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REVIEW

Dysregulation of ncRNAs located at the DLK1-DIO3 imprinted domain: involvement in urological cancers

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Abstract: Genomic imprinting has been found to be involved in human physical development and several diseases. The *DLK1-DIO3* imprinted domain is located on human chromosome 14 and contains paternally expressed protein-coding genes (*DLK1, RTL1, DIO3*) and numerous maternally expressed ncRNA genes (*MEG3, MEG8*, *antisense RTL1*, miRNAs, piRNAs, and snoRNAs). Emerging evidence has implicated that dysregulation of the *DLK1-DIO3* imprinted domain especially the imprinted ncRNAs is critical for tumor progressions. Multiple miRNAs and lncRNAs have been investigated in urological cancers, of which several are transcribed from this domain. In this review, we present current data about the associated miRNAs, lncRNAs, and piRNAs and the regulation of differentially methylated regions methylation status in the progression of urological cancers and preliminarily propose certain concepts about the potential regulatory networks involved in *DLK1-DIO3* imprinted domain.

Keywords: ncRNAs, *DLK1-DIO3* imprinted domain, epigenetics, regulatory network, urological cancers, *DMRs*, *MEG3*

Introduction

Genomic imprinting usually plays a critical role in human development and diseases. As one of the important imprinted domains, the *DLK1-DIO3* imprinted domain is located on human chromosome 14, which contains three paternally expressed protein-coding genes (DLK1, RTL1, and DIO3), maternally expressed lncRNAs (*MEG3, MEG8*, and antisense RTL1) genes, and numerous sncRNA genes. Emerging evidence implicates this domain in disease pathogenesis, especially in cancers.[1,2]

ncRNAs refer to RNAs that do not encode proteins but modulate the whole transcription and translation process in an epigenetic way. Currently, 98% DNA in human genome is nonprotein coding, which was once considered as "junk" DNA for a time, with the continuous deeper investigation of ncRNAs, the ENCODE project in 2012 discovered that at least 80% of the human genome is biologically active.[3] According to the length of RNAs, they are classified as lncRNAs (>200 bp), and sncRNAs (<200 bp), which include miRNAs, piRNAs, and snoRNAs, and recently, a kind of nonlinear ncRNAs has been detected and described as circular RNAs.[4,5]

Numerous studies have revealed that the differential distributions and significant roles of ncRNAs are involved in various diseases, especially in cancers, ncRNAs as biomarkers present important diagnostic values. As the largest miRNAs cluster, *DLK1-DIO3* imprinted domain is composed of 54 miRNAs, 11 lincRNAs, and several piRNAs

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and snoRNAs, and surging evidence has indicated that deregulation of these ncRNAs in this region is significantly associated with tumor progression.[1,6] Similarly, an amount of ncRNAs especially miRNAs is identified to be involved in urological cancers; besides, these miRNAs are partially transcribed from *DLK1-DIO3* imprinted domain.

In this review, we provide an overview of the representative ncRNAs at *DLK1-DIO3* imprinted domain and the regulation networks involved in urological cancers (RCC, BCa, and PCa).

DLK1-DIO3 imprinted domain

Genomic imprinting and ncRNAs at *DLK1-DIO3* imprinted domain

Genomic imprinting

Genomic imprinting is a critical epigenetics-regulating phenomenon that contributes to specific, monoallelic gene expression in diploid cells.[7] It has been a consensus that imprinted genes have profound effects on fetal development, placental biology, and controlling of the activities in neonates, such as the feeding, maintenance of body temperature, and regulation of metabolism. In addition, its regulatory values are gradually demonstrated in a wide range of common diseases, especially in obesity, diabetes mellitus, cancer, etc.[8] Nearly 150 imprinted genes have been identified in the mouse (data in MouseBook Imprinting Catalog), and ~50% of these genes have nearly also been detected in humans (data in Catalog of Parent of Origin Effects).[9] Furthermore, >80% of the known imprinted genes are characteristic of cluster, and about 2–15 genes which always contain two or more genes, and the size of each cluster vary from <100 kb to several megabases.[10] DLK1-DIO3 imprinted domain is one of the clustered genomic imprinting regions, which is located on distal mouse chromosome 12 and human chromosome 14.[11] Its critical regulatory role in physical development and several diseases are being uncovered step by step.

ncRNAs at DLK1-DIO3 imprinted domain

Currently, the paternally expressed genes *DLK1, RTL1, and DIO3*, and the maternally expressed ncRNAs *MEG3 (Gtl2), MEG8 (RIAN)*, and antisense *RTL1 (RTL1as)* are detected in this domain. Moreover, numerous sncRNAs also enrich the contents of this domain. So far, there are 53 miRNAs on the forward strand and one more (miR-1247) on the reverse strand, which consist of the largest human miRNAs cluster, another nine lncRNAs, of which six are on the forward strand (CTD-2561F5.1, RP11-168L7.3, RP11-909M7.2, AL132709.5, AL132709.7, and AL132709.8) and three on the reverse strand (AL132709.1, RP11-168L7.1, and DIO3OS), several snoRNAs downstream of *MEG3*, piRNAs, and several pseudogenes, which are all encoded at the *DLK1-DIO3* domain (Figure 1).

