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REVIEW

The roles of microRNAs in the progression of castration-resistant prostate cancer

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Prostate cancer (PCa) is one of the leading causes of cancer-related death in men. PCa is androgen-dependent, and androgen-deprivation therapy is effective for first-line hormonal treatment, but the androgen-independent phenotype of PCa eventually develops, which is difficult to treat and has no effective cure. Recently, microRNAs have been discovered to have important roles in the initiation and progression of PCa, suggesting their use in diagnosis, predicting prognosis and development of treatment for castration-resistant PCa (CRPC). Understanding the networks of microRNAs and their target genes is necessary to ascertain their roles and importance in the development and progression of PCa. This review summarizes the current knowledge about microRNAs regulating PCa progression and elucidates the mechanism of progression to CRPC.
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INTRODUCTION

Prostate cancer (PCa) is the most frequent malignant tumor and the second leading cause of cancer death in men in Western countries.[1] Recently, multimodality treatments have become available, but the prognosis is still poor once a patient progresses to castration-resistant PCa (CRPC). PCa is initially androgen-sensitive, and androgen-deprivation therapy is widely used for metastatic PCa patients. The disease is known to progress to CRPC about 12 months after androgen-deprivation therapy.[2] As new hormonal therapies, abiraterone (CYP-17A inhibitor)[3] and enzalutamide (second-generation anti-androgen)[4] have been developed to treat CRPC, and cabazitaxel (second-generation chemotherapy)[5] has been developed for patients who relapse to docetaxel. However, the effects of these new therapies are limited, and patients progress to the lethal stage, with overall survival increasing only 3–4 months after becoming CRPC compared with controls. Prostate-specific antigen (PSA) is a highly specific marker used to detect PCa, but multiple factors including genetic variations, growth factors and androgen receptor (AR) status affect disease progression and prognosis.[6]

MicroRNAs (miRNAs) are a class of small non-coding RNAs that have important roles in cell development, differentiation, signal transduction, cancer formation and progression by regulating the expressions of protein-coding genes by repressing translation or cleaving of RNA transcripts in a sequence-specific manner.[7] In addition, miRNAs are known to have important roles in the regulation of malignant transformation and development of PCa.[8]

Recent clinical priorities include the identification of biomarkers that discriminate between low- and high-risk diseases to select appropriate treatment for each patient. From the profiling of expressions of miRNAs in PCa, many miRNAs are consistently upregulated or downregulated, suggesting that certain biomarkers predict prognosis in PCa. It is important to determine the target genes of these differentially expressed miRNAs to elucidate their functions. The roles of miRNAs in PCa can be divided by their associations with cell proliferation, apoptosis, invasion and metastasis, epithelial–mesenchymal transition (EMT), cancer stemness, and AR status. First, we introduce the expression profiles of miRNAs in PCa, and we discuss miRNAs that have important effects on each categorized function in PCa. We also addressed possible therapeutic roles of miRNAs and significance of miRNAs as predictive biomarkers in CRPC.

EXPRESSION PROFILES OF MIRNAS IN PCA

To date, 2578 human mature miRNAs have been registered in the public database (miRBase, http://microrna.sanger.ac.uk/. release 21 June 2014). A growing body of evidence suggests that expression profiles of miRNAs in PCa are increasing in importance because of their usefulness for diagnosis, staging, progression, and predicting prognosis and response to treatment.[9] Differential expressions of miRNAs in PCa were analyzed using current genome-based technologies, which have been able to distinguish benign prostate tissue from PCa. Recently, miRNA signatures comparing expressions of miRNAs in benign prostate tissue and CRPC clinical specimens obtained from autopsy have been published.[10] Upregulation of *miR-96, -182, 182*, -183, -375, 32, -26a, -181a, -93, -196a, -25, -92* and *let-7i* and downregulation of *miR-16, -31, -125b, -145, -149, -181b, -184, -205, -221* and *-222* were confirmed in PCa tissue.[11,12] Many other biomarkers have been reported using hormone-sensitive PCa[13] or CRPC[10,14,15] and serum or urine of PCa patients.[16,17] It appears that miRNAs may function as oncogenes or tumor suppressors. Oncogenic miRNAs are upregulated, and tumor-suppressive miRNAs are

