

The microRNAs within the DLK1-DIO3 genomic region: involvement in disease pathogenesis

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Abstract The mammalian genome is transcribed in a developmentally regulated manner, generating RNA strands ranging from long to short non-coding RNA (ncRNAs). NcRNAs generated by intergenic sequences and protein-coding loci, represent up to 98 % of the human transcriptome. Non-coding transcripts comprise short ncRNAs such as microRNAs, piwi-interacting RNAs, small nucleolar RNAs and long intergenic RNAs, most of which exercise a strictly controlled negative regulation of expression of protein-coding genes. In humans, the DLK1-DIO3 genomic region, located on human chromosome 14 (14q32) contains the paternally expressed imprinted genes DLK1, RTL1, and DIO3 and the maternally expressed imprinted genes MEG3 (Gtl2), MEG8 (RIAN), and anti-sense RTL1 (asRTL1). This region hosts, in addition to two

long intergenic RNAs, the MEG3 and MEG8, one of the largest microRNA clusters in the genome, with 53 miRNAs in the forward strand and one (mir-1247) in the reverse strand. Many of these miRNAs are differentially expressed in several pathologic processes and various cancers. A better understanding of the pathophysiologic importance of the DLK1-DIO3 domain-containing microRNA cluster may contribute to innovative therapeutic strategies in a range of diseases. Here we present an in-depth review of this vital genomic region, and examine the role the microRNAs of this region may play in controlling tissue homeostasis and in the pathogenesis of some human diseases, mostly cancer, when aberrantly expressed. The potential clinical implications of this data are also discussed.

Keywords DLK1-DIO3 · Long non-coding RNA · microRNAs · Cancer · Signaling pathways · Transcriptional regulatory pathways

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Introduction

The mammalian genome is transcribed in a developmentally regulated manner, generating RNA transcripts ranging from long to short non-coding RNA (ncRNAs). NcRNAs represent up to 98 % of the human transcriptome and can be considered as a molecular marker in the evolution of complex organisms, since there is an association between organism complexity and non-coding transcripts [1]. Additionally, recent work has implicated ncRNAs in many aspects of gene regulation and various cellular processes [2]. The ncRNAs can be generated by both intergenic sequences and protein-coding loci [3] and are arbitrarily subdivided in long ncRNAs (lncRNAs) (longer than 200 nucleotides) and short ncRNAs (<200 nucleotides long).

Short non-coding RNAs include microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs) and other categories [4]. Short ncRNAs are involved in the regulation of protein biosynthesis, removal of intronic sequences, telomere synthesis, transcription control and posttranscriptional gene silencing [5]. SnoRNAs are highly conserved, intermediate-sized RNAs (60–300 nucleotides long) that are predominantly found in the nucleus [6]. Two major classes of snoRNAs have been identified, each possessing distinctive, evolutionary conserved sequence elements: the C/D box group and the H/ACA box group. SnoRNAs are components of the small nucleolar ribonucleoproteins (snoRNPs) and are involved in the post-transcriptional modification of rRNAs and some spliceosomal RNAs [7]. A large number of lncRNAs have also been identified and constitute the largest portion of the mammalian non-coding transcriptome. The biological role of lncRNAs as a class remains largely elusive, however several of them have been shown to be involved in the regulation of the epigenetic machinery by recruiting DNA methyltransferases, histone modifying enzymes, and chromatin remodeling complexes to their sites of action in specific cells at specific stages of differentiation [8]. LncRNAs are also involved in genomic imprinting [9]. Long intergenic RNAs (lincRNAs), and others with enhancer-like functions were described only recently [10, 11]. LincRNAs are transcripts that have been generated from intergenic regions with a specific chromatin signature. This signature consists of a short stretch of trimethylation of histone protein H3 at the lysine in position 4 (H3K4me3), which corresponds to promoter regions, followed by a longer stretch of trimethylation of histone H3 at the lysine in position 36 (H3K36me3), which covers the entire transcribed region, also known as “K4–K36 signature” [10]. In primary and metastatic tumors, lincRNAs have been found to be differentially expressed, and this aberrant expression was also found to be a powerful predictor of disease outcome [12, 13]. LncRNAs overlapping or antisense to protein-coding gene promoters may also control the expression of their cognate protein-coding genes through epigenetic modifications contributing to carcinogenesis [2]. ncRNAs can function either *in cis* or *in trans*. *Trans*-acting function of ncRNAs is associated with both lincRNAs and short ncRNAs, while *cis*-acting functions are associated with long ncRNAs. In fact, the majority of imprinted lncRNAs serve as host transcripts for *trans*-acting microRNAs (miRNAs) [9, 14].

microRNA biology

MiRNAs are the best-studied class of ncRNAs. MiRNAs are single-stranded RNAs of approximately 22 nucleotides

long that act as gene regulators at a post-transcription level. As part of a protein-to-RNA complex known as RISC or miRNP, they provide sequence specificity and by pairing with their target mRNAs anywhere along the length commonly but not only in the 3′ untranslated region leading mainly to translational repression [15]. Occasionally some miRNAs, such as miR369-3 can also activate translation of target mRNAs on cell cycle arrest [16]. While miRNAs’ loci are predominantly found in non-coding and intronic regions of the genome, recent work has identified miRNAs encoded from within protein-coding exons [17]. Additionally, miRNAs are sometimes arranged as clusters with the multiple members of the cluster being transcribed simultaneously [18]. MiRNAs’ biogenesis is a multistep process involving a complex circuit of proteins; the biogenesis typically requires the enzymes Drosha, DGCR8 and Dicer, although recently alternative pathways have also been described [19, 20].

MiRNAs have been shown to regulate complex cell signaling networks [21] in the context of different diseases including cancers [22, 23]. A widespread down-regulation of miRNAs is generally observed in human neoplasias [24]. On a related note, many miRNAs are located in cancer-associated genomic regions or in regions of chromosomal instability that are frequently affected in human cancer, suggesting potential roles in the onset and progression of cancer [25]. Deregulated miRNAs expression profiles have been observed in both solid and blood cancers [26–31] and can distinguish among human cancers according to the developmental lineage and differentiation status of the cancer [32].

The DLK1-DIO3 genomic region

DLK1 and *MEG3* (*Gtl2* in mouse) are imprinted genes located on mouse chromosome 12 and on human chromosome 14 (Fig. 1), which are paternally and maternally expressed, respectively [33]. In humans, the DLK1-DIO3 genomic region contains the paternally expressed genes *DLK1*, *RTL1*, and *DIO3*, and, the maternally expressed genes *MEG3* (*Gtl2*), *MEG8* (*RIAN*), and antisense *RTL1* (*RTL1as*) [34].