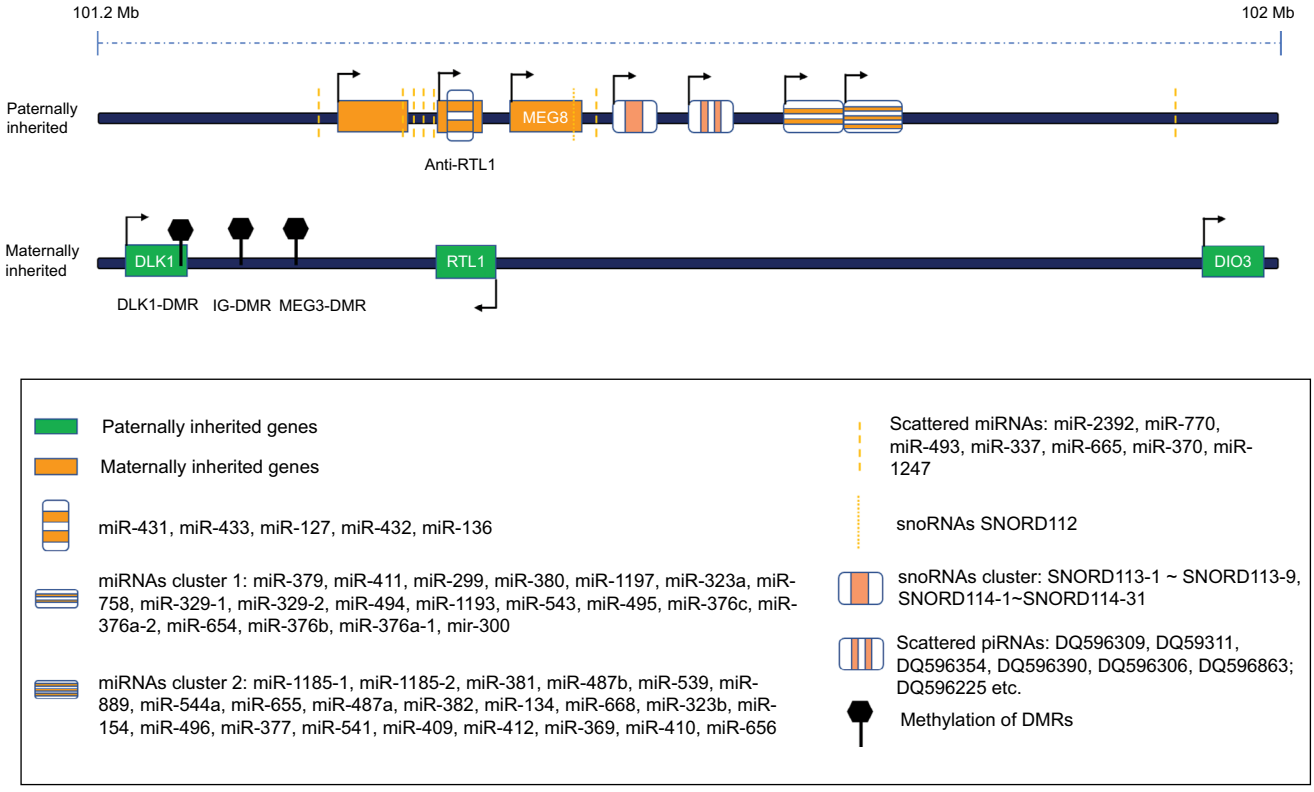

Figure 1 The schematic diagram of *DLK-DIO3* imprinted domain including multiple ncRNAs and upstream DMRs.
Abbreviation: DMR, differentially methylated region.

Regulations of *DLK1-DIO3* imprinted domain

Allele-specific expression of imprinted domain is mainly regulated by *ICRs*, most of which are *DMRs* in the *DLK1-DIO3* imprinted region. According to their origin, DMRs are classified as the *gDMRs* including *IG-DMR*, *H19-ICR, KvDMR1, Nespas-DMR*, and somatic DMRs including *GTL2-DMR*, and *DLK1-DMR*.[12,13] Three methylated DMRs: the *IG-DMR*, DLK1-DMR, and the *MEG3-DMR* are detected to regulate the *DLK1-DIO3* imprinted region in humans. Several critical regulatory proteins, histone modifications, and lncRNAs/miRNAs participate in the regulation of *DMRs* methylation.