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Table 1 Expression, gene location, target genes and functions of each miRNA in prostate cancer

miRNAs	*Expression*	*Chromosomal location*	*Targets in PCa*	*Functions and key findings*
let-7a-1,2,3	Down	9q22.32, 11q24.1, 22q13.31	E2F2,[100] CCND2,[100] IGF1R,[101] EZH2[59]	Inhibits cell proliferation and cell growth
let-7-b	Down	22q13.31	EZH2[59]	Inhibits cell proliferation and cell growth
let-7c	Down	21q21.1	c-MYC,[62] Lin28,[62] EZH2,[59] IGF1R[23]	AR antagonize
miR-125b-1 miR-125b-2	Up Up	11q24.1 21q21.1	P53,[21] BAK-1,[21,102] Puma,[21] NCoR,[68]EIF4EBP1,[46] IGF1R[23]	Increases cell proliferation and inhibits apoptosis, activates AR signaling
miR-133a-1 miR-133a-2	Down Down	18q11.2 20q13.33	EGFR,[103] PNP[104]	Inhibits cell proliferation, invasion and migration
miR-143/145	Down	5q32	KRAS,[105] ERK5,[106] FSCN1,[48] SWAP70,[49] MMP13, KLKs,[107] GOLM1,[52] CD133,[50] CD44,[50] MYC,[50] KLF4,[50] OCT4,[50] SOX2,[108] LMK1,[109] DAB2,[110] ZEB2,[51] ERG[111]	Inhibits cell proliferation, invasion and migration, reversing EMT
miR-15/16-1 miR-16-2	Down Down	13q14.2 3q25.33	BCL2,[25] CCDN1,[25] WNT3A,[25] CDK1,[26] CDK2,[26] FGF-2,[112] FGFR1[112]	Inhibits cell proliferation, invasion and migration, angiogenesis
miR-200a, 200b miR-200c	Down Down	1q36.33 12p13.31	ZEB1,[113] ZEB2,[113] SNAIL2,[113] SLUG[114]	Reversing EMT,[115] docetaxel-resistant[90]
miR-205	Down	1q32.2	c-SRC,[116] ErbB3,[117] E2F1,[117] ZEB2,[117] BCL-w,[118] E2F6,[118] MAPK and AR signaling,[119] TP53INP1[120]	Inhibits cell proliferation, invasion, migration and reversing EMT, AR signaling, docetaxel-resistant[118]
miR-21	Up	17q23.2	MARCKS,[36] RECK,[121] PTEN,[32] PDCD4[33]	Increases cell migration and invasion; EMT, docetaxel-resistant[83]
miR-221/222	Down	Xp11.3	Ecm29,[10] SIRT1,[122] IRF2,[123] SOCS3,[123] IRF2,[123] Bmi-1[124]	Inhibits cell proliferation, invasion and migration
miR-23b	Down	9q22.32	PTEN,[29] Scr kinase,[125] MAPK and JAK/STAT pathway[126]	Proliferation, invasion, migration
miR-34a	Down	1p36.22	AR,[127] NOTCH1 signaling, SIRT1,[128] CD44,[57] LEF1,[129]	Stimulates cancer stemness, tumorigenesis, proliferation, migration, AR regulation, associated with GS, LN metastasis
miR-34b, 34c	Down	11q23.1	c-MYC,[130] BCL2, HuR[131]	
miR-488*	Down	1q25.2	AR[66]	Inhibits AR-mediated cell growth

Abbreviations: AR, androgen receptor; EMT, epithelial–mesenchymal transition; GS, gleason score; LN, lymph nodes.

