.4 million new cases were diagnosed in 2020, culminating in approximately 375,000 deaths associated with this disorder (Siegel et al. 2023). In the United States and Brazil, it is the most common non-cutaneous malignancy and the second most common cause of death among men (Siegel et al. 2023; INCA 2023). Therefore, it is extremely important to have deep and detailed knowledge of the molecular mechanisms involved in prostate cancer progression to develop more effective therapeutic strategies.

NFκB (Nuclear Kappa Factor B) is part of important cell signaling pathways, which has been widely investigated in the PCa microenvironment. NFκB is a transcription factor with different components; NF-κB1 (known as p105), NF-κB2 (known as p100), RelA (known as p65), RelB and c-Rel (Staal and Beyaert 2018). The transactivating members (RelA, RelB and c-Rel) of the NFκB family are kept inactive in the cytoplasm by the binding of NFκB family member inhibitors, the IκBs. Thus, the IκBs prevent NF-κB from translocating to the nucleus and then participate in the transcription of important genes, which are responsible for regulating several biological processes, such as inflammation, apoptosis, cell proliferation and the immune response (Zinatizadeh et al. 2020).

This work was performed at University of Campinas (UNICAMP), at Institute of Biology (IB), Department of Structural and Functional Biology.

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Studies have explored therapeutic strategies that act on the NFκB pathway in order to attenuate inflammation and cell proliferation (Kido et al. 2022; Montico et al. 2023; Stark et al. 2015). Recently, Rossetto et al. (2023) demonstrated the ability of tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) to differentially modulate inflammation in PCa preclinical models, decreasing protein levels of NFκB p65, TNF-α, and increasing protein levels of iκBs. Also, tempol, considered a nitroxide of significant biological value as an antioxidant and redox compound, has shown anticancer activity in various experimental models (Wilcox 2010), however its role specifically in PCa is still not widely known (Rosseto et al. 2023, Lejeune et al. 2006; Thomas and Sharifii 2012). In addition, studies related to tempol action modulating gene expression and miRNAs expression are scarce (Li et al. 2023). MiRNAs are small non-coding RNA molecules that play a crucial role in the post-transcriptional regulation of gene expression (Shi et al. 2021). Aberrant miRNAs expression has been detected in different types of cancer, including prostate cancer, and is associated with uncontrolled cell proliferation, invasion, metastasis and resistance to therapy (Urabe et al. 2022).

Various experimental models have been used in PCa studies. One of these models is TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate), which involves the transgenic expression of the murine virus oncogene (SV40 T/t) under the control of the prostate-specific promoter (Greenberg et al. 1995). This model has biological similarities to the tumor progression observed in human prostate cancer and has been widely used to study the molecular mechanisms and targeted therapies for this disease (Kido et al. 2019).

In addition to the TRAMP model, the use of tumor cell lines derived from prostate cancer patients has also been an important strategy for studying this disease. These tumor cell lines are kept in culture and can be used to investigate the effects of different therapeutic approaches; test the efficacy of new drugs; and evaluate the individualized response of patients (Namekawa et al. 2019).

The aim of this study was to investigate the potential effects of tempol on prostate cancer (PCa), specifically at the levels of gene expression and miRNAs. We explored its impact on the NFκB pathway and the miRNAs associated with this pathway. Taking these factors into consideration, we observed distinct actions of tempol in the TRAMP model at both the early and late stages of PCa progression. Furthermore, its effects were examined in human tumor cell lines PC-3 and LNCaP, considering the characteristics of androgen independence and dependence of these cells.

Materials and methods

In silico analysis of miRNAs and target genes

Selection of miRNAs for experimental validation

Firstly, a bioinformatic search was performed for the identification of miRNAs that target key members of the NFκB pathway. To this end, the constituents of the NFκB pathway were first downloaded from the KEGG PATHWAY Database (Kyoto Encyclopedia of Genes and Genomes - https://www.genome.jp/kegg/). The list of pathway elements was entered into the miRWalk version 2.0 online software (http://mirwalk.umm.uni-heidelberg.de/) for the computational prediction of miRNAs for each member of the ontological categories of interest. All the miRNas interacting with the NFκB pathway elements were selected (based on the choice of 10 prediction algorithms, with the Targetscan algorithm (https://www.targetscan.org/vert_80) as a condition for prediction).

The miRNAs that potentially regulate key members of the ontological categories of interest were selected for experimental analysis in vivo and in vitro. Then, a literature search was conducted to search for the selection of miRNAs with known involvement in inflammation and cell proliferation.

Gene enrichment analysis and network construction

miRWalk version 2.0 (http://mirwalk.umm.uni-heidelberg.de) was used to predict miRNA-target sequence-based interactions using 12 different algorithms. Only interactions predicted by TargetScan and located on the 3'UTR were considered valid. From the resulting list, we created a protein-protein interactions network. For such we used the STRING protein query on Cytoscape (version 3.8.2). Confidence score was set to 0.8, 10 additional interactions were allowed, and singletons were not considered. The enrichment of these networks was performed using the STRING enrichment tool.

Cell culture and reagents

Human PCa cell lines PC-3 and LNCaP were obtained from American Type Culture Collection (ATCC) and Rio de Janeiro Cell Bank (Rio de Janeiro, SP, Brazil), respectively. Both cell lines were maintained in RPMI-1640 medium (Vitrocell, SP, Brazil) at 37 °C with 5% CO_2, supplemented with 10% fetal bovine serum (FBS; Vitrocell, SP, Brazil) and 1% penicillin/streptomycin (Vitrocell, SP, Brazil). Tempol (4-hydroxy-TEMPO, 176141, Sigma Aldrich, St. Louis, MO, USA) was dissolved directly in the medium at different concentrations.

In vitro treatment of cell lines

Sample preparation

PC-3 (3×10^5) and LNCaP (4×10^5) cells were incubated in *petri* dishes ($60mm^2$) and treated or not with tempol 24 h after the plating procedure. The dose and treatment time chosen for PC-3 and LNCaP were based on a previous study by Rossetto et al.[8]. In the end, the following groups were analyzed:

a. **PC-3 Control group (PC-3-C)**: PC-3 cell cultures maintained in culture medium for 48 h.
b. **PC-3 + tempol group (PC-3-TPL)**: PC-3 cell cultures treated 48 h with tempol added to the culture medium at a concentration of 1mM.
c. **LNCaP Control group (LNCaP-C)**: cultures of LNCaP cells maintained in culture medium for 48 h.
d. **LNCaP + tempol group (LNCaP-TPL)**: LNCaP cell cultures treated for 48 h with tempol added to the culture medium at a concentration of 2mM.

Animals and experimental procedures

25 male TRAMP mice (C57BL/6-Tg(TRAMP)8247Ng/J X FVB/NJ)F1/J) were obtained from the Multidisciplinary Center for Biological Investigation of the State University of Campinas (CEMIB/ UNICAMP). The animals were divided into five experimental groups ($n=5$) in order to evaluate the effects of tempol in the early (CT12 and TPL12 groups) and late-stage (CT20, TPL20-I and TLP20-II) of PCa development (Fig. 1). TPL groups were treated with 50 mg/Kg or 100 mg/Kg of tempol (4-Hydroxy-TEMPO, 97%, Sigma-Aldrich − 176141) diluted in water, 5 times a week, during 4 weeks. After that, the animals were euthanized. The ventral prostate lobes and plasma were collected and processed for subsequent analyses. The experiment was carried out in accordanceto Ethics Committee in the Use of Animals (CEUA 5115-1/2019, 5115-1(A)-2020).

Gene expression analysis

Total RNA extraction

Total RNA was extracted from cell lines and tissue samples according to the protocol described by Chomczynski and Sacchi (1987), using TRIzol reagent (Invitrogen), according to the manufacturer's instructions.

Reverse transcription for mRNAs

For the synthesis of cDNA (complementary DNA), reverse transcription was carried out using Thermo Fischer reagents (Applied Biosystems). Based on the concentration of RNA in the sample measured by the Implen P330 nanophotometer, the volume of sample needed to obtain 2 µg of RNA was calculated. After this, 1µL of Oligo(dt) (Exxtend, Brazil), 1µL of 10mM dNTPmix (Invitrogen, 18427013) and enough H2O for the solution to reach 12µL were added to each sample. This solution was incubated at 65ºC for 5 min, followed by immediate refrigeration. After that, 2µL of 0.1 DTT, 4µL of 5x first strand buffer, 1µL of RNAseOUT 40 U/µL (Invitrogen, 10777-019) and 1µL of M-MLV Reverse Transcriptase 200U/µL (Invitrogen, 280225-013) were added to each tube. The tubes containing the samples and the above-mentioned reagents were incubated in a dry bath for 50 min at 42ºC, with subsequent inactivation of the reaction at 70ºC for 15 min. The final content in tube were diluted in 180 µL of RNAse free water.