Several critical regulatory proteins

Several critical regulatory proteins are involved in the process of genomic imprinting. DNA methyltransferases (DNMT3A/B) are dispensable for the establishment step with their catalytic functions. Besides, certain indirect regulatory proteins are also crucial for the maintenance of this domain. As a KRAB-contained zinc finger protein, ZFP57 is vital to maintain the parent-of-origin-specific epigenetic marks and expression of the imprinted gene clusters by selectively binding to the methylated motif (TGC5mCGC).[14–17] Another key protein KAP1 is reported to selectively maintain the stability of *DMRs* that are effective in *H19-ICR* and *H19* promoter DMR but do not function in *IG-DMR* and *Peg3-DMR*.[18] Multiple ways may exist for PGC7 in the regulation of DMRs. Previous study has indicated that PGC7 recognizes and binds H3K9me2 and corresponding chromatins to protect the methylation of the imprinted domains.[19] But recently, its N-terminal DNA-binding domain has been detected to interact with TET2 and TET3 to suppresses the enzymatic activity of these two proteins, thus partially protecting the methylation status of *DMRs*.[20] The regulation mediated by the PRC2 is still controversial. Conventional theory proposes that PRC2 serves as a maintenance of the repressor to control domain silencing via catalyzing H3K2me3, and a further RIP sequencing assay confirms that the 9,000 lncRNAs are involved in this regulation (such as *Xist, H19, Igf2, MEG3*); thus, at *DIO3-DLK1* imprinted domain, PRC2 can repress *DLK1* expression by interacting with *MEG3* lncRNAs.[21,22] However, a recent study provides a novel mechanism of *PRC2* in the regulation of the maternal *Gtl2-Rian-Mirg* locus, suggesting that PRC2 maintains expression of the locus by counteracting DNMT3 to prevent their recruitments and subsequent DNA methylation at the *IG-DMR*,[23] which indicates the complicated regulations of *DLK1-DIO3* imprinting domain. In terms of the erasure of imprinted domain, previous findings detect the partial erasing role of 5-methylcytosine oxidizing TET1 enzyme at some imprinted regions.[24–27] However, silencing of TET1 does not appear to upregulate the methylation status at DLK1-DIO3 DMR, but significantly upregulate the methylation status of H19 DMR in uES, dES and EB cell lines.[28]

Histone modifications

Histone methylation is usually interacted with DNA methylation.[29] Substantial methylations of H3K4 induced by the histone lysine demethylase KDM1B deficiency block the formation of *DMR* methylations during oogenesis.[30] In addition, variable histone modifications were detected in paternally and maternally methylated *ICRs* during the stages preceding the global histone-to-protamine exchange. H3-lysine-4 methylation and H3 acetylation are abundant at maternally methylated *ICRs*, but absent at paternally methylated *ICRs*.[31]

Regulatory role of lncRNAs/miRNAs

A recent in vitro experiment reveals that ~30 variable genes including *DLK1, DIO3,* and *MEG3* are detected upregulated in *HOTAIR*-targeted deletion mice, and silencing of *HOTAIR* disables the binding to the PRC2 and LSD1 complex, consequently causing H3K4me3 gain and H3K27me3 loss at Dlk1 imprinted gene to upregulate its expression; however, no significant alterations of the *IG-DMR* methylation status are detected.[32] Interestingly, both the miR-127 and the miR-136 of this domain are detected to target RTL1, which reveals that self-regulation mediated by miRNAs transcribed from this domain also exist. In addition, *PRC2 (RBAP48, EED, and HDAC2)* are found targeted by miR-495 and miR-323-3p of this domain, presenting a feedback regulatory loop in regulating all the genes and ncRNAs encoded by this *DLK1-DIO3* domain in full pluripotent stem cells.

Deregulations of *DLK1-DIO3* imprinted domain are involved in various diseases especially cancers

DLK1-DIO3 imprinted domain and diseases

It has been established that *DLK1-DIO3* imprinted domain is critical not only for the normal growth but also the cognitions and behaviors after birth. The dysregulations of this domain are associated with several rare cognitive disorders and even common diseases ranging from obesity, diabetes mellitus, psychiatric disorders, and cancers.[2,8,33] In terms of cancers, dysregulations of ncRNAs in this domain, especially miRNAs and lncRNAs, the paternally expressed genes *DLK1*, *RTL1*, and *DIO3*, and the domain regulatory factors such as the

modification enzymes and key proteins (such as KAP1) are all involved in the tumorigenesis of cancers, which indicates a complicated regulation network in tumor progression.

Mechanisms of the *DLK1-DIO3* imprinted domain in regulating cancers

Reviewing the previous studies, we found that disordered DMR regulations are the concentrated mechanisms to dysregulate the expression of certain mRNA or ncRNA expression, subsequently inducing cancers. Although many studies found that the imprinted genes are associated with cancers, however, mechanisms by which imprinting is regulated in cancers are poorly understood. Apart from the above complicated regulations of *DMRs* from variable approaches that are involved in carcinogenesis, other points are listed as follows.