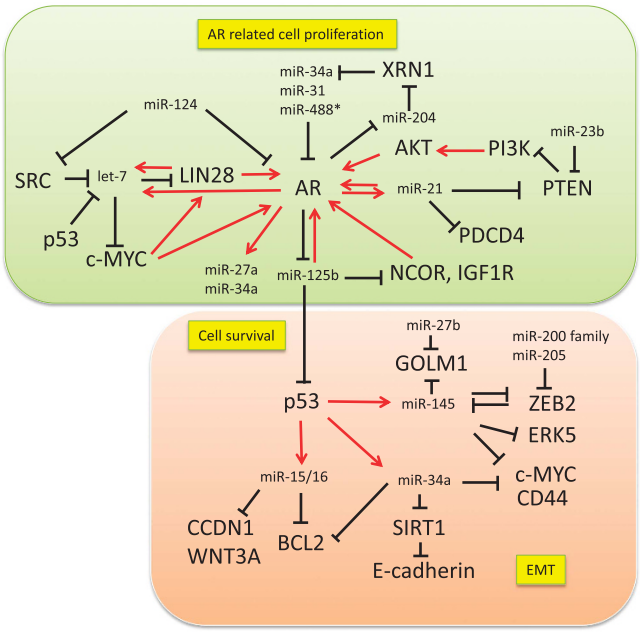

Figure 1 Physiological roles of miRNAs in prostate cancer. miRNA targets and functions are categorized into relation with androgen receptor, cell survival and EMT.

downregulated in cancers. The role of miRNAs in PCa is understood by elucidating the relationships of miRNAs and their target genes. The miRNAs that have been shown to have important functions in PCa and whose target genes have been determined are listed in Table 1, and the association of each miRNA and the target genes are shown in Figure 1.

MIRNAS ASSOCIATED WITH CELL PROLIFERATION AND APOPTOSIS IN PCA

p53 is a tumor suppressor gene, and loss of *p53* function has a critical role in the development of PCa.[18,19] *p53* mutation is a late event in the progression of PCa and is associated with advanced stage, loss of differentiation and the transition from androgen-dependent to androgen-independent growth.[20] Shi *et al.*[21] found that *miR-125b*, which is aberrantly expressed in PCa cells and tissues, promoted the growth of PCa xenograft through downregulation of three key pro-apoptotic genes, *p53,* p53 upregulated modulator of apoptosis (*PUMA*) and *Bak1*. Thus, increased expression of onco-*miR-125b* decreased p53 expression, resulting in survival of PCa cells. Insulin-like growth factor 1 receptor (*IGF1R*) is important for the tumorigenesis and progression of PCa because of its demonstrated roles in angiogenesis, transformation and mitogenesis.[22] Recently, it has been reported that *miR-99a/let-7c/125b* cluster, which is transcriptionally repressed by androgen-activated AR, has been shown to directly target IGF1R in androgen-dependent PCa cells.[23] This might be one of the mechanisms by which AR induces cell proliferation in PCa.

Two miRNAs, *miR-15a* and *miR-16-1,* are located at ch13q14, a locus in which complete or partial genomic loss is reported in advanced PCa and is associated with tumor initiation, progression and metastasis.[24] Downregulation of these miRNAs is significant in advanced PCa.[25] The *miR-15a-miR-16-1* cluster has been reported to regulate various oncogenes, such as B-cell lymphoma 2 (*BCL2*), cyclin D1 (*CCND1*) and wingless-related MMTV integration site 3A (*WNT3A*), through posttranscriptional repression in PCa.[25] Deregulated expression of Bcl2 and cyclins is commonly reported in PCa, which is thought to facilitate the survival of cells under androgen-depletion therapy. Overexpression of *miR-16* in PCa cell lines significantly inhibits the growth of prostate tumors through the downregulation of CDK1 and CDK2 in bone, suggesting that *miR-16* could represent a novel type of personalized therapy for treating metastatic PCa.[26]

Monoallelic loss or mutation of phosphatase and tensin homolog (*PTEN)* is detected in the early stages of many sporadic tumors, including PCa.[27] Downregulation of PTEN, allowing activation of phosphatidylinositol 3-kinase (PI3K)-AKT pathway, results in decreased cell apoptosis and provision of cell survival signals.[28] Recently, studies have increased to investigate the roles of miRNAs in PTEN regulation. *miR-19b, miR-23b, miR-26a* and *miR-92a* have been reported to promote prostate cell proliferation by targeting *PTEN* and inhibiting the PI3K/AKT pathway, and cyclin D1 *in vitro*.[29] Other studies have also confirmed that *miR-22* and the *miR-106b ~ 25* cluster are either directly or indirectly involved in the PTEN regulation in PCa.[30]

