Title
Small RNA and its application in andrology and urology

Journal
Translational Andrology and Urology, 1(1)

ISSN
2223-4683

Authors
Wang, Ji
Li, Long-Cheng

Publication Date
2012

DOI
10.3978/j.issn.2223-4683.2011.12.04

Peer reviewed

Review Article

Small RNA and its application in andrology and urology

Ji Wang, Long-Cheng Li

Department of Urology and Helen-Diller Comprehensive Cancer Center, University of California, San Francisco, CA 94158, USA
Correspondence to: Long-Cheng Li. Department of Urology and Helen-Diller Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA 94158, USA. Email: lilc@urology.ucsf.edu.
Submitted Dec 12, 2011. Accepted for publication Dec 29, 2011.

Abstract: Small non-coding RNAs such as small interfering RNA (siRNA), microRNA (miRNA) and piwi-interacting RNA (piRNA) exist in almost all kingdoms of organisms and have recently emerged as master regulators of gene expression to affect a diverse range of important biological processes. They exert their functions largely through two related but opposing mechanisms: RNA interference (RNAi) mediated by siRNA, miRNA and piRNA, and RNA activation (RNAa) mediated by small activating RNA (saRNA) and miRNA, leading to silencing and overexpression of target genes respectively. Dysregulation of these mechanisms have been implicated in a variety of human diseases including urological and andrological diseases. Importantly, both mechanisms can be readily harnessed for therapeutic purposes for a variety of diseases by using small RNA molecules as the "ribodrug". In this review, we highlight recent advances in the applications of small RNA as therapeutics for urological cancer, male infertile and erectile dysfunction.

Keywords: Small RNA; microRNA; RNAi; RNAa; urology; andrology; prostate cancer; bladder cancer; erectile dysfunction

Submitted Dec 12, 2011. Accepted for publication Dec 29, 2011.
doi: 10.3978/j.issn.2223-4683.2011.12.04
View this article at: http://dx.doi.org/10.3978/j.issn.2223-4683.2011.12.04

Introduction

RNA molecules can be divided into two major categories: protein-coding RNA and non-coding RNA (ncRNA). In human, around 98% of all transcriptional output is ncRNA (1). ncRNA can be further divided into two main subgroups based on their function: housekeeping ncRNA such as transfer RNA (tRNA), ribosomal RNA (rRNA), and regulatory ncRNA which includes both short and long ncRNA. It has become increasingly clear in recent years that regulatory ncRNA has profound biological function by regulating protein coding genes through a diverse array of molecular mechanisms. The best studied regulatory ncRNA is a class of tiny RNA molecules which have a size ranging from 20 to 30 nucleotides (nts) and highly depend on a family of conserved proteins known as Argonaute (Ago) for fuction. They include microRNA (miRNA), piwi-interacting RNA (piRNA) and small interfering RNA (siRNA). These small ncRNAs are mainly involved in an evolutionarily conserved gene silencing mechanism known as RNA interference (RNAi). Apart from their main function as silencers, we and other groups have recently reported that small RNA can also activate gene expression by targeting gene promoter sequences in a process termed RNA activation (RNAa) (2-4). These observations thus revealed a new class of small RNA molecules and a new layer of complexity of small RNA-mediated gene regulation (*Table 1*). It has become increasingly evident that various small RNA-guided gene regulations play important roles in normal physiological processes and in disease. It is also clear that these magnificent cellular mechanisms which were not known to us until recent years can be harnessed as powerful therapeutics for disease treatment. In this article, we review recent advances in the research of small RNA in urological and andrological diseases and focus on their potential application in the treatments of these diseases.

miRNA and its applications

miRNA and its biological functions

MicroRNA (miRNA) is a class of endogenous small RNA

Table 1 Classes of small RNA

Properties	microRNA (miRNA)	Piwi-interacting RNA (piRNA)	Small interfering RNA (siRNA)	Small activating RNA (saRNA)
Size (nt)	20-24	24-30	21-23	21
Source	Endogenous(ubiquitous)	Endogenous(restricted largely to spermatocytes and spermatids)	Exogenous or endogenous	Exogenous or endogenous
Conservation	Eukaryotes	Vertebrates and invertebrates	Eukaryotes	Mammals
Strandedness	Single-stranded	Single-stranded	Double-stranded	Double-stranded
Biogenesis	Dicer dependent	Dicer independent	Dicer dependent	Dicer dependent
Ago dependence	Ago subfamily	Piwi subfamily	Ago subfamily	Ago subfamily
Target	3'-UTR, 5'-UTR, gene promoter, coding regions, pseudogenes and competing endogenous RNA	Transposon	mRNA or gene promoter	Gene promoter
Functions	mRNA degradation,transcriptional silencing, posttranscriptional silencing, translation inhibition	Transposon silencing, transcriptional regulation, post-transcriptional regulation	mRNA degradation, transcriptional silencing, posttranscriptional silencing	Gene activation

(20-24 nt), which is transcribed from the genome and processed into its mature form by RNase III enzymes Drosha and Dicer via a multi-step process. Upon loaded by Ago proteins, miRNA binds to homologous sequences on the 3'untranslated regions (UTRs) of target messenger RNA (mRNA), resulting in translational repression and/or mRNA degradation (5). miRNA has also been shown to regulate gene expression by targeting gene promoters (6), 5'-UTR (7), coding regions (8), pseudogenes (9) and competing endogenous RNA (ceRNA) (10). A recent report showed that miRNA could regulate gene translation in a "seed" sequence-independent manner (11). Various models of action for miRNA revealed so far imply that the miRNA has diverse functions in biology and disease.

The application of miRNA in urological cancers

Urological malignancies including cancer of the prostate, bladder and kidney are the leading causes of urological patient death. The molecular mechanisms underlying their development and progression remain poorly understood. Human miRNA genes are frequently located at fragile sites and genomic regions implicated in cancers (12), suggesting the involvement of miRNAs in this complicated biological processes (*Table 2*). With the improvement of RNA-sequencing technology, hundreds of miRNAs have been identified and each miRNA are predicted to have hundreds of target genes.