This region has undergone important changes during the course of evolution, the most important being the acquisition of the Imprinting Control Region (ICR). In the process, the genes of this region have acquired their own distinct and important functions. For example, *RTL1* has evolved into a new gene with important functions in growth and development, while miRNAs and snoRNAs also evolved in this specific region. Finally, the intron size of *DLK1* was reduced and the entire imprinted cluster gained a sub-telomeric chromosomal position in eutherians [35]. Two distinct, differentially methylated regions (DMR) are

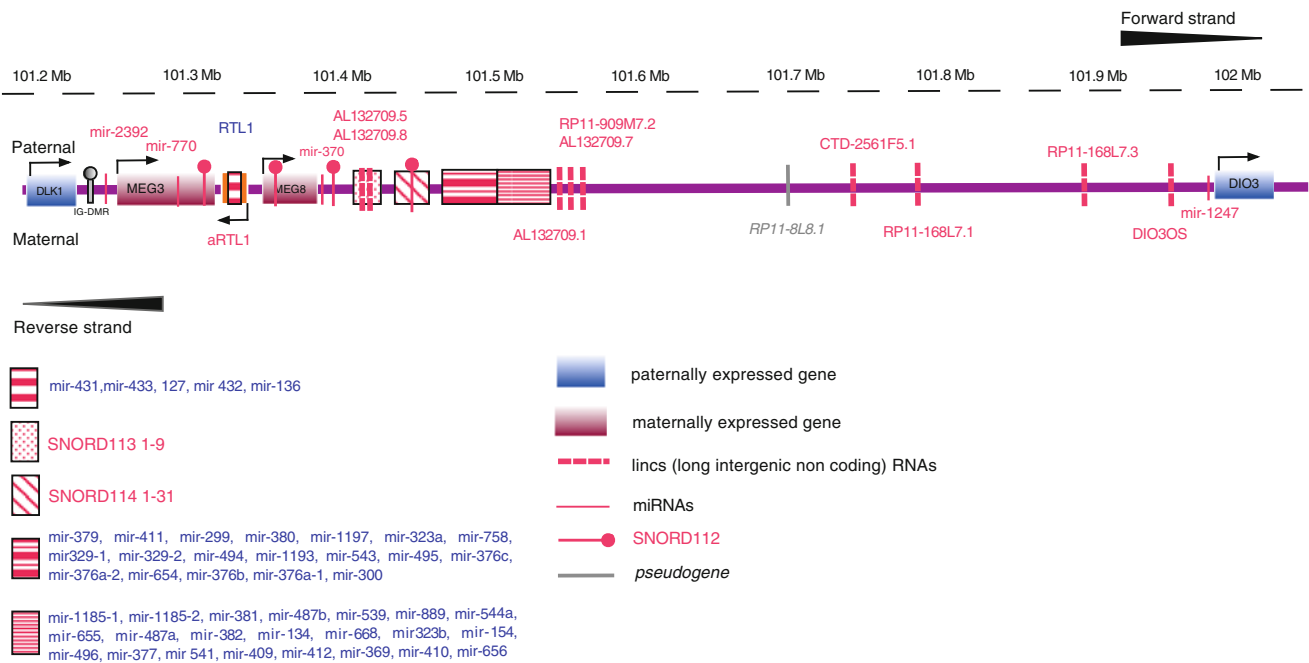


Fig. 1 Schematic representation of the DLK1-DIO3 genomic region in the parental and maternal chromosomes. Genes, miRNAs, lincRNAs, and pseudogenes are noted

present in the region and have separate functional properties: a primary, germline-derived DLK1-MEG3 intergenic DMR (IG-DMR) and a secondary, post-fertilization-derived MEG3-DMR. Both DMRs are hyper-methylated after paternal transmission and hypo-methylated after maternal transmission in somatic cells. Functionally, IG-DMR seems to be at the top of the hierarchy and controls the methylation pattern of the imprinting regulator of both paternally and maternally expressed genes, i.e., of the secondary MEG3-DMR on the maternally transmitted chromosome [36] (see also Fig. 1). Both MEG3 and MEG8 are lincRNAs.

DLK1 is a member of the Notch signaling pathway and is involved in cell differentiation of several tissues [37]. RTL1 is on the opposite strand from DLK1 and is a retrotransposon-like gene. RTL1 is essential for the normal development of placental and embryonic tissues whereas DIO3 encodes a type 3-iodothyronine deiodinase protecting developing tissues from excessive amounts of thyroid hormone [37].

This region also hosts 53 miRNAs (forward strand) and one more (mir-1247) on the reverse strand. This cluster of 54 miRNAs is the largest one currently known in the human genome (Fig. 1; Table 1). MiR-2392 and miR-770 are in the DLK1-MEG3 region whereas the rest of the miRNAs are located downstream of MEG3 (Fig. 1; Table 1). The region downstream of MEG3 also includes many snoRNAs, and in particular one, nine, and 31 paralogous copies of *SNORD112* [14q(0)], *SNORD113* [14q(I)] and *SNORD114* [14q(II)], respectively. In addition to *MEG3* and *MEG8*,

nine more lincRNAs are found in the DLK1-DIO3 locus, six of which are on the forward strand (AL132709.5, AL132709.7, AL132709.8, CTD-2561F5.1, RP11-168L7.3, RP11-909M7.2) and three on the reverse strand (RP11-168L7.1, AL132709.1 and DIO3OS). A pseudogene, *RP11-8L8.1* and a spliceosomal small nuclear RNA, U3 also lie in the reverse strand of DLK1-DIO3 locus (ENSEMBL release 67).

MEG3 is currently the most studied transcript of the region, however its function remains poorly understood. It is believed to be involved in both physiological and pathological processes of cell biology, to possess tumor suppressor properties [38], and to exert anti-proliferative function through interactions with p53 and MDM2, and also by regulating directly or indirectly the phosphorylation of Rb [39]. MEG3 also interacts with crucial signaling pathways involved in angiogenesis, cell proliferation and differentiation, survival regulation, and has been found deregulated in several types of tumors [39].

Recent studies in knock-out mice attempted to delineate the function of some of the components of DLK1-DIO3 region. Takahashi et al. [40] produced *Gtl2*-KO mutants harboring a deletion of exons 1–5 including *Gtl2*-DMR in an effort to gain a better understanding of the function of maternally expressed ncRNAs in this domain. They observed that the deletion of the *Gtl2* imprinted ncRNA with its DMR induced parental origin-dependent lethality in these mutants. Zhou et al. [41] also generated a knockout mouse model, in which the first five exons and adjacent

Table 1 miRNAs located in the DLK1-DIO3 region (MiRBase v. 18) (<http://www.mirbase.org/>), the predicted pathways that are involved in, GO terms analysis and also diseases that published work has shown they have been implicated in (PubMed search)

| microRNA (strand) | Predicted pathways involved | GO TERMS | Disease | References |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| miR-127-5p (+) | Type II diabetes mellitus, renal cell carcinoma, Toll-like receptor signaling pathway, natural killer cell-mediated cytotoxicity, T cell receptor signaling pathway, JAK-STAT signaling pathway | M band, ER-to-Golgi transport, regulation of microtubule depolymerization, negative regulation of microtubule depolymerization, calcium | AML, lymphomas, squamous esophageal carcinoma, colorectal Ca, hepatocellular carcinoma, ovarian Ca, cervical carcinoma, oligodendrogliomas, lung cancer, breast cancer, renal interstitial fibrosis, prostate, osteosarcoma, papillary thyroid carcinoma, bladder Ca, medulloblastoma, atherosclerosis, muscular dystrophy, gastric Ca | [73, 74, 77, 79–82, 85, 95, 97, 99, 101–103, 105, 110–119] |
| miR-134 (+) | Focal adhesion, endometrial cancer, prostate cancer, adherens junction | Cytoplasmic microtubule organization, regulation of microtubule depolymerization, dendrite development, response to axon injury | Lymphoma, tongue squamous carcinoma, esophageal squamous carcinoma, gastric cancer, colorectal Ca, GISTs, endometrial carcinomas, oligodendrogliomas, glioblastomas, lung Ca, osteosarcomas, fragile-X mental retardation, lupus nephritis, coronary artery disease, acute pulmonary embolism, mastocytosis | [68, 74–77, 84, 95, 110, 113, 120–125] |
| miR-136-5p (+) | Viral myocarditis, non-small cell lung cancer, glioma, ECM receptor interaction, prostate cancer, neurotrophin signaling pathway, adherens junction, small cell lung cancer, focal adhesion, tight junction, pathways in cancer | Substrate adhesion-dependent cell spreading, microfilament motor activity, actin filament-based movement, MAP kinase activity, cell–cell adhesion junction | Leukemia, glioblastomas, lung neoplasms, heart failure, muscular dystrophy, pheochromocytomas | [90, 97, 104, 118, 126, 127] |
| miR-154-5p (+) | Viral myocarditis, prostate cancer, ECM–receptor interaction, ABC transporters, glioma, adherens junction, pancreatic cancer, small cell lung cancer, focal adhesion, pathways in cancer, regulation of actin cytoskeleton | Protein phosphatase 2A binding, M band, myosin filament, microfilament motor activity, A band | AML, tongue squamous carcinoma, esophageal Ca, colorectal Ca, hepatocellular carcinoma, ovarian Ca, endometrial carcinomas, breast Ca, muscular disorders, salivary gland pleomorphic adenomas | [73, 74, 77, 80, 82, 84, 124, 128, 129] |
| miR-299-5p (+) | Notch signaling pathway, ECM–receptor interaction, regulation of actin cytoskeleton, focal adhesion | Dendrite morphogenesis, integrin complex, neuromuscular process controlling balance, basal plasma membrane, cerebellum development | Heart failure, muscular disorders AML, salivary gland adenomas, gastric Ca, colorectal Ca, ovarian Ca, lung Ca, breast Ca | [73, 75, 77, 95, 100, 126, 129, 130] |
| miR-300 (+) | Citrate cycle, regulation of actin cytoskeleton, focal adhesion, pathways in cancer | Protein phosphatase 2A binding, phospholipid-translocating ATPase activity, aminophospholipid transporter activity, extracellular glutamate-gated ion channel activity, A band | Heart failure, ovarian Ca | [126, 131] |