It has been shown that *miR-21* is one of the most common deregulated oncomiRs, which has an important role in cancer pathogenesis, and high expression of *miR-21* is found in almost all types of solid cancer tissues including PCa.[31] Furthermore, *miR-21* directly targets tumor suppressor genes such as *PTEN*[32] and programmed cell death 4 (*PDCD4*)[33] in PCa, and *miR-21* is induced by AR with binding to the promoter site of *miR-21*, resulting in the overexpression of *miR-21*, leading to the castration resistance phenotype of PCa.[34,35] Clinically, *miR-21* is a useful prognostic marker, which is associated with PCa recurrence after radical prostatectomy.[36,37]

A cell line-based genomic approach showed that the *miR-221/222* cluster was upregulated in LNCaP cells in the castration-resistant condition and was reported as oncogenic, promoting metastasis of PCa.[38] One of the mechanisms of *miR-221/222* on tumor cell proliferation in PCa cell lines is directly targeting *p27kip1*, a tumor suppressor gene.[39] The *miR-221/222* cluster is also reported to promote cell proliferation and repress apoptosis thorough suppressing caspase-10.[40] However, genome-wide miRNA expression signatures using clinical PCa and CRPC specimens showed significant downregulation of the *miR-221/222* cluster compared with normal prostate tissues, suggesting these miRNAs function as tumor suppressors in PCa patients, especially in CRPC.[10,12,13,41–45] These comprehensive approaches to miRNA expression in clinical specimens would help identify novel functional mechanisms of miRNAs.

MIRNAS ASSOCIATED WITH CELL MIGRATION, INVASION, EMT AND STEMNESS IN PCA

It was previously reported that *miR-145* was downregulated in many solid tumors, including PCa and functioned as a tumor suppressor,[15,46] and that *miR-145* was upregulated by wild-type p53.[47] *miR-145* directly targets fascin homolog 1 (*FSCN1*)[48] and switching B-cell complex 70kDa subunit (*SWAP70*).[49] Knockdown of *FSCN1* and *SWAP70* suppresses cell migration and invasion in PCa cells. Restoration of *miR-145* inhibits cell proliferation in PCa. The *miR-143/145* cluster is downregulated in PCa cells, resulting in enhanced cell migration and invasion through reduced expression of E-cadherin, thus promoting the EMT phenotype.[50,51] *miR-145* targets zinc-finger E-box binding homeobox 2 (*ZEB2*), as an EMT activator, and ZEB2 directly represses the transcription of *miR-145*. This double-negative feedback loop has important roles in suppressing tumor cell

invasion, migration and EMT.[51] Golgi membrane protein 1 (*GOLM1*) has also been identified as a target of *miR-143/145*, and it is considered to have roles in tumor cell proliferation, migration and invasion.[52] Moreover, another tumor-suppressive miRNA, *miR-27b,* regulates *GOLM1*, which indicates that several tumor-suppressive miRNAs regulate *GOLM1* in concert.[53]

Cancer stem cells (CSCs) are a subset of cancer cells that have important roles in tumor progression and metastasis in several cancers, including PCa.[54] *CD44,* an adhesion molecule, is a marker to identify CSCs, and the expression of *CD44* was found to be increased in a cell population with increased potential for tumor initiation and metastasis.[55] Studies have clearly shown that miRs are involved in promoting or inhibiting the stemness of CSCs;[56] *miR-143* and *miR-145* suppress tumor sphere formation and expression of CSC markers and stemness factors, including *CD133*, *CD44*, *Oct4*, *c-Myc* and *Klf4* in PC3 cells,[50] indicating that *miR-143* and *miR-145* may have a significant role in bone metastasis progression of PCa by regulating CSC characteristics.[50]

miR-34a has been shown to be downregulated in CD44+ PCa cells, and these cells have increased inhibition of clonogenic growth, metastatic behavior and tumor regenerator.[57,58] The tumor suppressor *p53* induces transcription of *miR-34a*, which is known to have strong anti-tumor effects. The *let-7* family also appears to have a key role in the recurrence and progression of PCa by regulating CSCs. The *let-7* family was found to be lost in PCa tissue specimens with Gleason score 7 or higher, with consistently increased expression of Enhancer of Zeste homolog 2 (*EZH2*).[59] *EZH2* is a putative target of the *let-7* family and was demonstrated to control stem cell function in PCa cells.[59]

As for neuroendocrine differentiation (NED), the *AR-miR-204-XRN1* (5′-3′ exoribonuclease 1) axis has been reported to contribute to NED. *miR-204* functions as a tumor suppressor in AR-positive LNCaP and 22Rv1 cells, but as an oncogene in PC3 and CL1 cells, and these dual functions of miRNAs provide insight into the importance of miRNAs in the NED mechanism in PCa.[60] Analyses of NED by miRNA approaches would reveal novel mechanisms of NED and a therapeutic approach to neuroendocrine-differentiated PCa.