According to their roles in cancer, miRNAs can be classified into oncogenic miRNAs (oncomiRs) and tumor suppressor miRNAs (tsmiRs). OncomiRs are generally upregulated in most tumor types and are able to promote malignant transformation and cancer progression. The well-known oncomiR is the miR-17-92 cluster consisting of several members including miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1. The miR-17-92 cluster is located at the 13q31 locus and amplified in many types of cancer (40). PTEN (phosphatase and tensin homologue) is one of the most frequently dysregulated tumor suppressors for several human urological cancers (41). Members of miR-17-92 cluster have been found to be able to inhibit PTEN expression via binding to its 3'UTR (13). mir-17-92 can also target and suppress the expression of BCL2L11(BIM) (13), TSP1 and CTGF genes (14). The miR-106b-25 cluster (miR-106b, miR-93 and miR-25), which shares high degree of homology with the miR-17-92 cluster, can promote cell proliferation and resistance to apoptosis by inhibiting BIM, p21 (15), PTEN (16) and p53 (17). The miR-221/222 locus, located on chromosome X, is another widely recognized oncomiR cluster. The two miRNAs in this cluster are transcribed from the same promoter and share identical seed sequences. Functional studies showed these oncomiRs negatively module many tumor suppressor genes, including p27, p57 (18), DDIT4 (19), PTEN and TIMP3 (20). Although different types of human cancer may have different miRNA expression signatures, some oncomiRs are consistently associated with a high risk for malignancies, such as miR-21 (40). Overexpression of miR-21 could suppress apoptosis, induce cell proliferation and survival, and facilitate cell migration and invasion by suppressing such target genes as PDCD4 (21), PTEN (22), RHOB (23), RECK and TIMP3 (24).

Table 2 Involvement of miRNA in urological cancers

Role	microRNA	Validated targets	References
OncomiRs	miR-17-92	PTEN, BIM, TSP1 and CTGF	(13,14)
	miR-106b-25	BIM, p21, PTEN and p53	(15-17)
	miR-221/222	p27, p57, DDIT4, PTEN and TIMP3	(18-20)
	miR-21	PDCD4, PTEN, RHOB, RECK and TIMP3	(21-24)
	miR-15a/miR-16-1	BCL2, CCND1, CCND3, CCNE1, CDK6, VEGF, FGF2 and FGFR1	(25-30)
	miR-143/145	RAS, c-Myc, BNIP3, FSCN1, OCT4, SOX2 and KLF4	(31-36)
TsmiRs	miR-200 family and miR-205	ZEB1 and ZEB2	(37)
	miR-101	EZH2	(38)
	miR-449a	HDAC-1	(39)

TsmiRs may inhibit cancers by suppressing oncogenes. Similar to protein-coding tumor suppressor genes, they are frequently deleted, mutated, or methylated in many human tumors (12,42). miR-15a and miR-16-1, located at human chromosome 13q14 and transcribed as a cluster, are the first identified tumor-suppressor miRNAs. Deletion or mutation of 13q14 locus was observed in many solid tumors and chronic lymphocytic leukemia (CLL) (12). Initially, miR-15a and miR-16-1 were found to be able to induce cell apoptosis by suppressing anti-apoptotic factor BCL2 in chronic lymphocytic leukemia (CLL) cells (25). Later studies showed that this cluster of miRNAs can also inhibit cell proliferation, induce apoptosis and suppress tumorigenicity by targeting multiple additional oncogenes in several signaling pathways, such as cell-cycle regulators (CCND1, CCND3, CCNE1 and CDK6) (26,27) and angiogenic factors and their receptors (VEGF, FGF2 and FGFR1) (28-30).

miR-143/145 is another important tsmiR cluster and is involved in suppressing the RAS and c-Myc signaling pathways (31,32). Systematic analysis of miRNA expression profile revealed that downregulation of miR-143 and miR-145 was associated with aggressive phenotype (43) in prostate cancer patients. BCL2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3) is widely upregulated in human tumors and miR-145 negatively regulates BNIP3 by targeting its 3'-UTR. Artificial restoration of miR-145 reduced cell growth in prostate cancer cells (33). In bladder tumor tissues, miR-145 is one of the most underexpressed miRNAs (44) and is capable of inhibiting tumor cell growth and invasion by targeting FSCN1 *in vitro* (34). Interestingly, three key pluripotency genes (OCT4, SOX2 and KLF4) essential for stem cell fate decision were demonstrated to be direct targets of miR-145 in both human embryonic stem cells (35) and cancer cells (36).

Beyond their role in cell growth control, miRNAs can also affect tumorigenesis and cancer progression via additional signaling pathways. For instance, miR-200 family and miR-205 which are frequently downregulated in invasive bladder and prostate cancer act as Epithelial-mesenchymal transition (EMT) repressors by targeting ZEB1 and ZEB2 (37). Moreover, miRNAs have been implicated in the regulation of epigenetics. Loss of miR-101 and miR-449a in prostate cancer cells led to overexpression of histone methyltransferase EZH2 (38) and histone deacetylase HDAC-1 (39), respectively.

Several studies have revealed that tumor suppressor factors (i.e. tumor suppressor genes, tsmiRs) and oncogenic factors (i.e. oncogenes, oncomiRs) are exquisitely regulated in organisms. On one hand, miRNAs are critical downstream effectors of classic oncogene/tumor suppressor networks. For examples, the transcription of miR-17-92 cluster and miR-34 family is directly activated by c-Myc and p53, respectively (45,46). And p53 can also module the biogenesis of miR-16-1, miR-143 and miR-145 (32,47). On the other hand, miR-145 suppresses c-Myc via targeting its 3'UTR (32), while mir-25 (a homologous miRNA of the miR-17-92 cluster) inhibits p53 by its 3'UTR (17).