Table 1 continued

| microRNA (strand) | Predicted pathways involved | GO TERMS | Disease | References |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|
| miR-323a-5p (+) | Endometrial cancer, ECM–receptor interaction, ABC transporters, small cell lung cancer, non-small cell lung cancer | Microfilament motor activity, fascia adherens, regulation of mitochondrial membrane permeability, Rac GTPase activator activity, sodium channel complex | AML, colorectal cancer, traumatic brain injury, papillary thyroid carcinoma, medulloblastoma, renal interstitial fibrosis | [73, 77, 103, 114, 116, 132] |
| miR-323b-5p (+) | ABC transporters, endometrial cancer, ECM–receptor interaction, arrhythmogenic right ventricular cardiomyopathy (ARVC), viral myocarditis | Intercalated disc, neuromuscular junction, microfilament motor activity, phospholipid-translocating ATPase activity, aminophospholipid transporter activity | | |
| miR-329 (+) | Pancreatic cancer, nuclear receptors in lipid metabolism and toxicity, progesterone-mediated oocyte maturation, pathways in cancer | Guanyl-nucleotide exchange factor activity, DNA replication, small GTPase regulator activity, nucleoside-triphosphatase regulator activity, GTPase regulator activity | GISTs, lung Ca | [76, 95] |
| miR-337-5p (+) | Beta-alanine metabolism, pathogenic Escherichia coli infection, butanoate metabolism | Apolipoprotein binding, neuromuscular process controlling balance, positive regulation of neurological system process, lamellipodium, microtubule-based movement | CLL, salivary gland pleomorphic adenomas, gastric Ca, colorectal Ca, ovarian Ca, lung Ca, breast Ca, prion disease | [77, 82, 97, 101, 102, 129, 133–135] |
| miR-369-5p (+) | Ribosome, intestinal immune network for IgA production | Myosin II complex, immune response-regulating cell surface receptor signaling pathway, myosin complex, cytosolic ribosome, actin binding | Gastric Ca, endometrial carcinosarcomas, renal interstitial fibrosis | [75, 84, 103] |
| miR-370 (+) | Role of Erk5 in neuronal survival, EPO signaling pathway, endometrial cancer, ECM–receptor interaction, arrhythmogenic right ventricular cardiomyopathy (ARVC) | Establishment or maintenance of epithelial cell apical/basal polarity, microfilament motor activity, actin filament-based movement, actin cytoskeleton reorganization, basal plasma membrane | AML, squamous esophageal carcinoma, gastric Ca, GISTs, colorectal Ca, cholangiocarcinoma, endometrial carcinosarcomas, prion disease, breast Ca, coronary artery disease | [73] [70, 74, 76, 77, 84, 123, 128, 135, 136], |
| miR-376a-5p (+) | Role of ERBB2 in signal transduction and oncology, long-term potentiation, Alzheimer's disease | Photoreceptor outer segment, regulation of microtubule depolymerization, negative regulation of microtubule depolymerization, negative regulation of microtubule polymerization or depolymerization, microfilament motor activity | Squamous esophageal carcinoma, GISTs, pancreatic Ca, endometrial carcinosarcomas, traumatic brain injury, lung Ca, breast Ca, pleomorphic adenomas of the salivary gland, primary pigmented nodular adrenocortical disease, medulloblastoma | [74, 76, 84, 95, 97, 101, 102, 116, 129, 132, 137, 138] |

Table 1 continued

| microRNA (strand) | Predicted pathways involved | GO TERMS | Disease | References |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|
| miR-376b (+) | Human cytomegalovirus and map kinase pathways, purine metabolism | Human cytomegalovirus and map kinase pathways, 3',5'-cyclic-nucleotide phosphodiesterase activity, cyclic-nucleotide phosphodiesterase activity, endocytic vesicle membrane, phosphoric diester hydrolase activity | Primary pigmented nodular adrenocortical disease, ovarian Ca, breast Ca | [82, 100, 137] |
| miR-376c (+) | 81.Structure_of_Caps_and_SMACs, neuroactive ligand-receptor interaction | Guanyl-nucleotide exchange factor activity, regulation of small GTPase mediated signal transduction, GTPase regulator activity, nucleoside-triphosphatase regulator activity, ion binding | AML, GISTs, colorectal Ca, ovarian Ca, lung Ca, renal interstitial fibrosis, osteosarcoma, hepatocellular carcinoma, chronic hepatitis, muscular disorders, primary pigmented nodular adrenocortical disease, muscular dystrophy, medulloblastoma | [72, 73, 76, 77, 83, 95, 103, 115, 118, 130, 137, 139] |
| miR-377-5p (+) | Primary immunodeficiency, Rho cell motility signaling pathway, endometrial cancer, apoptosis, focal adhesion | Histone methyltransferase activity (H3-K4 specific), smooth muscle tissue development, voltage-gated sodium channel complex, sodium channel complex, stereocilium | Gastric Ca, ovarian Ca, lung Ca, mesothelioma, breast Ca, diabetic nephropathy, bladder Ca, heart failure | [82, 98, 100, 105, 126, 134, 140, 141] |
| miR-379-5p (+) | ECM-receptor interaction, cytosolic DNA-sensing pathway, focal adhesion, natural killer cell mediated cytotoxicity | Detection of mechanical stimulus involved in sensory perception of sound, inner ear receptor stereocilium organization, transforming growth factor beta binding, detection of mechanical stimulus involved in sensory perception, endochondral ossification | Gastric Ca, glioblastomas, traumatic brain injury, lung Ca, renal interstitial fibrosis, muscular disorders, medulloblastoma hepatocellular carcinoma | [75, 90, 95, 97, 103, 116, 130, 132, 136, 142] |
| miR-380-5p (+) | Role of PPAR-gamma coactivators in obesity and thermogenesis, growth hormone signaling pathway, ECM-receptor interaction, Jak-STAT signaling pathway, axon guidance | Auditory receptor cell differentiation, inner ear receptor cell differentiation, I band, microtubule basal body, Z disc | Neuroblastoma | [89] |
| miR-381 (+) | Sonic Hedgehog (Shh) pathway, Hedgehog signaling pathway, neurotrophin signaling pathway, focal adhesion, calcium signaling pathway | Regulation of cholesterol storage, dendritic spine, Rho guanyl-nucleotide exchange factor activity, Ras guanyl-nucleotide exchange factor activity, regulation of Rho protein signal transduction | Mastocytosis, ovarian Ca, gliomas, heart failure, muscular disorders, autism | [68, 82, 88, 126, 130, 143] |
| miR-382-5p (+) | Inositol phosphate metabolism, ECM-receptor interaction, Focal adhesion, Regulation of actin cytoskeleton | Gamma-tubulin complex, negative regulation of translational initiation, cortical actin cytoskeleton, positive regulation of multicellular organism growth, cellular component maintenance | AML, ovarian Ca, glioblastomas, lung Ca, breast Ca, renal interstitial fibrosis, osteosarcoma, heart failure, massive macronodular adrenocortical disease, HIV infections | [72, 82, 90, 95, 103, 111, 115, 126, 144, 145] |