Reverse transcription step for miRNAs

For the miRNAs, reverse transcription was carried out using the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems) and the specific Taqman MiRNA Assays kit. For this, 10ng of total RNA was used for synthesis, in addition of 1.5µL of 10X buffer, 0.15µL of dNTP mix (100mM), 0.19µL of RNase inhibitor (20U/µL), 1µL of Multiscribe reverse transcriptase (50U/µL), and 3µL of primer specific for the miRNAs.

The mix was first prepared and kept on ice, then subjected to temperature variations in a Cyclogene thermal cycler (Techne, England) at 16 °C for 30 min, 42 °C for 30 min, followed by transcriptase inactivation at 85 °C for 5 min. The final content in tube were diluted in 86.5µL of RNAse free water.

Quantitative real-time PCR

5µL of the synthesized cDNA, 5µL of gene-specific mix of Forward and Reverse primers and 10µL of SYBR Green PCR MasterMix (Thermo Fischer, REF 4309155) were used to quantify the product formed during the mRNA PCR reaction (see Fig. 1).

To detect the products formed during the PCR reaction for miRNAs, 9µL of diluted cDNA synthesis, 10µL of TaqMan Universal Master Mix II, in UNG (Thermo Fischer, REF 4440040) (AppliedBiosystems) and 1µL of the mix containing probe and specific primers for the chosen miRNAs (see Fig. 1).

Fig. 1 Primer sequences of human and mouse genes and miRNAs

Specie	Gene	Sequency 5'-3'
Homo sapiens	BCL2	ATCGCCCTGTGGATGACTGAGT
		GCCAGGAGAAATCAAACAGAGGC
	BCL2L1	GCCACTTACCTGAATGACCACC
		AACCAGCGGTTGAAGCGTTCCT
	PTGS2	TGGTGGAGAAGTGGGTTTTC
		CGGGAAGAACTTGCATTGAT
	RELA	ATTGCGGACATGGACTTCTC
		GAACACACCCCACCAGAATC
	TNF	CTCTTCTGCCTGCTGCACTTTG
		ATGGGCTACAGGCTTGTCACTC
Mus musculus	BCL2	CCTGTGGATGACTGAGTACCTG
		AGCCAGGAGAAATCAAACAGAGG
	BCL2L1	TTTCCCCTAAACCAGCTCCT
		CCACCAACAAGACAGGCTCT
	PTGS2	GAGTGGGGTGATGAGCAACT
		TGGTTGAAAAGGAGCTCTGG
	RELA	GTGCCTACCCGAAACTCAAC
		TGGGGGAAAACTCATCAAAG
	TNF	CTTGTTGCCTCCTCTTTTGC
		TGGTCACCAAATCAGCGTTA

Specie	miRNA	Sequency 5'-3'	Ref
Homo sapiens	*hsa-miR-26a-5p*	UUCAAGUAAUCCAGGAUAGGCU	405
	hsa-miR-let-7c-5p	UGAGGUAGUAGGUUGUAUGGUU	379
	RNU6B	CGCAAGGATGACACGCAAATTCGT GAAGCGTTCCATATTTTT	1093
Mus musculus	*mmu-miR-26a-5p*	UUCAAGUAAUCCAGGAUAGGCU	405
	mmu-miR-let-7c-5p	UGAGGUAGUAGGUUGUAUGGUU	379
	mmu-miR-155-5p	UUAAUGCUAAUUGUGAUAGGGGU	2571
	U6 snRNA	GTGCTCGCTTCGGCAGCACATATA CTAAAATTGGAACGATACAGAGAA GATTAGCATGGCCCCTGCGCAAGG ATGACACGCAAATTCGTGAAGCGT TCCATATTTT	1973
C. elegans	*cel-miR-39-3p*	UCACCGGGUGUAAAUCAGCUUG	200

The reactions were carried out in an ABI 7300 Sequence Detection System® thermal cycler (Applied Biosystems) under the following conditions: 50ºC for 2 min, 95ºC for 10 min, and 40 cycles of 95ºC for 15 s and 60ºC for 1 min. After the end of the reaction, the specificity of the reaction was assessed by analyzing the dissociation of the amplified product. The 7300 SDS Software program was used to analyze the data obtained.

Detection of miRNAs in the plasma of TRAMP mice

Blood samples were collected in tubes containing EDTA and then centrifuged at 5000 rpm for 15 min. The miRNA fraction was isolated from all the samples using a specific kit for plasma extraction, the miRNeasy Serum/Plasma kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. For subsequent normalization, a Spike-in system (Spike-in control, REF 219610) was used, adding a known amount of the exogenous miRNA *cel-miR-39*, derived from the C. elegans organism, to each sample.

The Reverse Transcription step and quantitative real-time PCR for plasmatic miRNAs followed the same procedure as described above for tissue miRNA.

Differential gene expression calculation

The relative expression between the samples was calculated according to the method described by Pfaffl (2001). The *RPL19* gene, which showed stable expression under different treatment conditions in the laboratory, was chosen as the reference gene for normalizing qPCR reactions for mRNA analysis (Geraldo et al. 2012). The RNU6B miRNA (Thermo Fischer, REF 4427975) was used as the reference gene for the human tumor line samples. The miRNA U6 snRNA (Thermo Fischer, REF 001973) was used as the reference gene for mice prostate samples. The *spike-in cel-miR-39* was used as the exogenous reference gene for plasma samples.

miRNAs	BCL-2	BCL2L1	PTGS2	RELA	TNF
hsa-miR-448	10	2	2	1	1
hsa-let-7c-5p	4	9	5	2	3
hsa-miR-26a-5p	5	1	10	2	4
hsa-miR-124-3p	4	6	1	9	6
hsa-miR-181b-5p	9	1	9	1	7
hsa-miR-155-5p	7	1	5	7	1

Fig. 2 Interaction between target genes and miRNAs and number of algorithms that predict that interaction

Statistical analysis

In vitro analysis

Student's T Test was performed comparing control and treated groups for the chosen time points. ANOVA-One-way followed by Dunnett's test was carried out for cell viability assay.

In vivo analysis

the statistical analysis was considered separately for early-stage and late-stage groups. For the early-stage, Student's T Test was performed. For the late-stage, ANOVA-One-Way was performed, followed by Tukey's test.

All data was previously considered parametric after Shapiro-Wilk's Test. The statistical analysis was perfomed using GraphPad Prism and with the level of significance set at 5% (version 7.00).

Results

In silico analysis for the selection of target genes and miRNAs related to the NFκB pathway

The NFκB pathway downloaded from the KEGG PATHWAY Database showed a total of 87 genes. For these target genes, a total of 2578 miRNAs with predicted interaction with these genes were found, containing at least the TargetScan prediction algorithm. The selection of target genes for this study was based on the observed changes in protein levels within the markers already assessed in this pathway by previous studies in our laboratory. 5 genes of interest were selected and measured in the prostate of TRAMP mice and in the PC-3 and LNCaP cell lines.

To choose the miRNAs for analysis, we prioritized those with the highest number of interactions per target. In the end, the relationships established are detailed in Fig. 2. To refine the miRNAs for measurements, we conducted a literature review on the association between the listed miRs in the table and prostate cancer. Some of these were not significantly expressed in the prostate. Therefore, we selected 3 miRNAs that we considered interesting to analyze in this study: *miR-155-p*, *miR-let-7c-5p* and *miR-26a-5p*.

Tempol increased the miRNA expression levels in tumor cell lines

The androgen-independent PC-3 tumor cell line responded to tempol treatment by increasing the *miR-let-7c-5p* expression (Fig. 3A) and *miR-26a-5p* expression (Fig. 3B).