Methylation status of *DMRs*

Previous studies have confirmed that the hypermethylation status of *DMRs* at the *DLK1-DIO3* imprinted domain significantly reduced the expression of certain tumor suppressor miRNA clusters in several cancers.[34–37] It has been proven that *MEG3* and *DLK1* are deregulated in urothelial cancer due to the epigenetic silencing including hypermethylation of *IG-DMRs* and *DLK1* promoter and repressive histone modifications across the 14q32 imprinted gene cluster.[38] Similarly, in BCa, hypermethylated status of *IG-DMR* is also found to be an inhibitor for the expression of miR-323a-3p that repressed the EMT progression.

LOI

LOI induced by abnormal methylation status or mutations of DMRs has been described to contribute to the carcinogenesis and cancer progression.[39–41] LOI is identified as an important risk factor for colorectal carcinogenesis, and similarly, LOI of different loci has been confirmed to promote the progression in oligodendrogliomas, breast cancer, and hepatocellular carcinomas.[42–45] Upregulated *IGF2* induced by LOI has been reported to be significantly associated with few tumors such as the rhabdomyosarcoma, adrenal cancer, colon cancer and PCa, squamous cell carcinoma of the head and neck.[46–50] Because of the structural similarity between *IGF2 and DLK1*, the LOI of *DMR* is also consistently detected at the *DLK1-GTL2* locus in embryonal rhabdomyosarcomas but erasure of imprinting in alveolar rhabdomyosarcoma, which leads to a higher *GTL2/DLK1* mRNA ratio in alveolar rhabdomyosarcoma.[46] And LOI at the *DLK1-MEG3* domain generates the widespread epigenetic instability through deregulated expression in human hepatocellular carcinoma.[51] In addition, genome-wide miRNA profiles suggest that LOI changes in the 14q32 noncoding region are helpful to define and classify the osteosarcoma subtypes; however, the precise regulation network relationship needs further investigations.[52]

Dysregulation of ncRNAs at the *DLK1-DIO3* imprinted domain contributes to the carcinogenesis of urological cancers

As previous findings describe, numerous miRNAs are distributed in cluster at *DLK1-DIO3* imprinted domain. Besides, dysregulation of these miRNA clusters due to abnormal DMR methylations is involved in several cancers including urological cancers (RCC, BCa, and PCa). ncRNAs composed of lncRNAs, miRNAs, piRNAs, and snoRNAs are the important epigenetic regulators in multiple cancers. So far, the largest miRNAs cluster is detected in this domain. In this section, we will review the dysregulated ncRNAs of this cluster at the *DLK1-DIO3* imprinting domain in urological cancers (Table 1) (Figure 2).

RCC

Dysregulations of several ncRNAs have been confirmed to be associated with RCC, especially the disordered miRNAs of the *DLK1-DIO3* imprinted domain. In this section, we review the associated ncRNAs of this domain in regulating RCC.

Previously, certain studies have found that miR-136 is downregulated in ccRCC, which suggests to be a novel biomarker. Overexpression of miR-136 significantly induced apoptosis and inhibited the proliferation and migration.[53–55] Similarly, Kaplan–Meier survival analysis reveals that upregulated miR-379 predicts a poor overall survival in pRCC.[56] A significantly repressed proliferation, migration, and induced apoptosis is observed in RCC cell (786-O and ACHN) with miR-411 mimics.[57] Eleven miRNAs are screened and identified to be associated with the tumor progression from ccRCC clinical tumor samples, among which miR-299-3 p is obviously downregulated.[58] Forced expression of miR-494 inhibits the survival of RCC cell accompanied by increased lipid droplets and mitochondrial changes.[59] The tumor suppressing effect of miR-495 on proliferation in RCC has been confirmed and can be repressed by *lncRNA UCA1* via RNA sponging effect.[60] miR-381 has been proven to be a WEE inhibitor suppressing the proliferation and inducing the sensitivity to 5-FU by upregulating CDC2 level in RCC cell line (786-O).[61,62] It has been reported that miR-134 inhibits the cell proliferation and EMT progression by directly targeting *KRAS*.[63] miR-377 was significantly downregulated in ccRCC samples and gain of

Table 1 Targets and phenotypes of ncRNA at the DLK1-DIO3 imprinted domain in urological cancers