Supplementation of tsmiRs or inhibition of oncomiRs by antagomirs could be a new class of targeted molecular therapy. For instance, miR-15a and miR-16 are significantly decreased in cancer cells of advanced prostate tumors. Delivery of antagomirs specific for miR-15a and miR-16 to normal mouse prostate caused hyperplasia, but reconstitution of miR-15a and miR-16-1 expression led to growth arrest, apoptosis and regression of prostate tumor xenografts (27). Another independent study also reported that systemic delivery of synthetic miR-16 via tail vein injection significantly inhibited the growth of metastatic prostate tumors in a prostate cancer xenograft model in nude mice (48).

The application of miRNA in andrology

Although miRNAs have been intensively studied in cancer, their physiological roles in andrology are much less clear. Some recent works suggest that miRNAs may have regulatory role in testicular and epididymal development and spermatogenesis. A systematic cDNA array and miRNA array analysis showed that neonatal epididymis expressed more miRNAs than that of young adults and aged men. Some of the miRNAs expressing high in young adults include miR-143, miR-7a, miR-21, miR-23a, miR-24, miR-27a/b and miR-29a (49). In young adults, male infertility may be associated with dysexpression of certain miRNAs. Compared with fertile males, miR-34c-5p, miR-122, miR-146b-5p, miR-181a, miR-374b, miR-509-5p and miR-513a-5p are dramatically decreased in azoospermia patients, but increased in asthenozoospermia (50). Another work performed in non-obstructive azoospermia patients identified 19 differentially up-regulated and 154 downregulated miRNAs. Among the downregulated ones are some testicular miRNAs (miR-181a, miR-29c, and miR-34b*) and several members of the miR-17-92 cluster (51). It is interesting that miR-17-92 cluster is highly expressed in primordial germ cells and spermatogonia during development of mouse germ cells (52). It is known that single-nucleotide polymorphisms (SNPs) inside miRNA target sites (miR-TS-SNPs) may influence miRNA biogenesis and susceptibility to tumorigenesis (53). Recently, these SNPs were found to be implicated in male infertility. Some fertility related genes contain miR-TS-SNPs, and the most polymorphic were identified in the 3'UTR of KITL, ACTB, ACE, CAMK4, ESR1 and MTHFR (54). Ogorevc *et al*. also analyzed the relationships between selected infertile candidate genes and dysexpressed miRNAs in non-obstructive azoospermic patients and found 3 upregulated and 10 downregulated miRNA contained miR-TS-SNPs (54). These works suggested that miRNA expression profiles may serve as noninvasive molecular markers for male infertility diagnosis.

piRNA and its applications

piRNA and its biological functions

Piwi-interacting RNA (piRNA) is a class of endogenous single-stranded small RNAs (24-30 nt) found in both vertebrates and invertebrates. Distinct from miRNAs, piRNAs are transcribed from specific genomic regions containing repetitive elements, such as transposable elements, and processed into its mature form by a Dicer-independent pathway. At the assistance of Piwi and Aub proteins, piRNA is implicated in transposon silencing at both the transcriptional and post-transcriptional levels (55). Opposite to the well known piRNA-mediated silencing, piRNA is also able to function as an epigenetic activator. 3R-TAS1 piRNA, a piRNA transcribed from a region of the telomere-associated sequence (TAS) on the right arm of chromosome 3 (3R-TAS) in Drosophila, promotes the euchromatic character of 3R-TAS heterochromatin and its transcriptional activity (56).

The application of piRNA in andrology and urology

Different from miRNA's ubiquitous expression in different tissues and organs, piRNA is specifically present in spermatocytes and spermatids during spermatogenesis (57). Thus, it is believed that piRNA plays an essential role in germline development. In mice, PIWI-like proteins subfamily consists of MIWI, MIWI2, and MILI, and deficiencies of these members cause aberrant male germ cell development. MIWI-deficient mice display spermatogenic arrest at the beginning of the round spermatid stage (58). Spermatogenesis in the MILI-null mice is blocked at the early prophase of the first meiosis (59). MIWI2 -deficient mice display a defect in early prophase of meiosis I and a marked and progressive loss of germ cells with age (60). Despite considerable knowledge has been gained about the function of piRNA in Drosophila and mice, their function in human is obscure. Recently, nine SNPs of four human Piwi genes (PIWIL1/HIWI, PIWIL2/HILI, PIWIL3/HIWI3 and PIWIL4/HIWI2) were identified in patients with idiopathic azoospermia or oligozoospermia. Among them, an SNP in the 3'UTR region of HIWI2 and a non-synonymous SNP in HIWI3 were significantly associated with an altered risk of oligozoospermia (61). Furthermore, Hiwi (human Piwi ortholog) has been found to be aberrantly expressed in a variety of human cancers (62) and its overexpression correlates with poor clinical prognosis of soft-tissue sarcoma patients (63), while there has been no report of its association with urological cancers.

siRNA and its applications

siRNA and its biological functions

Small interfering RNA (siRNA) is a class of 21- to 23-nt double-stranded RNAs (dsRNAs) endogenously generated