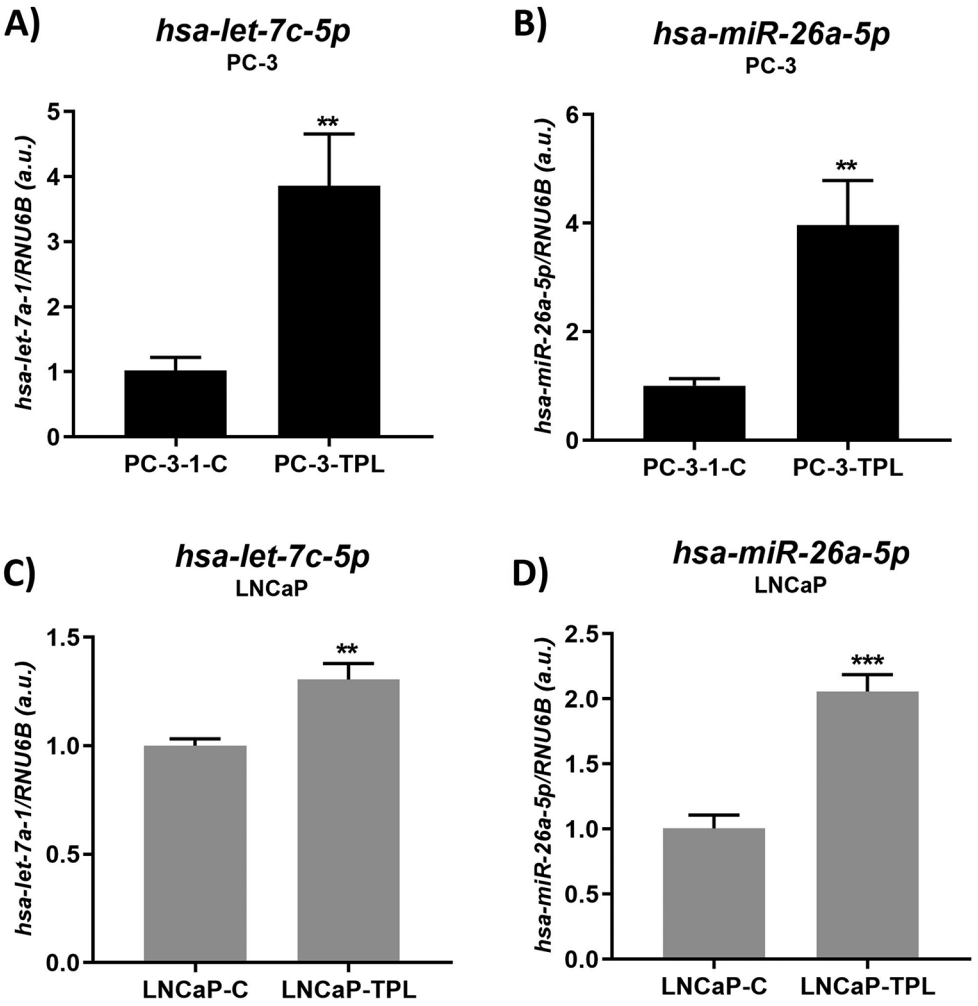

Fig. 3 Gene expression levels for the microRNAs *hsa-miR-let-7c-5p* and *hsa-miR-26a-5p* for the PC-3 and LNCaP cell lines (*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)

Similarly, the androgen-dependent LNCaP cell line also increased the miRNA expression levels after treatment (Fig. 3C and D).

Tempol influenced the target gene expression in the PC-3 and LNCaP cell lines

For the PC-3 cell line, tempol treatment was able to significantly decrease the RELA gene expression (Fig. 4D). For the other genes analyzed, there were no statistically significant changes.

For the LNCaP cell line, tempol treatment increased gene expression levels of BCL2 (Fig. 4E), BCL2L1 (Fig. 4F) and PTGS2 (Fig. 4I). In addition, a decrease in RELA gene expression was observed (Fig. 4G) in this cell line.

Tempol increased miRNA expression levels in prostate tissue

The TRAMP mouse model showed a tempol treatment response similar to that seen in the cell lines, leading to an elevation in gene expression. In particular, tempol presented a significant effect on the miRs *let-7c-5p* (Fig. 5A) and *mir-155-5p* (Fig. 5C) in early-stage PCa progression.

For the late stage, tempol was able to alter the expression levels of the miRs *let-7c-5p* (Fig. 5D), *miR-26a-5p* (Fig. 5E) and *miR-155-5p* (Fig. 5F), but only in the group that received the highest treatment dose (TPL20-II).

Tempol influenced target gene expression in early and late PCa stages in the TRAMP model

In the early stage, tempol led to decreased gene expression for BCL2L1 (Fig. 6B) and TNF (Fig. 6E). It also led to increased expression of the PTGS2 (Fig. 6D) and RELA (Fig. 6C) inflammatory genes.

In the late stage, tempol treatment led to an effective decrease in BCL2L1 (Fig. 7B) and RELA (Fig. 7C) gene expression for both treatment doses. The TPL20-II group showed decrease in BCL2 (Fig. 7A), PTGS2 gene expression (Fig. 7D) and decreased TNF expression (Fig. 7E).

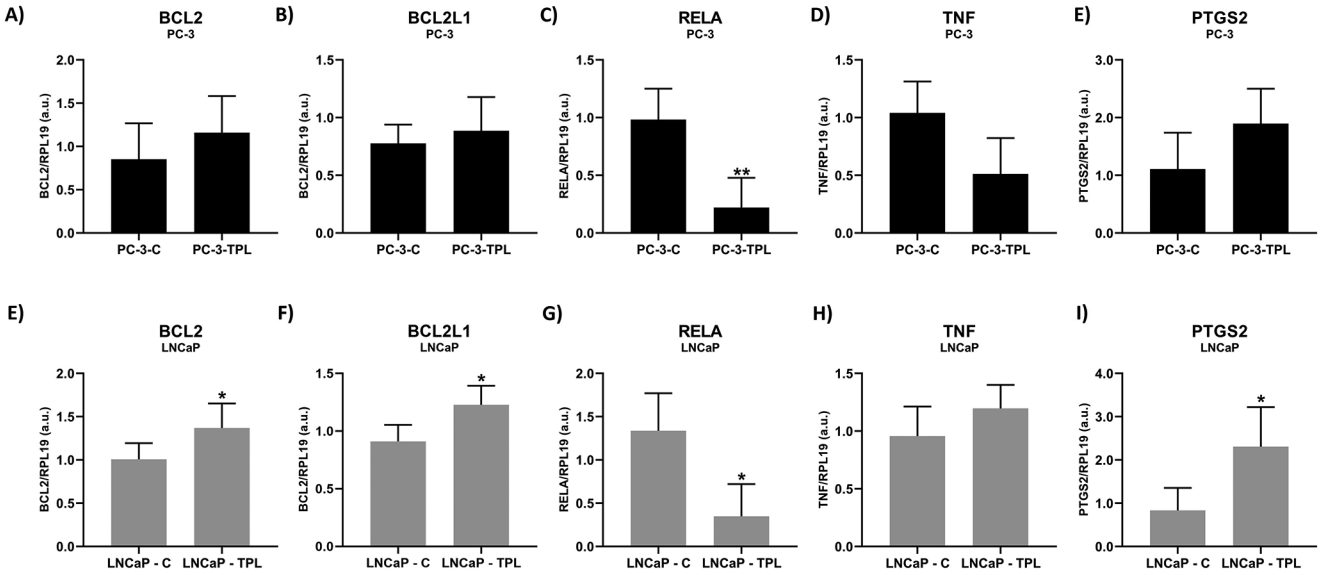

Fig. 4 Gene expression levels for BCL2, BCL2L1, RELA, TNF and PTGS2 for the PC-3 and LNCaP cell lines (*$p < 0.05$, **$p < 0.01$)