Tumor type	ncRNAs	Expression pattern	Targets or pathways	Phenotypes	Prognostic value	References
RCC						
	miR-494	Downregulation	Lipid droplet formation and mitochondrial changes (↑)	Proliferation (↑) and apoptosis (↓)	–	59
	miR-411	Downregulation	–	Proliferation, migration (↑) and apoptosis (↓)	–	57
	miR-381	Downregulation	*WEE* (↑)	Proliferation and 5-FU resistance (↑)	–	61, 62
	miR-134	Downregulation	*KRAS* (↑)	Proliferation and EMT (↑)	–	63
	miR-377	Downregulation	*ETS1* (↑)	Proliferation and migration (↑)	–	64
	miR-136	Downregulation	–	Proliferation, migration (↑) and apoptosis (↓)	–	53–55
	miR-379	Downregulation	–	–	–	56
	miR-299-3p	Downregulation	–	–	–	58
	miR-495	Downregulation	RNA sponging: *lncRNA UCA1* (↑)	Proliferation (↑)	–	60
	MEG3	Downregulation	Mitochondrial pathway (↑)	Apoptosis (↓)	–	65
BCa						
	miR-409	Downregulation	*c-MET* (↑)	EMT (↑)	–	67
	miR-433	Downregulation	*c-MET* and *CREB1* (↑)	Proliferation and EMT (↑)	–	68
	miR-323a-3p	Downregulation	*c-MET* and *SMAD3* (↑)	EMT (↑)	–	37
	miR-379-5p	Downregulation	*MDM2* (↑)	Proliferation and EMT (↑)	–	69
	miR-495	Upregulation	*PTEN* (↓)	Proliferation and migration (↑)	Higher MiR-495 predicts a larger tumor size, advanced TNM stage, and lymph node metastasis	70
	miR-300	Downregulation	–	–	–	71
	MEG3	Downregulation	–	Autophagy (↓) and proliferation (↑)	Lower MEG3 predicts a poor recurrence-free survival	72, 73
PCa						
	miR-379	Upregulation	–	Bone metastasis (↑)	Higher MiR-379 predicts a poorer overall survival	80
	miR-409	Upregulation	–/*UGT2B17 UGT* (↓)	EMT/androgen bioavailability (↑)	–	86
	miR-543	Upregulation	*RKIP* (↓)	EMT (↑)	–	88
	miR-154	Downregulation	*CCND2*, *HMGA2*, and *E2F5* (↑)	Proliferation and EMT (↑)	–	76, 81, 82
	miR-376c	Downregulation	*UGT2B15* and *UGT2B17*(↑)	Androgen bioavailability (↑)	–	76, 86
	miR-495	Downregulation	*Akt* and *mTOR* (↑)	Growth and migration (↑)	–	76–78
	miR-432	Downregulation	*TRIM29* and *PYGO2* (↑)	G1/S phase arrest and apoptosis (↓)	–	79
	miR-323	Downregulation	Adiponectin receptor (↑)	Vessel formation (↑)	–	83
	miR-494	Downregulation	*CXCR4*/*survivin*/*UGT2B17 UGT* (↑)	Growth and metastasis/growth/androgen bioavailability (↑)	–	84, 85
	miR-539	Downregulation	*SPAG5* (↑)	Proliferation and metastasis (↑)	–	89
	miR-382	Downregulation	*COUP–TFII* (↑)	Proliferation and metastasis (↑)	–	90
	miR-134	Downregulation	*RAS* (↑)	Carcinogenesis (↑)	–	91
	miR-493-5p	Downregulation	*c-MET, CREB1, EGFR* (↑)	Proliferation and migration (↑)	–	94
	MEG3	Downregulation	–	G0/G1 phase arrest and apoptosis (↓)	–	95–98

Abbreviations: BCa, bladder cancer; ETS1, E26 transformation specific-1; PCa, prostate cancer; RCC, renal cell carcinoma.

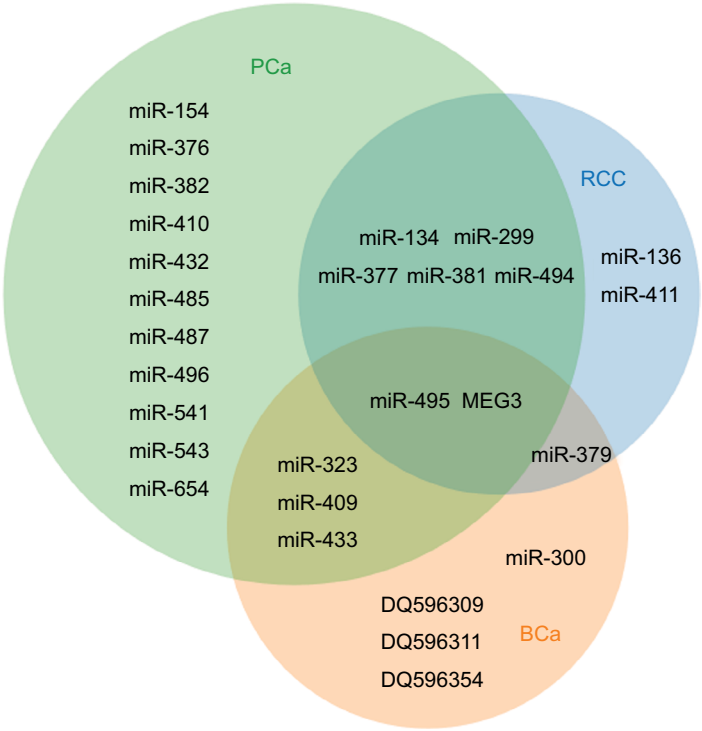

Figure 2 The common or specific ncRNAs in each class of urological cancers.
Abbreviations: BCa, bladder cancer; PCa, prostate cancer; RCC, renal cell carcinoma.