Table 3 The application of siRNA in urology and andrology

Disease	Target gene	Trigger RNA	Applications	References
Prostate cancer	Survivin	shRNA	*In vitro*, *in vivo* (xenograft)	(67)
	Stat3	shRNA	*In vitro*, *in vivo* (xenograft)	(68)
	FOXO3/*Foxo3*	siRNA	*In vitro*	(69)
	Integrin αv	siRNA	*In vitro*, *in vivo* (xenograft)	(70)
	PLK1 and BCL2	aptamer-siRNA chimeras	*In vitro*, *in vivo* (xenograft)	(71)
Bladder cancer	Survivin	siRNA	*In vitro*, *in vivo* (xenograft)	(72)
	BCL2	siRNA	*In vitro*	(73)
	EphB4	siRNA	*In vitro*, *in vivo* (xenograft)	(74)
	PLK1	siRNA	*In vitro*, *in vivo*	(75)
Renal cancer	SOCS3	siRNA	*In vitro*, *in vivo* (xenograft)	(76)
	PLK1	siRNA	*In vitro*	(77)
	Survivin	siRNA	*In vitro*	(78)
Erectile dysfunction	PDE5/ PDE5	shRNA/siRNA	*In vitro*, *in vivo*	(79-81)
	PIN	shRNA/siRNA	*In vitro*, *in vivo*	(82)
	CX43	shRNA	*In vitro*	(83)

or artificially designed and is the trigger of the well-known RNA interference (RNAi) mechanism. RNAi can be induced by exogenously introduced dsRNAs (64), or endogenous dsRNAs (65). In addition, plasmid-expressed short hairpin RNA (shRNA) can also be used to silence gene expression via RNAi pathway (66). It is well known that many human diseases are caused by abnormal overproduction of specific gene products, such as oncogenes. Therefore, siRNA or shRNA targeting disease-causing gene is promising therapeutic for many diseases (*Table 3*).

The application of siRNA in urological cancers

Apoptosis (programmed cell death) is a crucial biological process in cancer initiation and progression. Cancer cells overexpress many anti-apoptotic genes (i.e. survivin, BCL2, etc) or underexpress pro-apoptotic genes (i.e. caspase family) to maintain aggressive cell proliferation. Resisting cell death caused by dysregulation of apoptosis related genes is one of the fundamental hallmarks of cancer and is a major target for cancer therapy (84). Abnormal upregulation of survivin may inhibit caspase activity and enable cancer cells to escape programmed cell death and promote resistance to radiation and chemotherapy. Vector-based siRNA expression effectively suppressed survivin expression and led to decreased tumor formation in nude mice bearing prostate cancer xenografts and enhanced chemosensitivity (67). Knockdown of survivin Null by siRNA in bladder cancer cells also suppressed their growth *in vitro* and *in vivo* (72). Similarly, BCL2 downregulation by siRNA enhanced mitomycin C induced apoptotic cell death in bladder cancer cell lines (73). Stat3 belongs to the STAT transcription factor family and functions as an oncogenic transcription factor by promoting proliferation, anti-apoptosis and cell cycle progression. RNAi of Stat3 led to downregulation of its downstream genes including BCL2, cyclin D1 and c-Myc in prostate cancer cell lines and in tumors implanted in nude mice (68). EphB4 is a receptor protein tyrosine kinase aberrantly expressed in bladder cancer cell lines and tumor specimens. EphB4 knockdown *in vitro* using siRNA suppressed cell viability by inducing apoptosis via activation of caspase-8 pathway and by inhibiting anti-apoptotic factor, Bcl-xl. EphB4 siRNA delivered into tumors was able to alleviate xenograft tumor burden *in vivo* (74).

Overactivated mitosis is another essential cause for the aberrant proliferation signaling in cancer cells. Polo-like kinase-1 (PLK1) is a critical regulator of mitotic progression. Elevated PLK1 expression in patients with bladder cancer and renal cell carcinoma is frequently associated with poor prognosis. siRNA targeting PLK1 could disrupt cell mitosis and cell cycle progression and thus reduced cell proliferation in bladder cancer and renal cancer cells (75,77). In an orthotopic bladder cancer mouse model, PLK1 siRNA delivery successfully prevented bladder cancer

cell growth (77).

Evading immune destruction is also one of the hallmarks of cancer development and progression (84). Interferons are essential parts of the primary immune system by effectively triggering protective response, such as activating natural killer cells and macrophages, and facilitating antigen presentation to T lymphocytes. SOCS3 have been identified as an inhibitor of the IFN-mediated JAK/STAT signaling pathways. Suppression of SOCS3 by siRNA promoted IFN-α-induced cell death in renal cell carcinoma xenografts in nude mice (76). Dendritic cells (DCs) are the most potent antigen-presenting cells, while DCs can also induce tolerance, rather than immune activation. In tumors from human prostate cancer patients and transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, tumor-associated dendritic cells (TADCs) possess increased FOXO3/*Foxo3* expression resulting in suppressed T cell function. Silencing FOXO3/*Foxo3* with siRNAs could block its inhibitory effect on T cells, promote expression of costimulatory molecules and proinflammatory cytokines, and diminish expression of tolerogenic factors (69).

High pathological grades of malignancy is usually reflected in local invasion and distant metastasis. Integrins are one of major cell adhesion components, which can determine cells fate by controlling cell replication, migration and differentiation, especially in cancer metastasis (41). siRNA targeting Integrin αv, an ECM receptor gene, inhibited growth of human prostate cancer in a bone xenograft imaging model (70).

Clinical application of siRNA for cancer therapy highly depends on the efficiency and cell-type specificity of siRNA delivery. Sato *et al*. reported that Topotecan, a topoisomerase I inhibitor able to enhance survivin siRNA, caused renal cancer cell growth suppression by increasing cellular uptake of the siRNA (78). Furthermore, siRNAs targeting PLK1 and BCL2 were linked to an aptamer which specifically recognized prostate specific membrane antigen (PSMA) could inhibite tumor growth and mediate tumor regression in a xenograft prostate cancer model, by selectively binding to prostate cancer cells and tumor vascular endothelium (71).