MIRNAS ASSOCIATED WITH AR STATUS

PCa is initially AR-dependent, but it eventually acquires the AR-independent phenotype. Androgen signaling through AR is an important pathway for progression of PCa cells. During progression to CRPC, AR splice variant appears to increase in expression. AR-V7 is the most common AR splice variant without a ligand-binding domain, which is thought one of the mechanisms of progression of PCa to CRPC. It is known that PCa patients with AR-V7 in their circulating cancer cells do not respond to new hormonal agents (enzalutamide and abiraterone), because of androgen-independent proliferation of PCa cells with AR-V7.[61] Several miRNAs (*miR-21*, *-31*, *-34a*, *let-7c*, *-124*, *-205*, *-185*, *488** and so on)[9,23,62–66] have been reported to regulate AR expression (both full length and AR-V7), and AR can regulate the expression of several miRs (*miR-21, -27a, -34, -125b*, *-221*, *-204*, *let-7*).[9,60,67,68]

Modulation of the AR transcriptional complex and AR co-repressor is an important mechanism in the response to androgen-deprivation therapy and eventual development of CRPC.[69] *miR-125b* is reported to directly target the AR co-repressor *NCOR2* and subsequently activate AR signaling.[68] AR inhibition has been shown to drive *miR-125b*, suggesting that androgen-deprivation therapy eventually results in the activation of AR by suppressing *NCoR* by *miR-125b*.[68]

A recent study has shown that *miR-21* and AR positively regulate each other and exert their oncogenic effects by inhibiting TGFβ receptor II (*TGFBR2*) expression.[63] They showed that *miR-21*/AR mediates its tumor-promoting function by attenuating TGFβ-mediated Smad2/3 activation, cell growth inhibition, cell migration and apoptosis in PCa.[63] *miR-124* is also reported to inhibit proliferation of PCa cells *in vitro* and sensitize them to inhibitors of AR signaling.[61]

Let-7c has been reported to suppress AR expression by degradation of *c-Myc*.[62] The *c-Myc* is an oncogenic transcription factor that is pathologically activated in many human malignancies including PCa, and *c-Myc* activity is known to induce androgen-independent PCa growth.[70] *Lin28* is a highly conserved RNA-binding protein known to be overexpressed in PCa. *Lin28* derepresses *c-Myc* by repressing *let-7c*, and *c-Myc* transcriptionally activates *Lin28*.[71] Thus, the *let-7c-Myc-Lin28* loop may have important roles in regulating AR expression and may help target-enhanced and hypersensitive ARs in advanced PCa.[62]

MIRNAS AS PREDICTIVE BIOMARKERS IN PCA

In CRPC patients, PSA is not an appropriate marker to predict prognosis nor efficacy of treatment because poorly differentiated or neuroendocrine-differentiated PCa often show low levels of serum PSA. As a surrogate indicator, miRNAs can be attractive biomarkers because they are relatively stable in biological fluids, easy to measure and resistant to storage handling.[9] The aberrantly expressed miRNA levels in tumor tissue, serum or plasma, and urine can be a promising biomarker for PCa diagnosis, prognostic prediction or treatment efficacy.[9] In this review, we categorized miRNAs as predictive biomarkers by their origin, prostate tissue, serum or plasma, and urine.

The expression levels of miRNAs derived from prostate tissue have been analyzed and reported from multiple laboratories. Using radical prostatectomy specimens, *miR-21*, *-200a*, *-145*, *-30d*, *-301a*, *-449b* and *-182* have been mentioned as biomarkers to predict biochemical recurrence after prostatectomy.[36,72–77] Recently, combination of Gleason score and lymph node status with expression levels of *miR-4516* and *miR-601* has been reported to predict biochemical recurrence after post-prostatectomy salvage radiation therapy, supporting the use of miRNAs in clinically used predictive models.[78] Furthermore, Goto *et al.*[10,53,79] has reported that *miR-27b*, *miR-222* and *miR-452* could be potential biomarkers predicting progression time to CRPC.