function further validates that miR-377 significantly inhibits the proliferation and migration by targeting E26 transformation-specific-1.[64] Apart from the regulation of various miRNAs in this region, lncRNAs also play a great role. Previous research has reported that 2.5% Wilms' tumor samples present a hypermethylation status in *MEG3-DMR*, which significantly inhibits the expression of tumor-suppressed *MEG3*, thus triggering the tumor progression.[65] Further study demonstrate that inhibition of *MEG3* repressed the targeted imprinted genes (*IGF2 in Wilms tumor and DLK1 in RCC*), and DLK1 is lost in 78% primary RCC tissues and identified as a critical tumor suppressor in the regulation of RCC.[65] A consistent function assay confirms that overexpression of *MEG3* induces the cell apoptosis via mitochondrial pathway in 786-0 cell lines. To conclude, *MEG3* plays a crucial tumor suppressor role in RCC.

Bladder cancer

Previously, it has been proven that 14q loss is common in invasive BCa, and two potential suppressor loci at 14q12 and 14q32.1–32.2 are detected.[66] Numerous ncRNAs especially miRNAs have been reported to be involved in tumorigenesis of BCa, several of which belong to *DLK1-DIO3* region. The associated ncRNAs of this domain in regulating BCa are reviewed in this section.

Forced expression of miR-409 is found to inhibit the EMT progression by directly targeting c-MET.[67] Hypermethylation of miR-433 promoter has been confirmed to contribute to the downregulation of miR-433 in BCa, and further experiment reveals that both *c-MET* and *CREB1* are the direct targets of miR-433; besides, MITF is detected as an intermediate protein induced by CREB1 to indirectly regulate c-MET/AKT/GSK-3β/SNAIL signaling, thus a complicated network mediated by miR-433 is established to regulate the proliferation and EMT progression of BCa.[68] Interestingly, miR-323a-3p in this region is also identified to regulate the EMT progression by targeting *c-MET* and *SMAD3*. In addition, SNAIL is the last confocal protein found to induce EMT, thus, miR-323a-3p/c-MET/SMAD3/SNAIL circuit is detected.[37] We find that miR-409, miR-433, miR-323a-3p all target *c-MET* to inhibit EMT of BCa. In addition, overexpression or silencing of c-MET represses or induces the expression of above miRNAs in turn. However, its specific mechanisms still remain elusive, and a critical network may be involved. The tumor-suppressive role of miR-379-5p in tumor proliferation and metastasis is demonstrated to target *MDM2*, and overexpression of MDM2 partially reverses the inhibitory effects of miR-379-5p, which further validates the direct interaction between miR-379-5p and MDM2.[69] A

recent study analyses the 67 pairs of BCa and adjacent normal bladder tissues to find miR-495 as an onco-miRNA that is upregulated in BCa tissues and overexpression of miR-495 promotes BCa progression via targeting *PTEN*, which is significantly negatively correlated with miR-495.[70] By utilizing miRNA microarray, researchers detected the expression pattern of miRNAs in normal tissues (5 samples) and BCa (30 samples), and found that miR-300 is downregulated in all six groups (grade I, grade II, grade III, grade I+II+III, infiltrating, and noninfiltrating group) and further confirmed by q-RT-PCR in BCa cell line T24.[71] Similar to RCC, MEG3 also plays a tumor suppressor role in BCa progression. Previous function study proves that downregulation of *MEG3* induces autophagy but contributes to cell proliferation in BCa.[72] A recent lncRNAs doublecheck screening between 80 BCa with matched adjacent normal tissues, and 220 BCa patients' serum samples, suggests that the expression of *MEG3* is negatively correlated with recurrence-free survival by Kaplan–Meier analysis, which proposes that *MEG3* is recurrence-independent prognostic factor.[73] In terms of the mechanisms of dysregulation of *MEG3*, both hypermethylation of *IG-DMRs* and *DLK1* promoter and repressive histone modifications contribute to the loss or downregulation of *MEG3* and *DLK1*. Accumulating evidence has confirmed that piRNAs inhibit the target gene transcription or translation in cancer progression via targeting DNA sequence with piRNA/PIWI complex or targeting 3′-UTR with RNA-induced silencing complex. One hundred thirty-eight piRNAs are detected at the *DLK1-DIO3* imprinted domain. Recent study reports that seven piRNAs are differentially expressed and predict the patient outcome of lung cancer combined with several miRNAs in this domain. However, currently, no study refers to piRNAs of this domain in BCa progression.[74] However, a set of BCa piRNA microarray data have been reported, by analyzing the data, we found that three piRNAs transcribed from the *DLK-DIO3* domain, DQ596309 (fold change =17.2, P=0.017), DQ596354 (fold change =6.2, P=0.027), and DQ596311 (fold change =5.3, P=0.031) are downregulated in BCa,[74] which needs a further investigation of function and mechanisms. To conclude, piRNAs in this domain may serve as critical potential tumor regulators in BCa tumorigenicity.