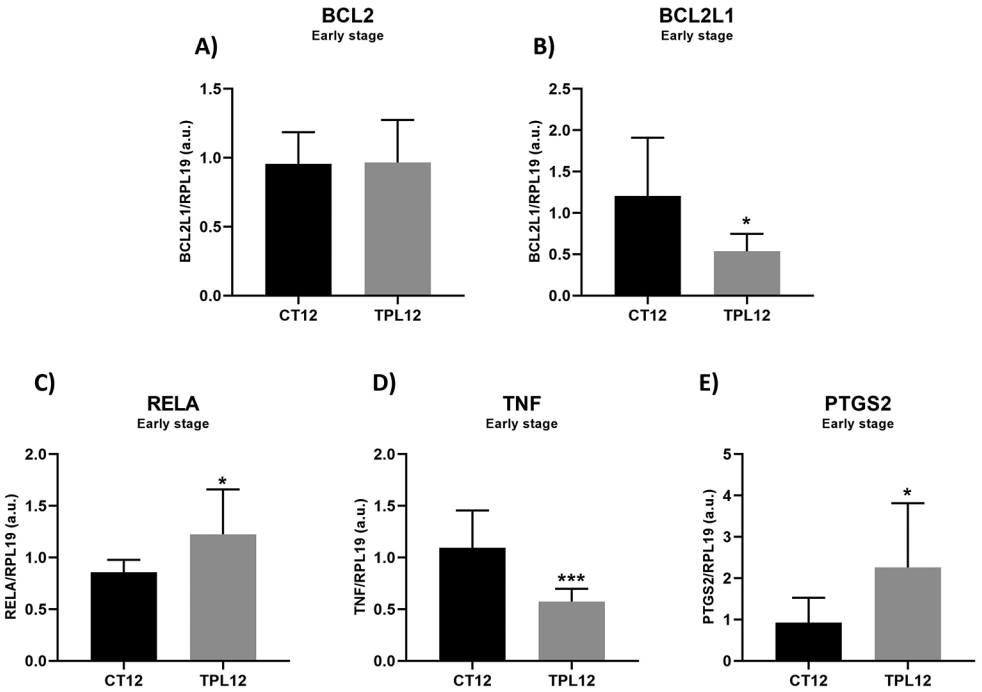

Fig. 5 In vivo gene expression levels for the microRNAs *mmu-miR-let-7c-5p*, *mmu-miR-26a-5p* and *mmu-miR-155-5p*. The letter "a" denotes a statistical difference in relation to CT20 (*$p < 0.05$)

Circulating miRNas were influenced by tempol in the TRAMP model

Tempol treatment influenced the expression of circulating miRNAs. In the early stage, there was a significant decrease in the miRs *let-7c-5p* (Fig. 8A) and *miR-26a-5p* (Fig. 8B).

In the late stage of PCa, tempol led to a decrease in *miR-let-7c-5p* (Fig. 8D) in the TPL20-I group and an increase in this same miRNA in the TPL20-II group. *miR-26a-5p* increased in the group treated with a double dose compared to the control group and the group treated with the lowest dose (Fig. 8E).

There was no change in *miR-155-5p* after treatment in any of the cancer stages analyzed (Fig. 8C and F).

Gene enrichment categories for *mir-let-7c-5p*

After bioinformatic analysis of gene enrichment for *miR-let-7c-5p*, interaction categories, possibly influenced by the increase in this miR, were predicted after treatment with tempol related to; (I) regulation of transcription by RNA

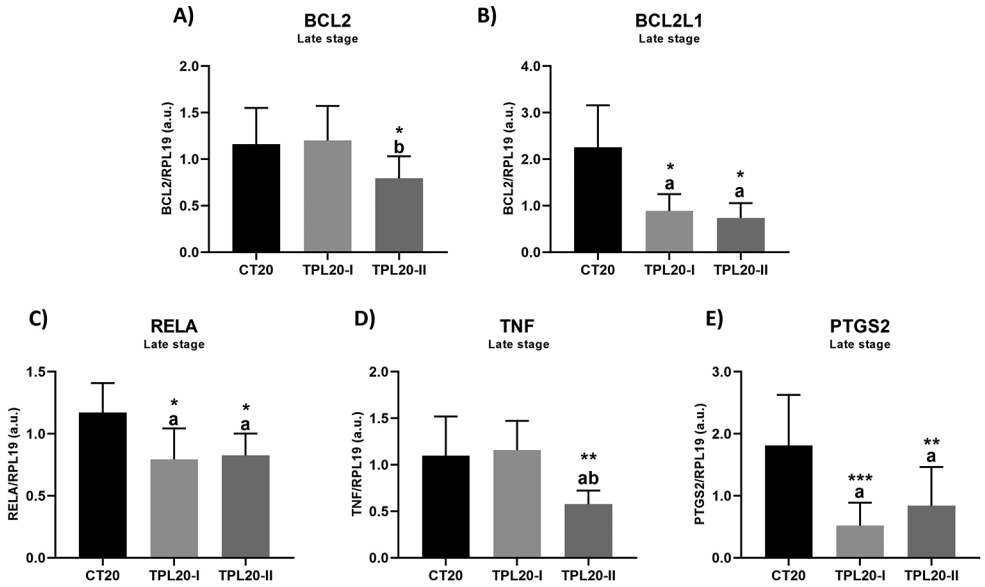

Fig. 6 Gene expression levels for BCL2, BCL2L1, RELA, PTGS2 and TNF for early-stage PCa in the TRAMP model (*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)

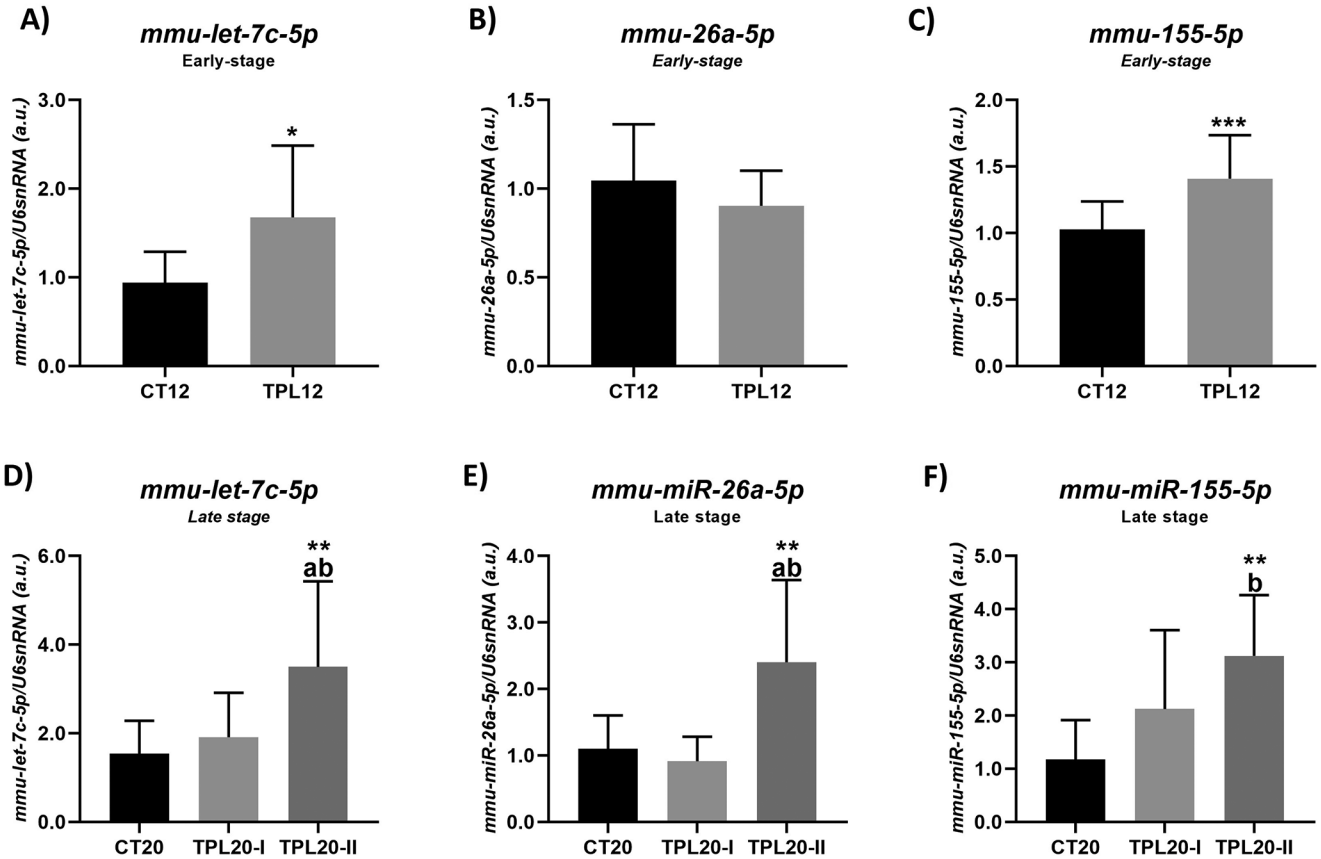

Fig. 7 Gene expression levels for BCL2, BCL2L1, RELA, PTGS2 and TNF for the later stage of PCa in the TRAMP model (*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)