A growing body of evidence indicates usefulness of circulating miRNAs in serum or plasma as biomarkers.[80,81] Circulating miRNAs can originate from tumor cells involved in tumor invasion or metastasis. Serum *miR-21*, *-375, -378*, 141, -201, miR-200c, -423-3p* and *-210* have been reported as upregulated miRNAs in CRPC patients.[82–88] Furthermore, recent RNA sequencing of exosomal miRNAs in peripheral blood of CRPC patients indicates that higher expression of *miR-1290* and *miR-375* predicted poor overall survival.[89] Several reports describe the association of expression levels of circulating miRNAs and docetaxel chemotherapy for CRPC patients. Lin *et al.*[90] have revealed that non-responders to docetaxel therapy had high pre-docetaxel levels of serum *miR-200b,* and pre-docetaxel levels of serum *miR-200b* and post-docetaxel change in *miR-20a* levels were independent prognostic factors for overall survival. In other analysis, higher expression levels of serum *miR-21* was mentioned as a predictive factor for response to docetaxel.[91] Clinically monitoring these miRNAs would be useful to predict prognosis and sensitivity to therapeutic modality in CRPC patients.

Excretion of miRNAs in urine has been reported in several cancers including bladder cancer, renal cell carcinoma and PCa.[92] As for long non-coding RNA in urine, urinary prostate cancer antigen 3 (PCA3)

test is the most successfully developed marker in clinical use for PCa diagnosis.[93] Recently, it has been reported that miRNA levels in urine from PCa patients are significantly altered compared with that from benign prostate hypertrophy patients.[94] They showed that higher levels of urinary *miR-100* and *miR-200b* were effective parameters to detect the presence of PCa with PSA levels in the gray zone.[94] Other investigators identified *miR-205, -214, -1825* and *-484* as potential urinary biomarkers for PCa diagnosis.[95,96] At present, no reports describe association of urinary miRNA levels and risk of CRPC.

Combination of miRNAs with other conventional markers including Gleason score, clinical stage and PSA would be a suitable practical biomarker for patients to determine the most appropriate strategy to treat PCa and CRPC; however, studies with larger sample size are warranted to use miRNAs for biomarkers in the clinical settings.

MIRNAS FOR TREATMENT OF PCA

Inhibition of oncogenic miRNAs or delivery of tumor-suppressive miRNAs could become a novel treatment strategy for PCa. As for tumor-suppressive miRNAs, adequate miRNA delivery system to the PCa tumors is required. However, the development of efficient *in vivo* miRNA delivery system has been challenging because of rapid degradation and excretion in serum condition and the lack of delivery system trapping miRNAs into the cancer cells.[97] A recent study has reported efficient miRNA delivery techniques using PCa-targeted nanoparticles (R11-SSPEI).[98] They showed R11-SSPEI/*miR-145* peptide inhibits intraperitoneal inoculated PC3 tumor growth *in vivo*. *miR-16*-conjugated atelocollagen has been shown to inhibit bone-metastatic human prostate xenograft growth in the mouse bone site *in vivo*.[26] Liposomal *miR-34* mimic (MRX34, Mirna Therapeutics Inc.) is under phase I clinical trial for liver cancer, lung cancer, malignant lymphoma, melanoma, multiple myeloma and renal cell carcinoma.[99] *miR-34* functions as tumor suppressor in PCa; thus, this *miR-34* delivery system will hopefully be useful for PCa in future. The optimization of the stability of miRNAs and improvement in delivery system of miRNAs are challenges for the future treatment of PCa.

CONCLUSION

Accumulating evidence on the roles of miRNAs and the interactions between miRNAs and their target genes would promote a better understanding of PCa oncogenesis and castration resistance. Furthermore, determining roles of miRNAs that could be used as new diagnostic or predictive biomarkers would enable individualized therapeutic management for PCa patients. Elucidation of molecular mechanisms of PCa by miRNAs would help improve the therapeutic strategy of PCa.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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