The application of siRNA in andrology

Erectile dysfunction (ED) is a common andrological disorder, especially in aged men or men with diabetes. During physiological process of erection, nitric oxide (NO) release triggers the accumulation of cyclic guanosine monophosphate (cGMP) in cavernous smooth muscle cells (CSMCs), which subsequently induces increased blood flows into the corpus cavernosum and finally stimulates an erection by loosing smooth muscle cells. Dysregulation of NO synthesis and cGMP accumulation may result in erectile dysfunction (ED). Phosphodiesterase type 5 (PDE5) can catalyze the degradation of cGMP and suppress erectile function. Lin *et al*. reported that a lentiviral vector-based siRNA expression caused the knockdown of *PDE5* and prolonged accumulation of cGMP in cultured rat CSMCs. Injection of the lentiviral vector into rat penis significantly enhanced erectile function (79). Synthetic siRNA or shRNA vector-mediated PDE5 silencing also led to enhanced cGMP in cultured human CSMCs (80,81). siRNA or shRNA-mediated knockdown of *PIN* (protein inhibitor of NOS), a key repressor of nNOS (neuronal nitric oxide synthase), elevated cGMP concentration *in vitro* and improved erectile dysfunction in aged rats (82). Additionally, aberrant upregulation of CX43 (connexin43) is usually associated with ED. CX43 siRNA could decrease GJIC (gap junction intercellular communication) in cultured human CSMCs (83). Taken together, siRNA may have a therapeutic application in ED treatment; however, delivery of siRNA into corpus cavernosum tissue may be challenging and awaits further development.

saRNA and its applications

SaRNA and its biological functions

RNA activation (RNAa) is a newly discovered mechanism of gene regulation triggered also by small dsRNA that targets gene promoter regions instead of coding sequences. These promoter-targeted dsRNAs that can activate gene expression are referred to as small activating RNA (saRNA) (2,4). saRNA has been shown to activate the expression of endogenous genes (2-4), presenting a novel and natural tool of overexpressing functionally important genes for disease treatment. Vector-based systems offer robust gene overexpression and are being tested as a promising gene therapy strategy for the treatment of a variety of diseases. However, serious concerns of insertional mutagenesis in clinical settings are associated with the use of viral vectors. As an alternative, RNAa may offer a safer approach for gene overexpression and disease treatment. Several recent studies have shown that saRNA-mediated gene activation can suppress cancer cell growth by upregulating tumor suppressor genes and improve smooth muscle function by

Table 4 The application of saRNA in urology and andrology

Disease	Species	Gene	Trigger RNA	Applications	References
Prostate cancer	Human	p21	saRNA	*In vitro*, *in vivo* (xenograft)	(2)
	Human	E-cadherin	saRNA and miRNA	*In vitro*	(2,6,85)
	Human	KLF4	saRNA	*In vitro*	(86)
	Human	NKX3.1	saRNA	*In vitro*, *in vivo* (xenograft)	(87)
	Mouse	*Ccnb1*	saRNA and miRNA	*In vitro*, *in vivo* (xenograft)	(87,88)
Bladder cancer	Human	p21	saRNA	*In vitro*, *in vivo* (xenograft)	(89,90)
	Human	E-cadherin	saRNA	*In vitro*	(91)
Renal cancer	Human	p21	saRNA	*In vitro*	(92)
ED	Human	VEGF	saRNA	*In vitro*	(93)

activating VEGF gene (*Table 4*).

The application of saRNA in urological cancers

It is well known that tumorigenesis and cancer progression correlates with inactivation of tumor suppressor genes. p21 is a cyclin-dependent kinase inhibitor that functions as a key mediator of cell-cycle arrest and down-regulation of p21 is frequently observed in different types of cancers. Several studies showed that restoration of p21 expression by saRNA can inhibit cell proliferation, cell cycle progression, and induce apoptosis in prostate, bladder and renal cancer cells (2,89,90,92). It is believed that EMT plays an important role in the initiation and progression of cancer. E-cadherin, a key mediator in EMT signaling, can serves as a potent inhibitor of cancer cell growth and invasion (84). Overexpression of E-cadherin by saRNAs led to inhibited cell proliferation and suppression of migration and invasion of prostate and bladder cancer cells (2,85,91). NKX3-1 is a prostate specific tumor suppressor gene and could be activated by its promoter-targeted saRNA (87).

Recently, our group has utilized RNAa as a laboratory tool to investigate the function of KLF4, a member of the Krüppel-like family of transcription factors, in prostate cancer cells in which KLF4 expression is significantly downregulated (86). *In vitro* studies indicated that saRNA-mediated overexpression of KLF4 inhibited prostate cancer cell proliferation and survival, and altered the expression of its downstream cell-cycle–related genes including p21, p27, p57 and CCNB1. Reactivation of KLF4 by saRNA also suppressed migration and invasion of prostate cancer cells (86).

It is largely recognized that miRNA functions as a silencer of gene expression. Our recent work indicates that miRNAs may also positively-regulate gene expression by targeting promoter sequences and thus act as saRNA. In human prostate cancer PC-3 cells, introduction of pre-miR-373/miR-373 could activate E-cadherin and CSDC2 (cold-shock domain-containing protein C2) (6). Cyclin B1 is an essential protein that drives cell cycle entry into mitosis and is a cancer antigen highly expressed in a majority of human cancer (88). In mouse prostate cells (i.e. TRAMP C1), we recently identified three miRNAs (miR-744, miR-1186 and miR-466d-3p) that target the cyclin B1 promoter to induce *Ccnb1* expression in a physiological context. Short-term overexpression of miR-744 and miR-1186 resulted in enhanced cell proliferation, while long-term expression caused chromosomal instability and tumor suppression *in vivo* (88). This study thus provided the first example that RNAa mechanism functions as an endogenous cellular process and is exploited by cancer cells to gain a growth advantage.

The application of saRNA in andrology

Vascular endothelial growth factor (VEGF) is a well-known cytokine with strong angiogenic properties and can also stimulate cell proliferation, delay senescence, suppress apoptosis and promote nerve regeneration. Vector-based overexpression of VEGF has been shown to improve erectile function (94). Our group identified a saRNA with the capacity to activate VEGF in human (2) and nonhuman primates cells (87). Very recently, this saRNA was demonstrated to induce endogenous VEGF expression in primary human corpus cavernosum smooth muscle cells (CCSMCs) *in vitro* (93). Delivering an saRNA targeting mouse VEGF by lentivirus into mice resulted in improved vascularity and blood flow in an ischemic mouse hindlimb model (95). These studies imply that saRNA-meditated

VEGF upregulation may present a new avenue for treating ED and other vascular disorders.