Table 1 continued

| microRNA (strand) | Predicted pathways involved | GO TERMS | Disease | References |
|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| miR-409-5p (+) | 99.NF- κ B_activation, type II diabetes mellitus, Apoptosis | Cytoskeletal anchoring at plasma membrane, regulation of microtubule depolymerization, negative regulation of microtubule depolymerization, negative regulation of microtubule polymerization or depolymerization, lateral plasma membrane | Salivary gland pleomorphic adenomas, medulloblastoma, gastric Ca, GISTs, ovarian Ca, glioblastomas, oligodendrogliomas, lung Ca, breast Ca | [75, 76, 82, 95, 99, 113, 116, 119, 129] |
| miR-410 (+) | Focal adhesion | Ligand-gated ion channel activity, ligand-gated channel activity, apoptosis, programmed cell death, cell death | Gastric Ca, ovarian Ca, lung Ca, pheochromocytomas, androgenetic alopecia | [75, 82, 97, 104, 146] |
| miR-411-5p (+) | Actions of nitric oxide in the heart, induction of apoptosis through DR3 and DR4/5 death receptors, small cell lung cancer, cell adhesion molecules (CAMs), ubiquitin mediated proteolysis | Anaphase-promoting complex, cell-matrix adhesion, cell-substrate adhesion, amine transport, cation channel activity | Preeclampsia, glioblastomas, breast Ca | [90, 111, 147] |
| miR-412 (+) | Keratan sulfate biosynthesis, ECM-receptor interaction, viral myocarditis, B cell receptor signaling pathway, prostate cancer | Vascular endothelial growth factor receptor activity, laminin complex, microfilament motor activity, regulation of embryonic development, sodium channel complex | Lung Ca, breast Ca | [128, 146] |
| miR-431-5p (+) | ABC transporters, ECM-receptor interaction, Inositol phosphate metabolism, control of gene expression by vitamin D receptor, viral myocarditis | Uropod, trailing edge, cytoskeletal anchoring at plasma membrane, laminin complex, metalloenzyme inhibitor activity | Hepatocellular carcinoma, lung Ca, malignant pheochromocytomas | [80, 97, 104] |
| miR-432-5p (+) | ECM-receptor interaction, ABC transporters, non-small cell lung cancer, small cell lung cancer, focal adhesion | Sodium channel complex, voltage-gated sodium channel complex, microfilament motor activity, regulation of Cdc42 protein signal transduction, regulation of Cdc42 GTPase activity | Squamous esophageal carcinoma, schizophrenia, lung Ca, osteosarcoma, heart failure, muscular disorders, autism | [74, 95, 122, 126, 130, 143, 148] |
| miR-433 (+) | Adherens junction, focal adhesion, prostate cancer, tight junction, regulation of actin cytoskeleton | Lamellar body, voltage-gated sodium channel complex, microfilament motor activity, sodium channel complex, intercalated disc | Ovarian Ca, gastric Ca, mesothelioma, breast Ca, traumatic brain injury, lupus nephritis, adenoviral infections, X-linked chondrodysplasia | [75, 82, 98, 121, 128, 132, 136, 141, 149, 150] |
| miR-485-p (+) | 79.B_cell_activation, ABC transporters, arrhythmic right ventricular cardiomyopathy (ARVC), axon guidance, ECM-receptor interaction | Voltage-gated sodium channel complex, sodium channel complex, pronucleus, voltage-gated sodium channel activity, negative regulation of actin filament depolymerization | ALL, ovarian adenocarcinoma, traumatic brain injury, lung Ca, mesothelioma, breast cancer, renal interstitial fibrosis, endometriosis | [69, 95, 98, 101-103, 111, 128, 132, 151, 152] |
| miR-487a (+) | Toll-like receptor signaling pathway, RIG-I-like receptor signaling pathway | Nuclear export, nucleocytoplasmic transport, nuclear transport, alkali metal ion binding, induction of apoptosis | - | - |

Table 1 continued

| microRNA (strand) | Predicted pathways involved | GO TERMS | Disease | References |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|
| miR-487b (+) | CD40L signaling pathway, EGF signaling pathway, signal transduction through IL1R, angiotensin II-mediated activation of JNK pathway via Pyk2 dependent signaling, keratinocyte differentiation | Activation of MAPKK activity, positive regulation of nitric oxide biosynthetic process, SH2 domain binding, regulation of nitric oxide biosynthetic process, histone acetyltransferase activity | Glioblastomas, lung Ca, osteosarcomas, muscular disorders | [90, 95, 122, 130] |
| miR-493-5p (+) | ECM-receptor interaction, focal adhesion, small cell lung cancer, regulation of actin cytoskeleton, pathways in cancer | Smoothed signaling pathway, Rho guanyl-nucleotide exchange factor activity, Ras guanyl-nucleotide exchange factor activity, regulation of Rho protein signal transduction, basement membrane | Esophageal Ca, renal interstitial fibrosis, male breast Ca | [74, 103, 128] |
| miR-494 (+) | AKT signaling pathway, non-small cell lung cancer, ErbB signaling pathway, viral myocarditis, chronic myeloid leukemia | Regulation of cytokine biosynthetic process, positive regulation of cell differentiation, positive regulation of developmental process | Lymphomas, squamous esophageal carcinoma, gastric Ca, colorectal Ca, cholangiocarcinoma, lung Ca, breast Ca, bladder Ca, heart failure, lupus nephritis, WM, prion disease, intestinal-type gastric Ca, medulloblastoma, cervical carcinoma | [61, 71, 74, 75, 79, 95, 97, 101, 102, 105, 116, 121, 122, 153–156] |
| miR-495 (+) | Phosphatidylinositol signaling system, ECM-receptor interaction, progesterone-mediated oocyte maturation, GnRH signaling pathway, oocyte meiosis | Focal adhesion, cell-substrate adherens junction, cell-substrate junction, adherens junction, anchoring junction | Gastric Ca, glioblastomas, traumatic brain injury, lung Ca, breast Ca, bladder Ca, muscular disorders, salivary gland adenomas, medulloblastoma | [75] [90, 95, 99, 105, 116, 129, 130, 132] |
| miR-496 (+) | Cytosolic DNA-sensing pathway, Small cell lung cancer, ECM-receptor interaction, focal adhesion, natural killer cell mediated cytotoxicity | Catenin complex, negative regulation of lymphocyte differentiation, cell-cell adherens junction, DNA-dependent ATPase activity, gliogenesis | Gastric cancer, fetal alcohol syndrome | [75, 87] |
| miR-539 (+) | Pathways in cancer | Tumor necrosis factor binding, gamma-tubulin complex, regulation of interleukin-2 biosynthetic process, basal plasma membrane, hindlimb morphogenesis | Mastocytosis, autism, medulloblastoma | [68, 116, 143] |
| miR-541-5p (+) | Apoptosis, calcium signaling pathway | Lamin binding, protein export from nucleus, cell-cell adherens junction, dendrite development, negative regulation of protein complex disassembly | Pheochromocytomas | [104] |
| miR-543 (+) | Cytokine-cytokine receptor interaction, axon guidance, nuclear receptors in lipid metabolism and toxicity, dilated cardiomyopathy, neuroactive ligand-receptor interaction | Transmembrane receptor protein tyrosine kinase activator activity, protein tyrosine kinase activator activity, kinase activator activity, receptor activator activity, protein kinase activator activity | Glioblastomas, mesothelioma | [90, 98] |