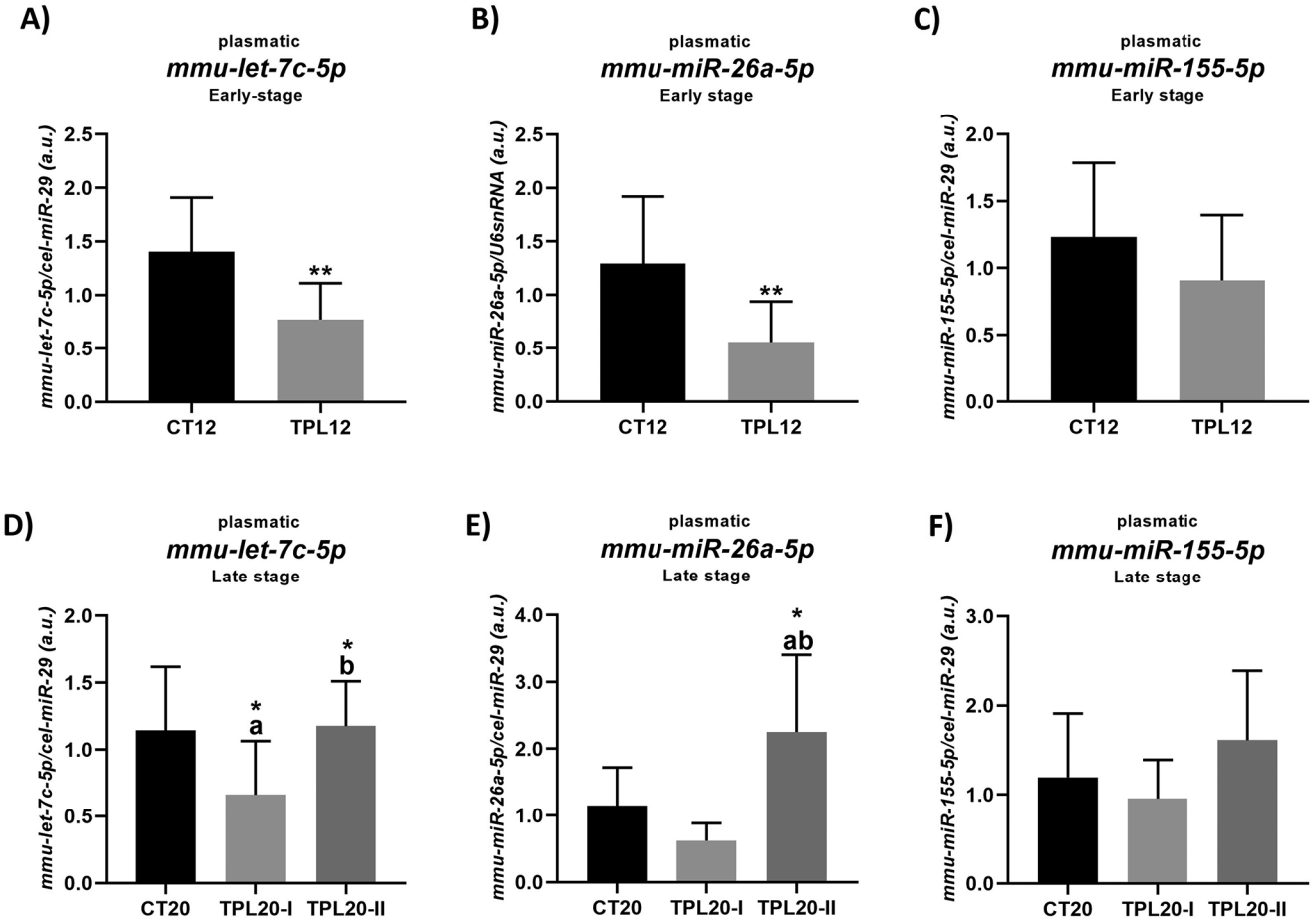

Fig. 8 Levels of plasmatic expression of miRNAs in vivo for the miRs *mmu- miR-let-7c-5p*, *mmu-miR-26a-5p* and *mmu-miR-155-5p*. The letter "a" denotes a statistical difference in relation to CT20 and the letter "b" denotes a statistical difference in relation to TPL20-I. (*$p < 0.05$)

polymerase II, (II) negative regulation of cell death, (III) regulation of the response to growth factors, (IV) regulation of the p53 signaling pathway, (V) regulation of the cellular response to oxygen levels (Fig. 9).

Gene enrichment categories for *miR-26a-5p*

After bioinformatic analysis of gene enrichment for *miR-26a-5p*, categories of interaction, possibly influenced by the increase in this miR, were predicted after treatment with tempol related to (I) regulation of transcription by DNA - templated, (II) regulation of transcription by RNA polymerase II, (III) regulation of cell death, (IV) regulation of the response to growth factors, (V) negative regulation of the apoptotic process (Fig. 10).

Gene enrichment categories for *miR-155-5p*

After bioinformatic analysis of gene enrichment for *miR-155-5p*, categories of interaction, possibly influenced by the increase in this miR, were predicted after treatment with tempol related to (I) positive regulation of gene expression, (II) regulation of the response to growth factors, (III) negative regulation of the apoptotic process, (V) regulation of signaling by TGF-beta, (V) regulation of the proliferation of cell populations (Fig. 11).

Discussion

This study demonstrated for the first time in literature that treatment with tempol alters mRNAs and miRNas expression in different experimental models for PCa. A noteworthy trend of tempol inducing an increase in the expression of the selected miRNAs was observed, both at the tissue and systemic levels, across nearly all the examined experimental conditions. Furthermore, despite the overall rise in miRNAs, a distinct and varied mRNA response of genes from the NFκB pathway was noted post-treatment, exhibiting alterations based on the type of tumor lineage or the stage of progression in the studied PCa.

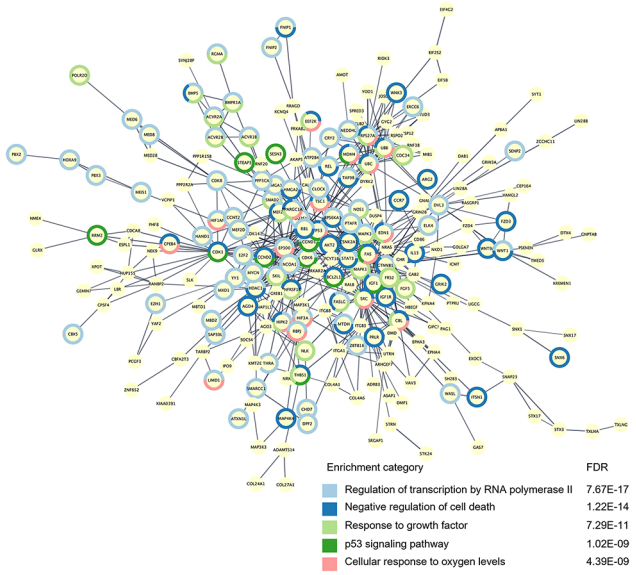

Fig. 9 Network annotates protein-protein interactions predicted by STRING database of our list of *miR-let-7c-5p* predicted targets. Nodes represent proteins and edges the predicted interaction. Colored borders of nodes represents enrichment categories. Confidence score: 0.8. Additional interactions:10

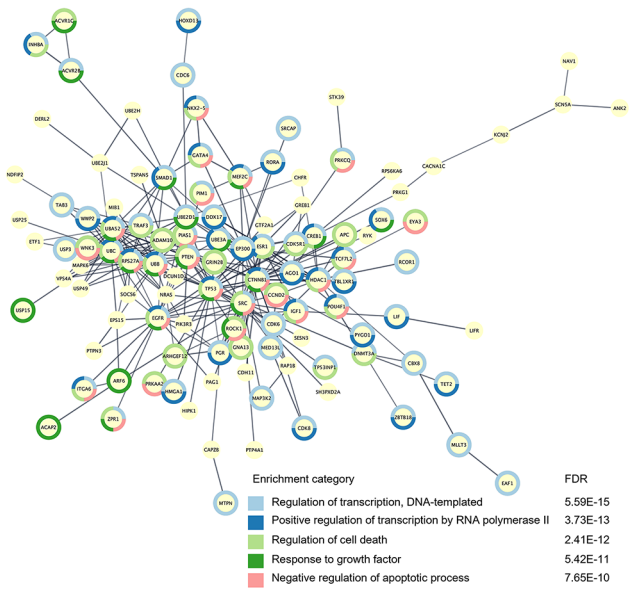

Fig. 10 Network annotates protein-protein interactions predicted by STRING database of our list of *miR-26a-5p* predicted targets. Nodes represent proteins and edges the predicted interaction. Colored borders of nodes represents enrichment categories. Confidence score: 0.8. Additional interactions: 10

These results are related to the reduced inflammatory protein levels, tissue proliferation and cell survival, previoulsy demonstrated by Rossetto et al. (2023). The *miRNAs let-7c-5p*, *26a-5p* and *155-5p* interact with different elements of the NFκB pathway, so now it is possible to figure out how indirectly tempol acts in the cellular machinery, mainly