Prospectives

Despite tons of efforts have been made to develop small RNA-based therapeutics, the scientific and pharmaceutical communities are still facing significant hurdles in bringing small RNA drugs (ribodrugs) to the clinic. Among them, efficient delivery to target tissue and cells is the greatest challenge (96). Recently, promising results have been reported from the first-ever phase I clinical trial of siRNA delivered by nanoparticles for treating melanoma (97). Many other clinical trials of siRNA-based drugs for a large array of diseases are ongoing with some having advanced into phase II. With the rapid progression in the development of small RNA delivery technology, we believe that in the future not far away from now ribodrugs will be an indispensable weapon in the arsenal of urologists and andrologists for fighting against a variety of diseases.

Acknowledgements

We thank Vera Huang for valuable help in manuscript preparation and Guiting Lin for critical reading of our manuscript.
Funding: This work was supported by grants from the National Cancer Institute at the National Institutes of Health (1R21CA131774-01 to L.C.L.), the National Institutes of Health (1R01GM090293-0109 to L.C.L.), Department of Defense (W81XWH-08-1-0260 to L.C.L.), California Institute for Regenerative Medicine (RL1-00660-1 to L.C.L.), and California Urology Foundation Award (2009 to J. W.).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Mattick JS. Non-coding RNAs: the architects of eukaryotic complexity. EMBO Rep 2001;2:986-91.
2. Li LC, Okino ST, Zhao H, et al. Small dsRNAs induce transcriptional activation in human cells. Proc Natl Acad Sci U S A 2006;103:17337-42.
3. Janowski BA, Younger ST, Hardy DB, et al. Activating gene expression in mammalian cells with promoter-targeted duplex RNAs. Nat Chem Biol 2007;3:166-73.
4. Portnoy V, Huang V, Place RF, et al. Small RNA and transcriptional upregulation. Wiley Interdiscip Rev RNA 2011;2:748-60.
5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281-97.
6. Place RF, Li LC, Pookot D, et al. MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc Natl Acad Sci U S A 2008;105:1608-13.
7. Lytle JR, Yario TA, Steitz JA. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5' UTR as in the 3' UTR. Proc Natl Acad Sci U S A 2007;104:9667-72.
8. Tay Y, Zhang J, Thomson AM, et al. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 2008;455:1124-8.
9. Poliseno L, Salmena L, Zhang J, et al. A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature 2010;465:1033-8.
10. Tay Y, Kats L, Salmena L, et al. Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell 2011;147:344-57.
11. Eiring AM, Harb JG, Neviani P, et al. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. Cell 2010;140:652-65.
12. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004;101:2999-3004.
13. Xiao C, Srinivasan L, Calado DP, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol 2008;9:405-14.
14. Dews M, Homayouni A, Yu D, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat Genet 2006;38:1060-5.
15. Petrocca F, Visone R, Onelli MR, et al. E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. Cancer Cell 2008;13:272-86.
16. Poliseno L, Salmena L, Riccardi L, et al. Identification of the miR-106b~25 microRNA cluster as a proto-oncogenic PTEN-targeting intron that cooperates with its host gene MCM7 in transformation. Sci Signal 2010;3:ra29.
17. Kumar M, Lu Z, Takwi AA, et al. Negative regulation of the tumor suppressor p53 gene by microRNAs. Oncogene 2011;30:843-53.
18. Fornari F, Gramantieri L, Ferracin M, et al. MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. Oncogene

2008;27:5651-61.
19. Pineau P, Volinia S, McJunkin K, et al. miR-221 overexpression contributes to liver tumorigenesis. Proc Natl Acad Sci U S A 2010;107:264-9.
20. Garofalo M, Di Leva G, Romano G, et al. miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. Cancer Cell 2009;16:498-509.
21. Frankel LB, Christoffersen NR, Jacobsen A, et al. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem 2008;283:1026-33.
22. Meng F, Henson R, Wehbe-Janek H, et al. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007;133:647-58.
23. Connolly EC, Van Doorslaer K, Rogler LE, et al. Overexpression of miR-21 promotes an in vitro metastatic phenotype by targeting the tumor suppressor RHOB. Mol Cancer Res 2010;8:691-700.
24. Gabriely G, Wurdinger T, Kesari S, et al. MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. Mol Cell Biol 2008;28:5369-80.
25. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 2005;102:13944-9.
26. Liu Q, Fu H, Sun F, et al. miR-16 family induces cell cycle arrest by regulating multiple cell cycle genes. Nucleic Acids Res 2008;36:5391-404.
27. Bonci D, Coppola V, Musumeci M, et al. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nat Med 2008;14:1271-7.
28. Hua Z, Lv Q, Ye W, et al. MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. PLoS One 2006;1:e116.
29. Wang J, Place R, Li L. Small RNA-mediated regulation of basic fibroblast growth factor (FGF2) in prostate cancer [abstract]. American Association for Cancer Research 100st annual meeting, Denver 2009;s2536.
30. Musumeci M, Coppola V, Addario A, et al. Control of tumor and microenvironment cross-talk by miR-15a and miR-16 in prostate cancer. Oncogene 2011;30:4231-42.
31. Kent OA, Chivukula RR, Mullendore M, et al. Repression of the miR-143/145 cluster by oncogenic Ras initiates a tumor-promoting feed-forward pathway. Genes Dev 2010;24:2754-9.
32. Sachdeva M, Zhu S, Wu F, et al. p53 represses c-Myc through induction of the tumor suppressor miR-145. Proc Natl Acad Sci U S A 2009;106:3207-12.
33. Chen X, Gong J, Zeng H, et al. MicroRNA145 targets BNIP3 and suppresses prostate cancer progression. Cancer Res 2010;70:2728-38.
34. Chiyomaru T, Enokida H, Tatarano S, et al. miR-145 and miR-133a function as tumour suppressors and directly regulate FSCN1 expression in bladder cancer. Br J Cancer 2010;102:883-91.
35. Xu N, Papagiannakopoulos T, Pan G, et al. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell 2009;137:647-58.
36. Fang X, Yoon JG, Li L, et al. The SOX2 response program in glioblastoma multiforme: an integrated ChIP-seq, expression microarray, and microRNA analysis. BMC Genomics 2011;12:11.
37. Gregory PA, Bert AG, Paterson EL, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008;10:593-601.
38. Varambally S, Cao Q, Mani RS, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 2008;322:1695-9.
39. Noonan EJ, Place RF, Pookot D, et al. miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. Oncogene 2009;28:1714-24.
40. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257-61.
41. Tamura M, Gu J, Tran H, et al. PTEN gene and integrin signaling in cancer. J Natl Cancer Inst 1999;91:1820-8.
42. Lopez-Serra P, Esteller M. DNA methylation-associated silencing of tumor-suppressor microRNAs in cancer. Oncogene 2012;31:1609-22.
43. Wang L, Tang H, Thayanithy V, et al. Gene networks and microRNAs implicated in aggressive prostate cancer. Cancer Res 2009;69:9490-7.
44. Ichimi T, Enokida H, Okuno Y, et al. Identification of novel microRNA targets based on microRNA signatures in bladder cancer. Int J Cancer 2009;125:345-52.
45. O'Donnell KA, Wentzel EA, Zeller KI, et al. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005;435:839-43.
46. He L, He X, Lim LP, et al. A microRNA component of the p53 tumour suppressor network. Nature 2007;447:1130-4.
47. Suzuki HI, Yamagata K, Sugimoto K, et al. Modulation of microRNA processing by p53. Nature 2009;460:529-33.
48. Takeshita F, Patrawala L, Osaki M, et al. Systemic delivery