Table 1 continued

| microRNA (strand) | Predicted pathways involved | GO TERMS | Disease | References |
|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|------------|
| miR-544a (+) | Hypertrophic cardiomyopathy (HCM), viral myocarditis, NOD-like receptor signaling pathway, dilated cardiomyopathy, epithelial cell signaling in <i>Helicobacter pylori</i> infection | Negative regulation of muscle cell apoptosis, muscle filament sliding, actin-myosin filament sliding, actin-mediated cell contraction, myosin filament | Breast Ca | [99, 128] |
| miR-654-5p (+) | Keratan sulfate biosynthesis, thyroid cancer, dorso-ventral axis formation, nuclear receptors in lipid metabolism and toxicity, ABC transporters | Plasma membrane part, cell junction, cytoskeleton, cytoskeletal protein binding, metal ion binding | Lupus nephritis, lung Ca | [95, 121] |
| miR-655 (+) | – | Helicase activity, purine NTP-dependent helicase activity, ATP-dependent helicase activity, stem cell differentiation | Adenoviral infections | [149] |
| miR-656 (+) | Role of Ran in mitotic spindle regulation, mismatch repair, eicosanoid metabolism | Cytosolic calcium ion homeostasis | Gastric Ca | [134] |
| miR-665 (+) | Multi-drug resistance factors, hypoxia and p53 in the cardiovascular system, ABC transporters, ECM–receptor interaction, non-small cell lung cancer | Transforming growth factor beta binding, cell-substrate junction assembly, patterning of blood vessels, collagen, regulation of mitotic metaphase/anaphase transition | GISTs | [76] |
| miR-668 (+) | ECM-receptor interaction, focal adhesion, calcium signaling pathway | Apoptotic cell clearance, calcium-transporting ATPase activity, calcium ion transmembrane transporter activity, regulation of dendrite development, regulation of dendrite morphogenesis | – | – |
| miR-758 (+) | ECM-receptor interaction, amyotrophic lateral sclerosis (ALS), viral myocarditis, focal adhesion, prostate cancer | <i>N</i> -methyl-D-aspartate selective glutamate receptor complex, endoplasmic reticulum calcium ion homeostasis, ionotropic glutamate receptor signaling pathway, suckling behavior, ionotropic glutamate receptor complex | Esophageal carcinoma | [153] |
| miR-770-5p (+) | ECM-receptor interaction, focal adhesion, ABC transporters, phosphatidylinositol signaling system, glioma | Actin-dependent ATPase activity, microfilament motor activity, sodium channel complex, spectrin, voltage-gated sodium channel complex | Glioblastomas | [90] |
| miR-889 (+) | – | – | Glioblastomas | [90] |
| miR-1185-5p (+) | Calcium signaling pathway, regulation of actin cytoskeleton | Calmodulin-dependent cyclic-nucleotide phosphodiesterase activity, homotypic cell–cell adhesion, Golgi-to-endosome transport, positive regulation of cell–matrix adhesion, organic anion transport | – | – |
| miR-1185-2 (+) | – | – | – | – |

Table 1 continued

| microRNA (strand) | Predicted pathways involved | GO TERMS | Disease | References |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|------------|
| miR-1193 (+) | ABC transporters, control of gene expression by vitamin D receptor, ECM-receptor interaction, nuclear receptors in lipid metabolism and toxicity, IL-2 receptor beta chain in T cell activation | Sodium channel complex, voltage-gated sodium channel complex, ionotropic glutamate receptor signaling pathway, cytoplasmic microtubule, voltage-gated sodium channel activity | - | |
| miR-1197 (+) | Heparan sulfate biosynthesis, ECM-receptor interaction, arrhythmogenic right ventricular cardiomyopathy (ARVC), dilated cardiomyopathy, hypertrophic cardiomyopathy (HCM) | Heparan sulfate proteoglycan biosynthetic process, collagen, clathrin-coated vesicle membrane, extracellular matrix part, extracellular matrix structural constituent | - | |
| miR-1247-5p (-) | Regulation of actin cytoskeleton, calcium signaling pathway | 1-phosphatidylinositol-4-phosphate 5-kinase activity, photoreceptor inner segment, sulfate transport, A band, negative regulation of angiogenesis | Adenoviral infections | [149] |

MIRNA targets were predicted using RNA22 (MIRBase v. 18, ENSEMBL release 65) [108]. Prediction of pathways and GO terms analysis was performed using DAVID 6.7 (<http://david.abcc.ncifcrf.gov/home.jsp>) [109], with default settings and EASE 0.01 (in case there were no results, EASE 0.1 was allowed). Only the top 5 results based on fold enrichment are listed for both predicted pathways and GO terms GO gene ontology. *CLL* chronic lymphocytic leukemia, *GISTs* gastrointestinal stromal tumors, *AML* acute myeloid leukemia, *WM* Waldenström's macroglobulinemia, *ALL* acute lymphoblastic leukemia, *Ct* cancer

promoter region of the *Gtl2* gene were deleted. In this study, maternal deletion of *Gtl2* resulted in perinatal death and skeletal muscle defects, completely abolished expression of downstream maternally expressed genes, activated expression of silenced paternally expressed genes and resulted in the methylation of the IG-DMR. By contrast, the paternally inherited deletion did not demonstrate such an effect. Such findings indicate that *Gtl2* plays an important role in embryonic development and in regulating *Dlk1-Gtl2* imprinting [41]. Gordon et al. [42] used *Meg3* knockout mice in order to identify targets and potential functions of this gene in embryonic development and tumorigenesis. They observed differences in signaling pathways and ontologies related to angiogenesis between wild-type and knock-out embryos. In addition, *MEG3*-null embryos were shown to have increased expression of vascular endothelial growth factor (VEGF) pathway genes and increased cortical microvessel density, suggesting important roles for *Meg3* in vascularization in the brain and in tumor suppression partly through angiogenesis inhibition [42].

The degree of activation of the *DLK1-DIO3* region has also been found to correlate with pluripotency levels of mouse stem cells. It has been found activated in fully pluripotent mouse stem cells and repressed in partially pluripotent cells. Additionally, several miRNAs from this cluster potentially target components of the Polycomb repressive complex 2 (PRC2) and therefore may form a feedback regulatory loop resulting in the expression of all genes and non-coding RNAs encoded by this region in full pluripotent stem cells [43].