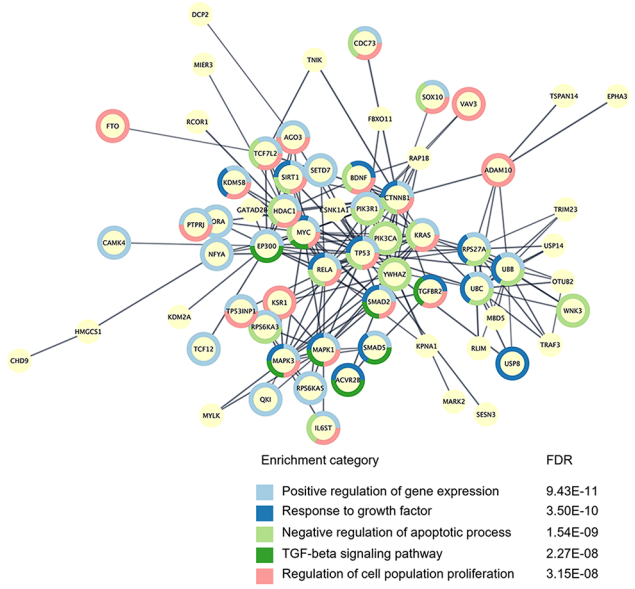

Fig. 11 Network annotates protein-protein interactions predicted by STRING database of our list of *miR-155-5p* predicted targets. Nodes represent proteins and edges the predicted interaction. Colored borders of nodes represents enrichment categories. Confidence score: 0.8. Additional interactions: 10

suggesting by gene enrichment analysis, that it exerts a gene silencing through the post-transcriptional control promoted by these miRNAs on the NFκB pathway.

Gene silencing mediated by miRNAs is an important part of the regulation of gene expression in many types of diseases (Hu et al. 2022). Considering cancer, some miRNAs may be completely deregulated, particularly those that control tumor suppressor genes and oncogenes (Khan et al. 2019). More specifically in PCa, several altered miRNAs have been identified which are associated with prostate cancer development and progression (Ouyang et al. 2015; Rajendiran et al. 2014).

In a study of *miR-let-7c-5p*, Nadiminty et al. (2012) showed that this miRNA is decreased in PCa and its presence in the cell suppresses cancer growth. Despite being, low, this same study showed that *miR-let-7c-5p* expression occurs at slightly higher levels for LNCaP when compared to PC-3 or DU145 cells, which are resistant to hormones (Nadiminty et al. 2012). Mulholland et al. (2019) pointed by bioinformatic searches that *miR-let-7c-5p* low expression has a recognized role in PCa pathogenesis, proposing that it is necessary to re-establish its miR normal level in the tissue. Confirming that, Kurniawati et al. (2022) suggested that exosomes derived from mesenchymal cells may serve as a therapeutic delivery system for *let-7c* to target castration-resistant prostate cancer, by exogenously supplementing these miR levels.

Taking in consideration the results herein and the specialized literature in relation to *miR-let-7c-5p*, it could

be suggested that the experimental models studied here showed decreased *miR-let-7c-5p* levels and that tempol treatment, by increasing the expression of *let-7c*, was able to re-establish its gene expression levels. *MiR-let-7c-5p*, as demonstrated by in silico analysis, acts mainly in the regulation of BCL2L1. The protein encoded by this gene, BCL-XL/S, belongs to the BCL-2 family of proteins and is located in the outer mitochondrial membrane and is responsible for regulating the opening of the outer mitochondrial membrane channel (Warren et al. 2019). The longer isoform of this protein acts as an apoptotic inhibitor. Thus, the increase in *miR-let-7c-5p* tends to canonically inhibit the BCL-XL/S transcription, preventing the inhibition of apoptosis generated by its presence in the cell (Warren et al. 2019). For the PC-3 and LNCaP tumor cell lines, particularly for LNCaP, BCL2L1 gene expression levels increased after tempol treatment. Considering that in these cells there was increased repression in the transcription of this gene, we could suggest a compensatory cellular response to try to maintain the levels of this marker. This is possible in the in vitro treatment because the exposure time to tempol can be considered short for the effects of mRNA destabilization and degradation (Eichhorn et al. 2014). However, the same could not be said for the BCL2L1 response in animals, where the period of experimental treatment was enough to significantly reduce mRNA levels at both stages and doses of treatment.

The tempol effects on *miR-26a-5p* showed that treatment altered the expression levels of this miR in the cell lines and particularly in the late stages of PCa progression (TPL20-II group). This miR also has a recognized antiproliferative role in cancer (Li et al. 2020; Zhu et al. 2020; Ye et al. 2020). It is recognized as a tumor suppressor miRNA, often downregulated in tumor tissues as well as in tumor cell lines (Chen et al. 2016). In PCa, Guo et al. (2016) demonstrated that the loss of *miR-26a-5p* promoted proliferation, migration and invasion through the negative regulation of SERBP1. On the other hand, re-expression of *miR-26a-5p* in the androgen-independent PC-3 tumor cell line rescued the activity of this tumor suppressor, directly repressing WNT5A and inhibiting cell proliferation (Zhao et al. 2014). In broad terms, it could be said that tempol was able to re-establish the *miR-26a-5p* levels, as well as those observed for *miR-let-7c-5p*. Rizzo et al. (2017) demonstrated the benefits of re-establishing *miR-26a-5p* expression levels in DU145 PCa tumor cells.

MiR-26a-5p shows a strong predicted interaction with PTGS2. This gene encodes a key enzyme in the biosynthesis of prostaglandins involved in inflammation and mitogenesis, known as cyclooxygenase-2 (COX-2) (Hashemi et al. 2019). Thus, an increase in *miR-26a-5p* implies COX-2 supression in the tissue. Rossetto et al. (2023) reported that tempol decreased tissue immunolocalization levels of COX-2 for the early stage of PCa progression in the TRAMP model. Despite this, *miR-26a-5p* gene expression levels did not increase after treatment at this stage. In the present study, an increase in PTGS2 gene expression was observed after tempol treatment, in both cell lines and in vivo model.

Despite the low predicted interaction with the RELA gene, Zhang et al. (2019) observed this possible interaction between this mRNA and *miR-26a-5p* in the PC-3 cell line. Since it interacts mainly with genes related to inflammation, *miR-26a-5p* also has an important anti-inflammatory action (Chen et al. 2022; Lu et al. 2023). Chen et al. (2022) showed that blocking *miR-26a-5p* promoted NF-κB-dependent renal inflammation in mouse renal tubular cells.

MiR-155-5p plays a role in tissue inflammation and, in general, its action in this process depends on the immune localization of its expression (Hu et al. 2022). Despite being one of the best-characterized miRNAs in literature, some studies have verified its action as a pro-inflammatory agente (Yang et al. 2020) and others as an anti-inflammatory agente (Liu et al. 2019) depending on the interaction with other cellular elements or different pathways. Particularly in PCa, Yao et al. (2020) demonstrated that particularly in PCa, *miR-155-5p* decreased cell invasion and migration by modulating the SOCK1 target. Santo et al. (2022) identified key miRNAs in prostate cancer progression based on the construction of the miRNA-mRNA network and found that *miR-155-5p* was stage-specific, increasing only in Gleason 6 PCa samples.

MiR-155-5p is usually regarded as an NFκB-dependent miRNA (Xiaodong et al. 2011). Yang et al. (2020) hypothesized that increased *miR-155* expression may restrict the NFκB signaling pathway in order to effectively reduce IL-1β-induced apoptosis, inflammation and oxidative stress in rat nucleus pulposus cells. In the present study, tempol treatment increased the gene expression levels of *miR-155-5p* in the early stage and for the TPL20-II group, in the later PCa stage. Simultaneously to the miR increase, there was na increase in RELA gene expression in the early stage of PCa progression in TRAMP mice, and a decrease in RELA expression after both treatments in the late stage.

Despite the *miR-155-5p* increase and the congruent increase in RELA expression observed in the early stage in the present study, Rossetto et al. (2023) previously demonstrated a significant decrease in NFκB p65 levels in both early and late PCa stages in TRAMP model after tempol treatment. Along with NFκB p65 protein levels, other important inflammatory markers decreased in prostate tissue, and the morphological parameters confirmed the reduction in the inflammatory process after treatment. The present study raises a hypothesis that, despite the increase in *miR-155-5p*

brought about by tempol, it did not lead to an increase in the inflammatory process in the tissue. Thus, it is suggested that inflammation regulation observed after tempol treatment may not depend on *miR-155*/NFκB signaling, suggesting that other miRNAs with a predominant anti-inflammatory role may be acting in the prostate tissue.