of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. Mol Ther 2010;18:181-7.
49. Zhang J, Liu Q, Zhang W, et al. Comparative profiling of genes and miRNAs expressed in the newborn, young adult, and aged human epididymides. Acta Biochim Biophys Sin (Shanghai) 2010;42:145-53.
50. Wang C, Yang C, Chen X, et al. Altered Profile of Seminal Plasma MicroRNAs in the Molecular Diagnosis of Male Infertility. Clin Chem 2011;57:1722-31.
51. Lian J, Zhang X, Tian H, et al. Altered microRNA expression in patients with non-obstructive azoospermia. Reprod Biol Endocrinol 2009;7:13.
52. Hayashi K, Chuva de Sousa Lopes SM, Kaneda M, et al. MicroRNA biogenesis is required for mouse primordial germ cell development and spermatogenesis. PLoS One 2008;3:e1738.
53. Nicoloso MS, Sun H, Spizzo R, et al. Single-nucleotide polymorphisms inside microRNA target sites influence tumor susceptibility. Cancer Res 2010;70:2789-98.
54. Ogorevc J, Dovc P, Kunej T. Polymorphisms in microRNA targets: a source of new molecular markers for male reproduction. Asian J Androl 2011;13:505-8.
55. Siomi MC, Sato K, Pezic D, et al. PIWI-interacting small RNAs: the vanguard of genome defence. Nat Rev Mol Cell Biol 2011;12:246-58.
56. Yin H, Lin H. An epigenetic activation role of Piwi and a Piwi-associated piRNA in Drosophila melanogaster. Nature 2007;450:304-8.
57. Klattenhoff C, Theurkauf W. Biogenesis and germline functions of piRNAs. Development 2008;135:3-9.
58. Deng W, Lin H. miwi, a murine homolog of piwi, encodes a cytoplasmic protein essential for spermatogenesis. Dev Cell 2002;2:819-30.
59. Kuramochi-Miyagawa S, Kimura T, Ijiri TW, et al. Mili, a mammalian member of piwi family gene, is essential for spermatogenesis. Development 2004;131:839-49.
60. Carmell MA, Girard A, van de Kant HJ, et al. MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline. Dev Cell 2007;12:503-14.
61. Gu A, Ji G, Shi X, et al. Genetic variants in Piwi-interacting RNA pathway genes confer susceptibility to spermatogenic failure in a Chinese population. Hum Reprod 2010;25:2955-61.
62. Siddiqi S, Matushansky I. Piwis and piwi-interacting RNAs in the epigenetics of cancer. J Cell Biochem 2012;113:373-80.
63. Taubert H, Greither T, Kaushal D, et al. Expression of the stem cell self-renewal gene Hiwi and risk of tumour-related death in patients with soft-tissue sarcoma. Oncogene 2007;26:1098-100.
64. Fire A, Xu S, Montgomery MK, et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998;391:806-11.
65. Watanabe T, Totoki Y, Toyoda A, et al. Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. Nature 2008;453:539-43.
66. Paddison PJ, Caudy AA, Bernstein E, et al. Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. Genes Dev 2002;16:948-58.
67. Shen J, Liu J, Long Y, et al. Knockdown of survivin expression by siRNAs enhances chemosensitivity of prostate cancer cells and attenuates its tumorigenicity. Acta Biochim Biophys Sin (Shanghai) 2009;41:223-30.
68. Gao L, Zhang L, Hu J, et al. Down-regulation of signal transducer and activator of transcription 3 expression using vector-based small interfering RNAs suppresses growth of human prostate tumor in vivo. Clin Cancer Res 2005;11:6333-41.
69. Watkins SK, Zhu Z, Riboldi E, et al. FOXO3 programs tumor-associated DCs to become tolerogenic in human and murine prostate cancer. J Clin Invest 2011;121:1361-72.
70. Bisanz K, Yu J, Edlund M, et al. Targeting ECM-integrin interaction with liposome-encapsulated small interfering RNAs inhibits the growth of human prostate cancer in a bone xenograft imaging model. Mol Ther 2005;12:634-43.
71. McNamara JO 2nd, Andrechek ER, Wang Y, et al. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat Biotechnol 2006;24:1005-15.
72. Hou JQ, He J, Wang XL, et al. Effect of small interfering RNA targeting survivin gene on biological behaviour of bladder cancer. Chin Med J (Engl) 2006;119:1734-9.
73. Duggan BJ, Maxwell P, Kelly JD, et al. The effect of antisense Bcl-2 oligonucleotides on Bcl-2 protein expression and apoptosis in human bladder transitional cell carcinoma. J Urol 2001;166:1098-105.
74. Xia G, Kumar SR, Stein JP, et al. EphB4 receptor tyrosine kinase is expressed in bladder cancer and provides signals for cell survival. Oncogene 2006;25:769-80.
75. Nogawa M, Yuasa T, Kimura S, et al. Intravesical administration of small interfering RNA targeting PLK-1 successfully prevents the growth of bladder cancer. J Clin Invest 2005;115:978-85.
76. Tomita S, Ishibashi K, Hashimoto K, et al. Suppression of SOCS3 increases susceptibility of renal cell carcinoma to interferon-α. Cancer Sci 2011;102:57-63.
77. Ding Y, Huang D, Zhang Z, et al. Combined gene