It has been demonstrated that *MEG3* is under-expressed in hepatocellular carcinoma cells compared to normal hepatocytes or adjacent cirrhotic tissue, due to altered DNA methylation, which is consistently found in several types of cancer [39, 44]. Transfection of miR-134, which was one of the significantly downregulated miRNAs, resulted in cell cycle arrest in the human pituitary-derived cell line (PDFS). Below, we examine the function of these miRNAs in disease etiology and possible implications as a result of their proximity to the imprinted *MEG3* lncRNA.

DLK1-DIO3 clustered microRNAs biology and involvement in disease pathogenesis

The clustered miRNAs of the DLK1-DIO3 genomic region

As already mentioned, the *DLK1-DIO3* genomic region contains 54 miRNAs and represents one of the largest miRNA-containing clusters of the human genome. In mouse, some of these miRNAs are expressed as products of a large non-coding transcript named *Mirg* [45, 46]. Hagan et al. [47] have reported the presence of at least ten genes in

the Dlk1-Meg3 mouse imprinted domain and cloned for the first time a novel RNA named Irm (Imprinted RNA near Meg3); Irm partially overlaps Rian (the mouse homologue of Meg8), raising questions about the *in vivo* existence of Rian based on their results [47].

MiR-127 and miR-433, which belong to the same cluster, are transcribed from independent promoters in overlapping genomic regions in a 5' → 3' direction [48], and are under a common transcriptional regulatory mechanism among different mammalian species depending on the transcription factors nuclear estrogen-related receptor gamma (ERR γ) and small heterodimer partner (SHP) [49, 50]. As both miRNAs possess a conserved gene structure, it seems that the miR-433/127 loci might have evolved from an ancient common origin in mammals and are now transcribed from two separate promoters among species.

Biology and functions of the DLK1-DIO3 region miRNAs

Collective data suggest that the miR-127/136 cluster has a potential role in the silencing of the Rtl gene. Seitz et al. [51] originally showed that miR-127 and miR-136 display perfect complementarities with the Rtl mRNA [51]; subsequently, it was shown that silencing of the miR-127/136 cluster correlated with an increase in the expression of Rtl1. Moreover, in knock-out mice lacking expression of miR-127/136, an increased expression of Rtl1 was noted [52]. Study of the expression levels of miR-127 and miR-136 in fertilized mouse embryos, parthenotes, androgonotes, and cloned embryos developing *in vitro*, showed the two miRNAs to be highly expressed in parthenotes, but rarely in androgonotes. The Rtl1 promoter region was hyper-methylated in blastocyst stage parthenotes and its methylation decreased during development. Conversely, the promoter region of miR-127 was hypo-methylated in parthenogenetically activated embryos but heavily methylated in cloned blastocysts, suggesting a role for miR-127 and miR-136 in the epigenetic reprogramming of the Rtl1 imprinting process [53]. Moreover, Seitz et al. [51] suggested that miR-136 also acts as a small interfering RNA silencing Rtl1 expression in a mouse model.

MiR-134 is specifically expressed in the brain and controls dendritic spine formation *in vitro*. It also regulates the expression of CREB through a post-transcriptional mechanism and thus is linked to synaptic plasticity. MiR-134 over-expression in specific brain areas of mutant mice contributed to the impaired long-term memory formation in the contextual fear-conditioning paradigm, negatively regulating synaptic plasticity through translational inhibition of target genes such as CREB in mice [54]. MiR-134 also acts through inhibition of the translation of Lim-domain-containing-protein-kinase 1 (Limk1) responsible for spine development, thus indicating miRNA are

important players in the maturation process of the mammalian brain [55]. MiR-134 expression increased in mouse embryonic stem cells (mESC) after treatment with retinoic acid. It also significantly reduced the promoter activity of LRH1, Sox2, Oct4, and Nanog promoters, which are known essential transcription factors for the maintenance and differentiation of embryonic stem cells status, acting at a post-transcription control level [15, 56]. MiR-134 could modulate mESC differentiation irrespectively of Oct4 and Nanog, and together with miR-470 could regulate pluripotency, self-renewal, and differentiation of stem cells [15, 56].

MiR-495 was the only miRNA that presented a similar expression in prefrontal and parietal cortex and exhibited laminar specificity in human prefrontal cortex [57]. MiR-541 over-expression negatively affected cell proliferation and differentiation, neurite outgrowth, synapse formation and modulated expression levels of the target mRNA synapsin I, conferring a role in neurocyte differentiation and axon maintenance [58]. Fiore et al. [59] demonstrated that the entire miR-379/410 cluster is most likely transcribed as single polycistronic unit, and is co-regulated by neuronal activity in a Mef 2-dependent manner. In particular, miR-329, miR-381, and miR-134 promoted dendritogenesis and dendritic growth in hippocampal neurons by down-regulating the RNA-binding protein Pum2 [59]. MiR-495 expression was also observed in embryonic liver and pancreas cells where it represses the expression of the HNF-6/Onecut transcription factor, suggesting a role in liver and pancreas development [60]. MiR-494 behaves as a pro-apoptotic post-transcriptional nucleolin regulator, thus probably possessing tumor suppressor properties, and competes with the RNA-binding protein HuR involved in nucleolin expression, therefore modulating cell proliferation and survival [61].

A subset of the miRNAs located in the DLK1-DIO3 genomic region, including miR-134, miR-154, miR-299, miR-323, miR-337, miR-370, and miR-376c, have been shown to be differentially expressed during human and mouse lung development [62]. MiRNAs of this particular domain are also involved in erythropoiesis [63]. In CD34+ cells derived from umbilical cord blood, miR-127, miR-136, miR-154, and miR-379 exhibited deregulated expression throughout erythropoiesis showing lineage commitment [63]. MiR-376a negatively modulated early human erythropoiesis in the K562 cell line with the ability to erythroid differentiation [64]. Enforced expression of miR-376a suppressed the hemin-induced erythropoiesis by repressing the erythroid differentiation of CD34+ stem/progenitor cells, while inhibition of the aforementioned miRNA promoted erythroid differentiation [64]. Lastly, miR-376a over-expression also negatively regulated Argonaute 2 (Ago2) and cyclin-dependent kinase (CDK) 2,

two factors known to affect erythropoiesis, and to result in the arrest of erythroid differentiation [64]. MiR-377 in combination with miR-217 are involved in the regulation of the expression of the heme oxygenase-1 enzyme, which metabolizes the excess heme generated during hemolysis [65].

DLK1-DIO3 region miRNAs in disease pathogenesis

In vitro Teferedegne et al. [66], using microarray-expression and qRT-PCR assays, found that increases in the expression of miR-376a, miR-543, miR-654-3p, miR-382 correlated with the acquisition of tumorigenic phenotypes in cell lines of non-human primates. In another *in vitro* study, it was found that the overexpressed p16^{ink4a} down-regulated CDK1 through modulation of miR-410 expression [67]. It also seems that miR-410 expression is under the negative control of the E2F4 transcription factor, and its expression may increase in response to p16^{ink4a} overexpression and consequent inhibition of the Rb/E2F pathway [67].

In recent animal model studies, it was reported that miRNAs jointly with KIT and the microphthalmia-associated transcription factor (MITF) comprise a novel regulatory pathway in mast cell transformation [68]. Among the miRNAs whose expression is repressed by KIT activation are miR-134, miR-539, and miR-381. Also, retroviral expression of miR-539 and miR-381 resulted in decreased expression of MITF, suggesting that KIT indirectly modulates MITF protein levels through regulation of these particular miRNAs [68].

MiR-485-3p was significantly under-expressed in the human lymphoblastic leukemia teniposide-resistant cell line compared to the sensitive counterpart. Decreased miR-485-3p expression inversely correlated to the NF-Y transcription factor increased expression, which in turn was accompanied by down-regulation of DNA topoisomerase II α [69]. In cholangiocarcinoma cell lines, miR-370 and miR-494 were found down-regulated [70, 71].