Tempol also had a significant inhibitory effect on the TNF gene. This gene encodes a multifunctional pro-inflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily (Balkwill 2006). This cytokine i.a. a soluble inflammatory mediator that can influence the entire dynamics of the tumor microenvironment (Schröder et al. 2020) and it is one of the major supra-regulators of NFκB expression. (Hayden and Gosh 2014). In the present study, tempol decreased TNF gene expression for PC-3 cells in the early stage and for the TPL20-II group in the late PCa stage. Together with what was reported by Rossetto et al. (2023), that demonstrated tempol significantly decreasing TNF-α protein levels for PC-3, LNCaP and also for the TRAMP model, it is understood that the attenuation of the expression of this cytokine is one of the most important points of inflammatory regulation resulting from tempol treatment. It is strongly suggested that tempol led to a decrease or delay in the onset of the inflammatory cascade. The production of TNF-α itself is amplified by the activation of NF-κB, leading to an intensification of the inflammatory process, due to the increased production of inflammatory cytokines, in a positive feedback (Kalliolias and Ivashkiv 2016).

Finally, tempol significantly altered some circulating miRNAs in the plasma of TRAMP mice. Once present in the bloodstream, miRNAs have systemic actions and are directed to the different organs and tissues. Taken as a whole, for the *let-7c-5p* and *26-5p* miRNAs, it can be said that 50 mg/kg tempol dose decreased the circulating levels of these miRNAs in plasma at both stages of progression and that the 100 mg/kg dose increased these miRNAs for the later stage. Therefore, it can be suggested that twice the dose induced an increase in circulating miRs, excepto for *miR-155-5p.* Cochetti et al. (2016) that circulating levels of *let-7c-5p* and *miR-26a-5p* had significantly decreased expression in PCa patient when compared to patients with benign prostatic hiperplasia. This is an interesting divergence from the present study, which used TRAMP mouse model. Despite the decrease in circulating miRs, tempol did not increase the aggressiveness of prostate lesions in the TRAMP model.

Conclusion

Taking into consideration both in vitro and in vivo PCa evaluation, we were able to conclude that tempol upregulated the miRNA expressions related to the NFκB pathway in the prostate tissue and human tumor cell lines. Despite its steadfast action in relation to the miR responses, mRNAs underwent differential changes according to hormone dependence or the PCa lesion stage.

Double tempol dose was more effective in modulating miRNAs in the tissue. Systemic miRNAs also changed with treatment and their expression in plasma did not necessarily correspond to that observed in the prostate tissue. Thus, it could be concluded that the molecular response to tempol, in terms of gene expression, was dose-dependent.

In general, regulated miRNAs are mainly linked to cell survival and their increase is linked to increased cell death and delayed PCa aggressivenes. Thus, tempol's capacity for miRNA-mediated gene silencing to decrease tissue proliferation and cell survival processes is part of its tissue mechanics.

By increasing the *miR-155-5p* levels, it is suggested that this miR does not seem to mediate the inflammatory cellular response to tempol. The main point of tempol action as an anti-inflammatory was to decrease the TNF gene expression and thus suppress the initiation of the inflammatory cascade of NFκB activation.

This exploratory study into the tempol action on gene expression in PCa reinforces its anti-inflammatory and anti-proliferative effects. Tempol can be recommended as a PCa therapy. However, there is a need for individualized consideration about the cancer characteristics for its application, taking into account the dose to be used, the stage of lesion progression, and its response to hormones.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

Balkwill F (2006) TNF-alpha in promotion and progression of cancer. Cancer Metastasis 25(3):409–16. https://doi.org/10.1007/s10555-006-9005-3. PMID: 16951987

Chen J, Zhang K, Xu Y, Gao Y, Li C, Wang R, Chen L (2016) The role of *microRNA-26a* in human cancer progression and clinical application. Tumour Biol 37(6):7095–7108. https://doi.org/10.1007/s13277-016-5017-y

Chen Y, Zhou X, Wu Y (2022) The *miR-26a-5p*/IL-6 axis alleviates sepsis-induced acute kidney injury by inhibiting renal inflammation. Ren Fail 44(1):551–561. https://doi.org/10.1080/0886022X.2022.2056486

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162(1):156–159. https://doi.org/10.1006/abio.1987.9999

Cochetti G, Poli G, Guelfi G, Boni A, Egidi MG, Mearini E (2016) Different levels of serum microRNAs in prostate cancer and benign prostatic hyperplasia: evaluation of potential diagnostic and prognostic role. Onco Targets Ther 13:9:7545–7553. https://doi.org/10.2147/OTT.S119027

Eichhorn SW, Guo H, McGeary SE, Rodriguez-Mias RA, Shin C, Baek D, Hsu SH, Ghoshal K, Villén J, Bartel DP (2014) mRNA destabilization is the dominant effect of mammalian microRNAs by the time substantial repression ensues. Mol Cell. 2;56(1):104–15. https://doi.org/10.1016/j.molcel.2014.08.028

Geraldo MV, Yamashita AS, Kimura ET (2012) MicroRNA *miR-146b-5p* regulates signal transduction of TGF-β by repressing SMAD4 in thyroid cancer. Oncogene. 12;31(15):1910-22. https://doi.org/10.1038/onc.2011.381

Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM (1995) Prostate cancer in a transgenic mouse. Proc Natl Acad Sci U S A. 11;92(8):3439-43. https://doi.org/10.1073/pnas.92.8.3439

Guo K, Zheng S, Xu Y, Xu A, Chen B, Wen Y (2016) Loss of *miR-26a-5p* promotes proliferation, migration, and invasion in prostate cancer through negatively regulating SERBP1. Tumour Biol 37(9):12843–12854 Epub 2016 Jul 23. PMID: 27449037

Hashemi GN, Najafi M, Salehi E, Farhood B, Mortezaee K (2019) Cyclooxygenase-2 in cancer: a review. J Cell Physiol 234(5):5683–5699. https://doi.org/10.1002/jcp.27411

Hayden MS, Ghosh S (2014) Regulation of NF-κB by TNF family cytokines. Semin Immunol 26(3):253–266. https://doi.org/10.1016/j.smim.2014.05.004

Hu J, Huang S, Liu X, Zhang Y, Wei S, Hu X (2022) miR-155: an important role in inflammation response. J Immunol Res 6:2022–7437281. https://doi.org/10.1155/2022/7437281

INCA (2023) In: < (ed) Câncer De Próstata. june, Acess, p 4. https://www.inca.gov.br/tipos-de-cancer/cancer-de-prostata>

Kalliolias GD, Ivashkiv LB (2016) TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nat Rev Rheumatol 12(1):49–62. https://doi.org/10.1038/nrrheum.2015.169

Khan S, Ayub H, Khan T, Wahid F (2019) MicroRNA biogenesis, gene silencing mechanisms and role in breast, ovarian and prostate cancer. Biochimie 167:12–24. https://doi.org/10.1016/j.biochi.2019.09.001

Kido LA, de Almeida Lamas C, Maróstica MR Jr, Cagnon VHA (2019) Transgenic adenocarcinoma of the mouse prostate (TRAMP) model: a good alternative to study PCa progression and chemoprevention approaches. Life Sci 15:217:141–147. https://doi.org/10.1016/j.lfs.2018.12.002

Kido LA, Rossetto IMU, Baseggio AM, Chiarotto GB, Alves LF, Santos FR, Lamas CA Jr, Cagnon MRM VHA (2022) Brazilian berry extract differentially induces inflammatory and Immune responses in androgen dependent and independent prostate Cancer cells. J Cancer Prev 30(3):182–191. https://doi.org/10.15430/JCP.2022.27.3.182

Kurniawati I, Liu MC, Hsieh CL, Do AD, Sung SY (2022) Targeting Castration-Resistant Prostate Cancer Using Mesenchymal Stem Cell Exosomes for Therapeutic *MicroRNA-let-7c* Delivery. Front Biosci (Landmark Ed). 6;27(9):256. https://doi.org/10.31083/j.fbl2709256

Lejeune D, Hasanuzzaman M, Pitcock A, Francis J, Sehgal I (2006) The superoxide scavenger TEMPOL induces urokinase receptor (uPAR) expression in human prostate cancer cells. Mol Cancer 6:5:21. https://doi.org/10.1186/1476-4598-5-21