Prostate cancer

In this section, we conduct a whole review of ncRNAs in the regulation of PCa. Previous study has conducted a systematic screening about the miRNA expression at 14q32.31 in four cell lines (normal prostate epithelial cells, three PCa cell lines DU-145, PC3, and LNCaP), and the results found that almost all miRNAs in this region are downregulated except for miR-656 in PC3 cell line.[75,76] In addition, after the treatment of AZA and the histone deacetylase inhibitor trichostatin, an obvious elevation of above miRNA cluster is detected, which indicates a potential epigenetic silencing results in the deregulations of miRNAs in this region. Further analysis of the expression pattern of these miRNAs (miR-154, miR-299-5 p, miR-376a, miR-376c, miR-377, miR-381, miR-487b, miR-485-3 p, miR-495, and miR-654-3 p) correlates with clinical and pathological variables.[76] Circulating miR-433 and miR-495 from PCa serum samples present their capability in differentiating indolent (low-risk) and aggressive (high-risk) PCa, and the results suggest that in high-risk PCa, circulating miR-433 is upregulated but miR-495 is downregulated.[77] In addition, another study reports that AKT and mTOR can be downregulated by miR-495 to inhibit the growth and migration.[78] miR-432 upregulated by LncRNA625 has been found to inhibit PCa cell proliferation by targeting *TRIM29* and *PYGO2* to regulate Wnt/β-catenin pathway.[79] Elevated miR-154-3 p and miR-379 is observed to promote PCa bone metastasis, and its expression level is significantly negatively correlated with progression-free survival of PCa patients.[80] However, several studies have confirmed that miR-154 inhibits proliferation via targeting *CCND2* and inhibits EMT progression via *HMGA2*.[81,82] E2F5 can also be downregulated by miR-154 to reduce the proliferation and migration,[82] which present the multitargets of miRNAs in the regulation of PCa. miR-323a is found to be upregulated in PCa tissues and overexpression of miR-323 increases the VEGF-A level and the vessel formation by targeting *adiponectin receptor*.[83] Downregulation of *CXCR4* mediated by miR-494 significantly represses the PCa growth and metastasis, which may be a potential therapeutic target for the treatment of PCa.[84] Synergistic effects are obtained after cotransfecting miR-494 (survivin targeted) with survivin short hairpin RNA to inhibit the growth of PC3 cell line.[85] miRNAs in this domain are also associated with androgen bioavailability. UGT is defined as a critical modulation factor to control androgen bioavailability, and interestingly, miR-409 and miR-494 are detected to target *UGT2B17 UGT* and miR-376c target both *UGT2B15* and *UGT2B17*.[86] Besides, ectopic expression of miR-409 induced by stromal fibroblast promotes the tumor induction and EMT.[87] miR-543 has been identified a EMT promotor in PCa LNCAP and C4-2B cell lines via directly downregulating Raf kinase inhibitory protein.[88] Downregulation of *SPAG5* induced by miR-539 drastically blocked PCa progressions, which maybe a therapeutic target.[89] miR-382 also shows its tumor suppressor role in cell proliferation

and metastasis by targeting the chicken ovalbumin upstream promoter transcription factor II.[90] miR-134, an RAS-targeted miRNA, has been reported to be repressed with other several miRNAs by inorganic arsenic during the transformation from the human prostate epithelial line to CAsE-PE and from the derivative normal stem cell line to As-cancer stem cell line.[91] Recently, a meta-analysis of PCa miRNA expression profiles suggest that 22 miRNAs including miR-496 and miR-541 are downregulated in recurrent PCa samples compared with nonrecurrent PCa samples, which may provide new candidate biomarkers for predicating recurrent PCa.[92] A study enrolling 149 PCa patients and 178 controls indicates that circulating miR-410 is drastically upregulated in PCa patients and may serve as a specific diagnostic biomarker of PCa (area under curve of 0.8097 (95% CI, 0.7371–0.8823; $P<0.001$).[93] Recently, miR-493-5p is found to be dysregulated by the hypermethylation of its promotor, and ectopic expression of miR-493-5p targets *c-MET*, *CREB1*, and *EGFR* to inhibit cell proliferation and migration via downregulating AKT/GSK-3β/SNAIL signaling.[94] Interestingly, *MEG3* is also involved in the progression of PCa. Previous analysis of 14 microarray has indicated that *MEG3* combined with eight other imprinted genes are downregulated, which was further validated by q-RT-PCR assay.[95,96] A function study demonstrates that forced expression of *MEG3* significantly induces G0/G1 phase arrest and apoptosis of PCa cell.[97] One of the critical mechanism of LncRNA is RNA sponge effect that functions as a ceRNA. And numerous imbalanced ceRNA pairs are found to be involved in the progression of PCa, among which loss of *MEG3-AQP3* ceRNA pair is identified as having potential prognostic value.[98]