Blood cancers Li et al. [72], conducted a genome-wide miRNA expression analysis in 52 acute myeloid leukemia (AML) samples with common translocations, including t(8;21)/AML1(RUNX1)-ETO(RUNX1T1), inv(16)/CBFB-MYH11, t(15;17)/PML-RARA, and MLL rearrangements. Several miRNAs were differentially expressed and clustered in the various AML subtypes. Among them, miR-382 was found over-expressed almost exclusively in leukemias harboring the t(15;17) translocation. Moreover miR-382 and miR-376c, along with miR-224, were found to represent a signature of t(15;17) AML with a very high diagnostic accuracy, suggesting their involvement in the pathogenesis of acute promyelocytic leukemia (APL).

Similar findings were also reported by another group who performed quantitative expression profile analysis of 157 mature miRNAs on 100 AML patients of a whole spectrum of known karyotypes common in AML [73]. The study identified a set of miRNAs, all of which located in the DLK1-MEG3 cluster, to be significantly associated with APL cases. The set comprised the up-regulated miR-127, miR-154, miR-370, miR-299, miR-376c and miR-323, which suggests that miRNAs hosted in the DLK1-MEG3 domain are highly specific for the APL subtype.

Solid cancers Serum miRNA levels have been evaluated in esophageal squamous cell carcinoma and at least nine miRNAs of the DLK1-MEG3 cluster (miR-127-3p, miR-134, miR-154, miR-370, miR-376a, miR-493, miR-494, miR-432, and miR-665) were found deregulated. MiR-127-3p was among a panel of seven serum miRNAs found up-regulated in the blood of patients with esophageal squamous cell carcinoma (ESCC) and could classify esophageal squamous cell carcinoma [74]. Several other miRNAs of the cluster have been implicated in the pathogenesis and progression of gastric cancer. In particular, miR-495, miR-134, miR-409-3p, miR-496, miR-379, miR-369-3p are linked to the tumor depth of invasion [75], while miR-376a expression was associated with nodal metastasis [75]. MiR-494 expression was found to correlate significantly with both staging variables [75]. Peritoneal dissemination was also associated with the deregulated expression of miR-299-5p, miR-409-3p, and miR-410. Moreover, miR-495, miR-433, and miR-410 predicted both disease-free survival (DFS) in gastric cancer patients and overall survival (OS) [75]. Such findings further emphasize the potential for miRNAs to serve as prognostic markers in cancer [31, 75].

In a recent study on gastrointestinal stromal tumors (GIST), Haller et al. [76] profiled miRNA expression for a relatively small series of GIST samples. They found 32 of 734 studied miRNAs to be differentially expressed according to localization and mutation status. Overexpression of miR-376c, miR-376a, miR-370, miR-409-3p, miR-665, and miR-329 was found to be correlated with the presence of *KIT* or *PDGFR* mutations and also gastric or intestinal localization. Moreover, decreased expression of miR-134 and miR-370 was associated with loss of the 14q chromosomal region. Although the single 14q loss does not confer shorter DFS in GISTs, it seems that low expression of miR-370 and miR-134 is associated with tumor progression and a shorter DFS irrespective of the 14q status [76].

In colon cancer, miR-154-3p, miR-323, miR-134, miR-376c, and miR-337 were all down-regulated in colorectal cancer cell lines and patient samples, probably indicating specific biological markers for such type of tumors [77].

On the contrary, miR-134 was not differentially expressed in the plasma of colorectal cancer patients compared to the control subjects [78]. MiR-494 had the highest fold change among over-expressed miRNAs in colorectal cancers whereas miR-127-3p was significantly over-expressed in colorectal cancer samples harboring *KRAS* mutations compared to wild-type *KRAS*, known downstream target of the EGFR signaling pathway [79].

Regarding hepatocellular carcinoma, Luk et al. [80], using a TRE-c-Met-driven transgenic HCC mouse model identified a cluster of 23 miRNAs that is encoded within the *Dlk1-Gtl2* imprinted region on chromosome 12qF1 overexpressed in all of the isolated liver tumors. Further to that, they examined the expression the miRNA cluster of the DLK1-DIO3 on chromosome 14q32.2, which corresponds to *Dlk1-Gtl2* imprinted region in mouse, in a cohort of 97 hepatitis B virus-associated HCC patients. Interestingly, overexpression of the DLK1-DIO3 miRNA cluster was positively correlated with HCC stem cell markers (CD133, CD90, EpCAM, Nestin) and associated with a high level of serum alpha-fetoprotein, a conventional biomarker for liver cancer, and poor survival rate in HCC patients [80]. The data are also supported by a rat model study showing that down-regulation of expression of miR-127 and consequent decreased apoptosis rates resulted in the formation of hepatocellular carcinoma. The expression of miR-127 was not restored in methyl re-fed rats, suggesting a non-methylation-dependent control mechanism, and also correlated with the different stages of rat hepatocarcinogenesis [81].

Recently, Shih et al. [82] evaluated the prognostic value of 470 miRNAs in advanced serous ovarian cancer. They found 29 miRNAs that were associated with disease outcome. Interestingly, 11 of the 29 miRNAs were located in the DLK1-MEG3 cluster, and included miR-433, miR-337, miR-127, miR-410, miR-381, miR-377, miR-299-3p, miR-409-3p, miR-154, miR-382, and miR-376b. Among them, three miRNAs (miR-337, miR-410, and miR-645) were negatively associated with overall survival. In another study, overexpression of miR-376c in ovarian cancer cells was found to block cisplatin-induced cell death: patients with serous ovarian cancer who responded well to chemotherapy had low miR-376c expression whereas chemotherapy resistant patients had high levels of miR-376c [83]. The investigators suggested that miR-376c enhances proliferation, survival, and chemoresistance by targeting, at least in part, activin receptor-like kinase 7 (ALK7) [83].

MiR-370, miR-369-5p, miR-154, miR-376a, and miR-134 were all up-regulated in the sarcomatous component of endometrial carcinosarcomas compared to the epithelial component, and could represent a potential miRNAs signature, for distinguishing epithelial-to-mesenchymal

transdifferentiation in this type of tumor [84]. In invasive cervical cancer, miR-127 and miR-134 were found significantly over-expressed compared with normal samples and miR-127 was significantly associated with lymph node metastasis making it a good candidate as a prognostic biomarker [85]. Zhang et al. [86] also showed that down-regulation of the chromosome 14 miRNA cluster is an event common to many human epithelial tumors and suggested that eight miRNAs (mir-337, mir-432, mir-495, mir-368, mir-376a, mir-376b, mir-377, and mir-419) located in DLK1-DIO3 domain could play a role as tumor suppressor genes in solid tumors.