Li Y, Wang P, Wu LL, Yan J, Pang XY, Liu SJ (2020) *miR-26a-5p* Ingastricastric Cancer Cell Proliferation and Invthroughhmediateddiated Wnt5a. Onco Targets Ther 27:13:2537–2550 PMID: 32273724; PMCID: PMC7108881

Li T, Zhang T, Gao H, Wang H, Yan H, Wan Z, Liu R, Yin C (2023) Tempol modulates lncRNA-miRNA-mRNA ceRNA networks in ovaries of DHEA induced PCOS rats. J Steroid Biochem Mol Biol 226:106175. https://doi.org/10.1016/j.jsbmb.2022.106175

Liu ZQ, Feng J, Shi LL, Xu J, Zhang BJ, Chen L (2019) J. influences of *miR-155*/NF-κB signaling pathway on inflammatory factors in ARDS in neonatal pigs. Eur Rev Med Pharmacol Sci 23(16):7042–7048. https://doi.org/10.26355/eurrev_201908_18746

Lu Y, Liu M, Guo X, Wang P, Zeng F, Wang H, Tang J, Qin Z, Tao T (2023) miR-26a-5p alleviates CFA-nduced chronic inflammatory hyperalgesia through Wnt5a/CaMKII/NFAT signaling in mice. CNS Neurosci Ther 29(5):1254–1271. https://doi.org/10.1111/cns.14099

Montico F, Lamas CA, Rossetto IMU, Baseggio AM, Cagnon VHA (2023) Lobe-specific responses of TRAMP mice dorsolateral prostate following celecoxib and nintedanib therapy. J Mol Histol. 2023. https://doi.org/10.1007/s10735-023-10130-z

Mulholland EJ, Green WP, Buckley NE, McCarthy HO (2019) Exploring the potential of MicroRNA *Let-7c* as a therapeutic for prostate Cancer. Mol Ther Nucleic Acids 6:18:927–937. https://doi.org/10.1016/j.omtn.2019.09.031

Nadiminty N, Tummala R, Lou W, Zhu Y, Shi XB, Zou JX, Chen H, Zhang J, Chen X, Luo J, deVere White RW, Kung HJ, Evans CP, Gao AC (2012) MicroRNA *let-7c* is downregulated in prostate cancer and suppresses prostate cancer growth. PLoS ONE 7(3):e32832. https://doi.org/10.1371/journal.pone.0032832

Namekawa T, Ikeda K, Horie-Inoue K, Inoue S (2019) Application of prostate Cancer models for preclinical study: advantages and limitations of Cell lines, patient-derived xenografts, and three-Dimensional Culture of patient-derived cells. Cells 20(8):1–74. https://doi.org/10.3390/cells8010074

Ouyang Y, Gao P, Zhu B, Chen X, Lin F, Wang X, Zhang H (2015) Downregulation of microRNA-429 inhibits cell proliferation by targeting p27Kip1 in human prostate cancer cells. Mol Med Rep 11:1435–1441. https://doi.org/10.3892/mmr.2014.2782

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 1;29(9):e45. https://doi.org/10.1093/nar/29.9.e45

Rajendiran S, Parwani AV, Hare RJ et al (2014) MicroRNA-940 suppresses prostate cancer migration and invasion by regulating MIEN1.Mol. Cancer 19:13250. https://doi.org/10.1186/1476-4598-13-250

Rizzo M, Berti G, Russo F, Fazio S, Evangelista M, D'Aurizio R, Pellegrini M, Rainaldi G (2017) Discovering the *miR-26a-5p* Targetome in Prostate Cancer Cells. J Cancer. 22;8(14):2729–2739. https://doi.org/10.7150/jca.18396

Rossetto I, Santos F, Kido L, Lamas C, Montico F, Cagnon V (2023) Tempol differential effect on prostate cancer inflammation: *in vitro* and *in vivo* evaluation. Prostate 83(5):403–415. https://doi.org/10.1002/pros.24473

Santo GD, Frasca M, Bertoli G, Castiglioni I, Cava C (2022) Identification of key miRNAs in prostate cancer progression based on miRNA-mRNA network construction. Comput Struct Biotechnol J 7:20:864–873. https://doi.org/10.1016/j.csbj.2022.02.002

Schröder SK, Asimakopoulou A, Tillmann S, Koschmieder S, Weiskirchen R (2020) TNF-α controls Lipocalin-2 expression in PC-3 prostate cancer cells. Cytokine 135:155214. https://doi.org/10.1016/j.cyto.2020.155214

Shi Y, Liu Z, Lin Q, Luo Q, Cen Y, Li J, Fang X, Gong C (2021) MiRNAs and Cancer: Key Link in Diagnosis and Therapy. Genes (Basel). 23;12(8):1289. https://doi.org/10.3390/genes12081289

Siegel RL, Miller KD, Wagle NS, Jemal A (2023) Cancer statistics, 2023. CA Cancer J Clin 73(1):17–48. https://doi.org/10.3322/caac.21763

Staal J, Beyaert R (2018) Inflammation and NF-κB signaling in prostate Cancer: mechanisms and clinical implications. Cells 29(9):122. https://doi.org/10.3390/cells7090122

Stark T, Livas L, Kyprianou N (2015) Inflammation in prostate cancer progression and therapeutic targeting. Transl Androl Urol 4(4):455–463. https://doi.org/10.3978/j.issn.2223-4683.2015.04.12

Thomas R, Sharifi N (2012) SOD mimetics: a novel class of androgen receptor inhibitors that suppresses castration-resistant growth of prostate cancer. Mol Cancer Ther 11(1):87–97. https://doi.org/10.1158/1535-7163.MCT-11-0540

Urabe F, Yamamoto Y, Kimura T (2022) miRNAs in prostate cancer: intercellular and extracellular communications. Int J Urol 29(12):1429–1438. https://doi.org/10.1111/iju.15043

Warren CFA, Wong-Brown MW, Bowden NA (2019) BCL-2 family isoforms in apoptosis and cancer. Cell Death Dis. 21;10(3):177. https://doi.org/10.1038/s41419-019-1407-6

Wilcox CS (2010) Effects of tempol and redox-cycling nitroxides in models of oxidative stress. Pharmacol Ther 126(2):119–145. https://doi.org/10.1016/j.pharmthera.2010.01.003

Xiaodong M, Buscaglia LEB, Barker JR, Yong L (2011) MicroRNAs in NF-κB signaling. J Mol Cell Biol 3(3):159–166. https://doi.org/10.1093/jmcb/mjr007

Yang L, Yang L, Wang S, Zhao E, Lu T, Liang Q (2020) Effects of *miR-155* on IL-1β-induced apoptosis, inflammatory response and oxidative stress of nucleus pulposus cells in rats. Acta Universitatis Medicinalis. 2020;55(7):1046–1051

Yao LY, Ma J, Zeng XM, Ou-Yang J (2020) *MicroRNA-155-5p* inhibits the invasion and migration of prostate cancer cells by targeting SPOCK1. Oncol Lett 20(6):353. https://doi.org/10.3892/ol.2020.12215

Ye MF, Lin D, Li WJ, Xu HP, Zhang J (2020) *MiR-26a-5p* Serves as an Oncogenic MicroRNA in Non-Small Cell Lung Cancer by Targeting FAF1. Cancer Manag Res 11:12:7131–7142. https://doi.org/10.2147/CMAR.S261131

Zhang YQ, Chen X, Fu CL, Zhang W, Zhang DL, Pang C, Liu M, Wang JY (2019) FENDRR reduces tumor invasiveness in prostate cancer PC-3 cells by targeting CSNK1E. Eur Rev Med Pharmacol Sci 23(17):7327–7337. https://doi.org/10.26355/eurrev_201909_18838

Zhao S, Ye X, Xiao L, Lian X, Feng Y, Li F et al (2014) *MiR-26a* inhibits prostate cancer progression by repression of Wnt5a. Tumour Biol. 2014;35:9725-33

Zhu WJ, Yan Y, Zhang JW, Tang YD, Han B (2020) Effect and Mechanism of *miR-26a-5p* on Proliferation and Apoptosis of Hepatocellular Carcinoma Cells. Cancer Manag Res 30:12:3013–3022. https://doi.org/10.2147/CMAR.S237752

Zinatizadeh MR, Schock B, Chalbatani GM, Zarandi PK, Jalali SA, Miri SR (2020) The Nuclear Factor Kappa B (NF-kB) signaling in cancer development and immune diseases. Genes Dis 18(3):287–297. https://doi.org/10.1016/j.gendis.2020.06.005