Conclusion

To conclude, accumulating evidence has implicated the significant role of ncRNAs at the *DLK1-DIO3* imprinted domain in the regulation of urological cancers tumorigenicity. From the above review, we find that miRNAs and lncRNAs are the dominant regulators among all regulatory factors, while studies on the piRNAs and snoRNAs in urological cancers are still few. Besides, the regulatory mechanisms are especially complicated, which indicates that the potential networks may exist in the whole progression and needs further investigations.

Throughout the review, we propose that several individual concepts or problems should be solved. 1) We review three common cancers of urology; however, the functions of the same miRNA may differ in different cancers. For example, miR-409 is a tumor suppressor in BCa, but it promotes the EMT progression in PCa, which indicate the tumor specificity and the regulation complexities of this domain. 2) In terms of the dysregulation of ncRNAs in this region, we find that hypermethylation of *DMRs* mainly accounts for this phenomenon of urological cancers. It is the same condition of the normal physiological process that *DMRs* are hypermethylated after paternal transmission and hypomethylated after maternal transmission in somatic cells. In addition, *IG-DMR* appears at the control of methylation pattern of the imprinting regulator of both paternally and maternally expressed genes, ie, of the secondary *MEG3-DMR* on the maternally transmitted chromosome.[99,100] Recently, MEF2A was found to induce the expression of miRNAs in *DLK-DIO3* involved in skeletal muscle regeneration via binding to *MEG3* promoter.[101,102] The molecular signaling network may contribute to carcinogenesis and tumor progression. We find that miR-409, miR-433, and miR-323a-3p own the common target *c-MET*, and c-MET can also negatively modulate the expression of these miRNAs in BCa. Consistently, *c-MET* is also one of the targets of miR-493-5 p; however, whether c-MET can similarly regulate miR-493-3 p has yet to be elucidated. Interestingly, Kumar et al identify a negative feedback loop between miR-493 and IGF1R via SNAIL in head and neck cancers, which means that miR-493 can target IGF1R/AKT/GSK-3β/NF-KB/SNAIL to inhibit SNAIL on the one hand, while on the other hand, SNAIL can bind to the promoter of miR-493 to inhibit the expression of miR-493 in turn.[103] Concerning that miR-493, miR-409, miR-433, and miR-323a-3p are located at the close positions of *DLK1-DIO3* imprinted domain, which gives us a hint as to whether c-MET can consistently inhibit the expression of miR-409/miR-433/miR-323a-3p by inducing SNAIL to bind to the promoter region of them. Thus, miRNAs/c-MET/SNAIL negative feedback loop maybe involved in the *DLK1-DIO3* imprinted domain of urological cancers; however, above concepts may need more studies to demonstrate. 3) We find most of the miRNAs in this region of PCa show downregulation, which indicates the tumor suppressor role. We wonder if these are potential therapeutic targets that can be activated by certain relatively mature genomic technique. 4) A large number of miRNAs have been detected; however, more concentrations need to be attached to the studies of snoRNAs and piRNAs, which may play a great role in the regulation of the whole imprinted region. 5) Although numerous ncRNAs and their functions have been confirmed, it is still a puzzle as to how the expression pattern and the epigenetic regulations are involved in urological cancers. No doubt *DLK1-DIO3* presents a significant effect on the progressions of cancers including urological cancer,

but it is still a long way to explore the profound mechanism and clinical therapeutic values in the future.

Abbreviations

AZA, 5-aza-2′-deoxycytidine; BCa, bladder cancer; ccRCC, clear cell renal cell carcinoma; ceRNA, competing endogenous RNAs; CAsE-PE, chronic arsenic-exposed prostate epithelial; DMR, differentially methylated region; EMT, epithelial–mesenchymal transition; ETS1, E26 transformation specific-1; gDMRs, germline DMRs; IG-DMR, intergenic DMR; ICR, imprinting control region; IGF2, insulin-like growth factor 2; LOI, loss of imprinting; lncRNAs, long noncoding RNAs; miRNAs, microRNAs; ncRNAs, noncoding RNAs; PCa, prostate cancer; piRNA, PIWI-interacting RNA; PRC2, polycomb repressive complex 2; pRCC, papillary renal cell carcinoma; RCC, renal cell carcinoma; RIP, RNA immunoprecipitation; sncRNA, small noncoding RNA; snoRNAs, small nucleolar RNAs; UGT, UDP-glucuronosyltransferases conjugating enzymes.

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Disclosure

The authors report no conflicts of interest in this work.

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