MiRNAs clustered in the DLK1-DIO3 locus are also involved in central nervous system diseases [87]. MiR-381 has been found to play a major role in human glioma progression through suppression of the tumor suppressor *LRRC4*, which is associated with decreased inhibition of MEK/ERK and AKT signaling [88]. MiR-380-5p has been found to be developmentally restricted, characteristic of undifferentiated cells, also expressed through neuronal specification, and possess oncogenic properties in vivo through interaction with H-RAS, p53, and p21^{waf1} [89]. Inhibition of miR-380-5p in embryonic stem cells leads to up-regulation of p53 enhancing apoptosis whereas ectopic expression of the specific miRNA results in direct translation suppression of p53. MiR-380-5p expression was high in primary human neuroblastoma tumors, suppressed p53 expression, and predicted a poor outcome [89]. By high-throughput sequencing, nine miRNAs were found down-regulated in glioblastomas compared to normal brain tissues and included miR-495, miR-543, miR-770-5p, miR-379, miR-487b, miR-889, miR-382, miR-136, and miR-411-3p [90]. On the contrary, no miRNAs of the specific cluster were found up-regulated. Again, some of these miRNAs demonstrated 5'-end structural modifications reflecting fluctuations in strand dominance and likely processing variations [90]. Recently, differential expression of a set of 17 miRNAs was found to discriminate high-risk from low-risk neuroblastoma [91]. Notably, a total of 15 of the 17 miRNAs belonged to the imprinted human 14q32.31 miRNA cluster and two, miR-487b and miR-410, were significantly down-regulated in the high-risk group. Multivariate analysis showed that expression of miR-487b and miR-410 carried a predictive value beyond the classical high-/low-risk stratification and therefore could be a potential biomarker of relapse in favorable neuroblastoma [91]. The silencing of 18 genes, including 13 miRNAs at the DLK1-MEG3 locus has also been reported in human clinically non-functioning pituitary adenomas [92].

In human bronchial epithelial cell lines, miR-494 was shown to target and regulate PTEN. Overexpression of miR-494 led to a decreased expression of PTEN resulting in attenuated apoptosis, and also directly promoted colony

formation acting as an oncomir [93]. Similarly, over-expressed levels of miR-494 were observed in primary murine bronchial epithelial cells exposed to the carcinogen benzo[a]pyrene, and resulted in G1 arrest of the cell cycle [94].

Guo et al. [95] evaluated the expression of 856 miRNAs and approximately 22,000 genes using miRNA microarray and cDNA microarray in sensitive (NCI-H69) and resistant to chemotherapy cell lines (NCI-H69AR) of small cell lung carcinoma (SCLC). They found 24 up-regulated and 37 down-regulated miRNAs. Among the miRNAs in the latter group, miR-134, miR-299, miR-329, miR-376a, miR-376c, miR-379, miR-382, miR-409, miR-485, miR-487b, miR-494, miR-495, miR-654, miR-127, miR-432, were found to be down-regulated with a concomitant modulation of the expression of their target mRNA [95]. In addition, miR-495 was found significantly up-regulated in *K-RAS* mutated lung adenocarcinomas compared to healthy lung tissue [96]. A novel 10 over-expressed miRNAs signature in murine lung cancers has been recently reported [97]. Interestingly, seven out of the 10 miRNAs belong to the DLK1-DIO3 domain and included miR-136, miR-376a, miR-337, miR-410, miR-127, miR-379, and miR-431. However, in human non-small cell lung cancers only miR-136 and miR-376a were over-expressed in contrast to murine [97]. MiR-377, miR-433, miR-543 and miR-485 have been identified in malignant mesothelioma differentially expressed in each histopathological subtype [98].

Five miRNAs including miR-485-5p, miR-544, miR-409-3p, miR-127, and miR-495 were up-regulated in two different breast cancer stem cell sub-populations. Most interestingly, miR-495 was the only one over-expressed in both breast cancer stem cell sub-populations evaluated while the remaining four were unique to each sub-population. MiR-495 has been suggested to promote colony formation, tumor growth, and cancer cell invasion through downregulation of E-cadherin expression [99]. It has also been shown to inhibit *REDD1* and therefore promote cancer cell proliferation under hypoxia [99]. MiR-495 expression is under the positive control of the transcription factors E12 and E47 involved in the cell cycle regulation of the hematopoietic stem cells [99]. Three distinct miRNAs signatures have been proposed in order to differentiate estrogen receptor, progesterone receptor, and HER2/Neu breast cancer. In all three instances, miRNAs of the DLK1-DIO3 cluster were identified, including miR-299-3p, miR-377, and miR-376b, respectively [100]. MiR-127 and miR-376a were found down-regulated in breast cancer samples compared to adjacent normal tissues, while miR-127-3p presented higher expression in basal cells than luminal cells and was significantly elevated in malignant myoepithelioma compared to luminal and basal-like cancer subtypes. Differentially expressed miRNAs in basal cells

and luminal cells included miR-337-3p, miR-485-3p, and miR-494 [101, 102].

In an effort to better understand the development of renal interstitial fibrosis, Kriegel et al. [103] evaluated the association of *TGF β 1* and miRNAs expression. Half of the up-regulated miRNAs after treatment with *TGF β 1* belonged to the DLK1-DIO3 domain such as miR-127-5p, miR-493, miR-376c, miR-379, miR-323-3p, miR-382, miR485-3p, and miR-369, while no miRNA of the cluster was down-regulated.

Lastly, miR-541 over-expression was observed in von Hippel-Lindau disease-associated pheochromocytomas compared to sporadic benign pheochromocytomas, and miR-376b was overexpressed in recurrent or metastatic/malignant pheochromocytomas. On the contrary, miR-431, miR-541, miR-410, and miR-136 were under-expressed in malignant pheochromocytoma [104]. Microarray analysis on bladder cancer cells and on normal fibroblast cells after treatment with azacytidine and/or phenylbutyric acid identified over-expression of miR-127, miR-134, miR-494, miR-495, and miR-377 [105]. In particular, miR-127 showed prominent up-regulation compared to the other miRNAs of the same cluster, after combinational therapy, and negatively regulated its target, the protooncogene *BCL6*. The particular miRNA was also down-regulated in primary human cancers of the colon, bladder and prostate as well as the other counterparts of the cluster [105].

Conclusions

A growing body of evidence suggests that ncRNAs are molecules of previously unrecognized major biological importance. LncRNAs can induce local gene silencing in *cis*, or repress multiple genes activity throughout the genome in *trans*, bind chromatin modifiers complexes, and be implicated in genomic reprogramming of somatic cells to induced pluripotent stem cells through global remodeling of the epigenome [106]. They can also activate transcription factors expression and enhance stem cell differentiation by recruiting epigenetic activators to chromatin [107].

MiRNAs are recognized as essential post-transcriptional regulators of gene expression in both physiological and pathological processes. Their expression is deregulated in many diseases and in cancer [2, 24, 26]. Deregulated miRNA expression affects most of the hallmarks of cancer and could also influence cancer cell chemosensitivity and therefore cancer curability.

The DLK1-DIO3 genomic region is dominated by the lncRNA *MEG3*, which is involved in several important signaling cascades and comprises one of the largest miRNA clusters encoded from non-coding transcription units. The findings reported here indicate that these

miRNAs are involved in a wide spectrum of human diseases, mostly cancers. They may modulate important signaling pathways like mTOR, MAPK, Wnt, JAK-STAT, p53, and they are related to cytokine signaling cascades, DNA methylation, oncogenic kinases expression, and many more. Given the quantitative assessment of these miRNAs, they can be used as biomarkers for the diagnosis, progression and prognosis of several cancers and can identify cancer sub-classes of different clinical behavior. Moreover, they are capable of early characterizing latent malignant cells in the bone marrow stroma that can pass through the gap junctional intercellular communication and move from stroma to cells altering cell cycle. The fact that some miRNAs (e.g., miR-127) are more up-regulated when azacytidine is co-administered with phenylbutyric acid [105] might in part explain the synergistic therapeutic effect observed in diseases such as myelodysplastic syndromes. Perhaps, strong re-expression of the suppressed miRNA might lead in higher down-regulation of the target oncogenes [105].

Our knowledge on long and short ncRNAs biology and function is increasing every day. A better understanding of miRNA clusters that are implicated in human diseases and malignant cell transformation could help lead to the discovery of new therapeutic strategies.

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Conflict of interest The authors declare no potential conflicts of interest.

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