expression profiling and RNAi screening in clear cell renal cell carcinoma identify PLK1 and other therapeutic kinase targets. Cancer Res 2011;71:5225-34.
78. Sato A, Ito K, Asano T, et al. Synergistic effect of survivin-specific small interfering RNA and topotecan in renal cancer cells: topotecan enhances liposome-mediated transfection by increasing cellular uptake. Int J Oncol 2007;30:695-700.
79. Lin G, Hayashi N, Carrion R, et al. Improving erectile function by silencing phosphodiesterase-5. J Urol 2005;174:1142-8.
80. Chen GQ, Bai WJ, Wang XF, et al. [Phosphodiesterase type 5 siRNA increases cGMP in the smooth muscle cells of human corpus cavernosum]. Zhonghua Nan Ke Xue 2006;12:979-81, 984.
81. Pan YG, Liu JH, Zhan Y, et al. [Impact of rAd5-shRNA-PDE5A3 on cGMP in the smooth muscle cells of human corpus cavernosum]. Zhonghua Nan Ke Xue 2009;15:689-92.
82. Magee TR, Kovanecz I, Davila HH, et al. Antisense and short hairpin RNA (shRNA) constructs targeting PIN (Protein Inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. J Sex Med 2007;4:633-43.
83. Cao ZG, Zhu YP, Sun YW, et al. [Small interfering RNA inhibits the expression of connexin43 in the human corpus cavernosum penis smooth muscle cells]. Zhonghua Nan Ke Xue 2007;13:440-3.
84. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
85. Mao Q, Zheng X, Yang K, et al. Suppression of migration and invasion of PC3 prostate cancer cell line via activating E-cadherin expression by small activating RNA. Cancer Invest 2010;28:1013-8.
86. Wang J, Place RF, Huang V, et al. Prognostic value and function of KLF4 in prostate cancer: RNAa and vector-mediated overexpression identify KLF4 as an inhibitor of tumor cell growth and migration. Cancer Res 2010;70:10182-91.
87. Huang V, Qin Y, Wang J, et al. RNAa is conserved in mammalian cells. PLoS One 2010;5:e8848.
88. Huang V, Place RF, Portnoy V, et al. Upregulation of Cyclin B1 by miRNA and its implications in cancer. Nucleic Acids Res 2012;40:1695-707.
89. Chen Z, Place RF, Jia ZJ, et al. Antitumor effect of dsRNA-induced p21(WAF1/CIP1) gene activation in human bladder cancer cells. Mol Cancer Ther 2008;7:698-703.
90. Yang K, Zheng XY, Qin J, et al. Up-regulation of p21WAF1/Cip1 by saRNA induces G1-phase arrest and apoptosis in T24 human bladder cancer cells. Cancer Lett 2008;265:206-14.
91. Mao Q, Li Y, Zheng X, et al. Up-regulation of E-cadherin by small activating RNA inhibits cell invasion and migration in 5637 human bladder cancer cells. Biochem Biophys Res Commun 2008;375:566-70.
92. Whitson JM, Noonan EJ, Pookot D, et al. Double stranded-RNA-mediated activation of P21 gene induced apoptosis and cell cycle arrest in renal cell carcinoma. Int J Cancer 2009;125:446-52.
93. Chen R, Wang T, Rao K, et al. Up-regulation of VEGF by small activator RNA in human corpus cavernosum smooth muscle cells. J Sex Med 2011;8:2773-80.
94. Rogers RS, Graziottin TM, Lin CS, et al. Intracavernosal vascular endothelial growth factor (VEGF) injection and adeno-associated virus-mediated VEGF gene therapy prevent and reverse venogenic erectile dysfunction in rats. Int J Impot Res 2003;15:26-37.
95. Turunen MP, Lehtola T, Heinonen SE, et al. Efficient regulation of VEGF expression by promoter-targeted lentiviral shRNAs based on epigenetic mechanism: a novel example of epigenetherapy. Circ Res 2009;105:604-9.
96. Pecot CV, Calin GA, Coleman RL, et al. RNA interference in the clinic: challenges and future directions. Nat Rev Cancer 2011;11:59-67.
97. Davis ME, Zuckerman JE, Choi CH, et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature 2010;464:1067-70.

Cite this article as: Wang J, Li LC. Small RNA and its application in andrology and urology. Transl Androl Urol 2012;1(1):33-43. doi: 10.3978/j.issn.2223-4683.2011.12.04

