Epigenetics 7:12, 1341–1348; December 2012; © 2012 Landes Bioscience

Small regulatory RNAs controlled by genomic imprinting and their contribution to human disease

Michael Girardot,[1,*] Jérôme Cavaillé[2,3] and Robert Feil[1,*]

[1]Institute of Molecular Genetics; CNRS and the University of Montpellier; Montpellier, France; [2]Laboratoire de Biologie Moléculaire Eucaryote; Université Paul Sabatier; Université de Toulouse; Toulouse, France; [3]CNRS; Toulouse, France

Keywords: epigenetic, genomic imprinting, microRNA, snoRNA, piRNA, cancer

More than a hundred protein-coding genes are controlled by genomic imprinting in humans. These atypical genes are organized in chromosomal domains, each of which is controlled by a differentially methylated "imprinting control region" (ICR). How ICRs mediate the parental allele-specific expression of close-by genes is now becoming understood. At several imprinted domains, this epigenetic mechanism involves the action of long non-coding RNAs. It is less well appreciated that imprinted gene domains also transcribe hundreds of microRNA and small nucleolar RNA genes and that these represent the densest clusters of small RNA genes in mammalian genomes. The evolutionary reasons for this remarkable enrichment of small regulatory RNAs at imprinted domains remain unclear. However, recent studies show that imprinted small RNAs modulate specific functions in development and metabolism and also are frequently perturbed in cancer. Here, we review our current understanding of imprinted small RNAs in the human genome and discuss how perturbation of their expression contributes to disease.

Imprinted Small Regulatory RNAs

Genomic imprinting is an epigenetic mechanism in mammals that gives rise to mono-allelic, parent-of-origin specific, gene expression during development. About 130 genes have been discovered to be controlled by imprinting in mice and humans. Mammalian imprinted genes are not randomly distributed in the genome, but are clustered in evolutionarily conserved domains that can comprise up to several megabases of DNA. Each of these imprinted domains is controlled by an essential regulatory region referred to as the "imprinting control region" (ICR). These CpG-rich control elements are several kilobases in length and are differentially methylated, with DNA methylation being present on the paternally- or the maternally-inherited allele only. These allelic "imprints" are acquired in either the sperm or the oocyte and, rather exceptionally, are resistant to the global waves of DNA demethylation and de novo methylation that occur during the early stages of development.[1] Most ICRs acquire their DNA methylation imprint during oogenesis. These so-called "maternal ICRs" include the ICRs controlling the imprinted *KCNQ1* domain on human chromosome 11, the *GNAS* imprinted domain on human chromosome 20 and the Prader-Willi Syndrome (PWS) imprinted domain on human chromosome 15. Only two imprinted domains in humans are controlled by an ICR that acquires its DNA methylation imprint during spermatogenesis. These are the growth-related *IGF2-H19* imprinted domain on human chromosome 11 and the *DLK1-DIO3* domain on human chromosome 14. Through studies on genetic mutations in humans, and from functional studies in the mouse, it has become evident that ICRs use different strategies to bring about the allelic gene expression at their imprinted domain. At the *GNAS* domain, the *KCNQ1* domain, the *IGF2R* domain and several other domains, this developmentally regulated epigenetic process involves the action of long non-coding RNAs (lncRNAs) that are transcribed from one of the two parental chromosomes only.[2]

It is generally less well appreciated that imprinted domains transcribe also hundreds of small non-coding RNA genes (that are < 105 bases in size) including microRNAs and small nucleolar RNAs (snoRNAs). This striking phenomenon is the focus of the current review. Almost seven percent of the known human microRNAs are encoded by imprinted domains[3] and nowhere in the genome there is such a high density of small regulatory RNAs as at imprinted domains, some of which comprise more than 50 small RNAs per Mb. The regulation and roles of imprinted small RNAs are actively being explored by many laboratories. Besides the finding that imprinted small RNAs play specific roles in development, behavior and metabolic processes, recent studies show that imprinted microRNAs are often perturbed in cancer. An emerging question is whether the latter could be related to their unusual transcriptional regulation and processing. Importantly, recent studies have suggested for some imprinted microRNAs that they contribute to tumorigenesis (**Table S1**), whereas imprinted snoRNAs may contribute to specific imprinting-related diseases as well. This review summarizes the rapidly growing literature on imprinted small RNAs and their roles in development and disease. It also discusses to which extent imprinted small RNAs might contribute to the imprinting process itself.

*Correspondence to: Michael Giradot and Robert Feil;
Email: michael.girardot@igmm.cnrs.fr and robert.feil@igmm.cnrs.fr
Submitted: 10/11/12; Accepted: 11/13/12
http://dx.doi.org/10.4161/epi.22884

Role of Imprinted Small RNAs in Development and Disease

Both snoRNAs and microRNAs are generated from large precursor RNAs through post-transcriptional processing. snoRNAs are 60–300 nucleotides long and are mostly present as ribonucleoproteins in the nucleolus, where they function in the post-transcriptional maturation of 5.8S, 18S and 28S rRNAs (rRNAs). They can be grouped into two main families: the so-called C/D snoRNAs and the H/ACA snoRNAs that guide the acquisition of 2'-O-methylation and pseudo-uridylation, respectively, on rRNAs, but also at small nuclear RNAs and tRNAs. So far, only C/D snoRNA genes have been shown to be controlled by genomic imprinting. snoRNAs are produced by exonucleolytic trimming of RNA polymerase II (RNAP II)-transcribed large precursor RNAs.[4] Likewise, microRNAs are small RNAs that are generated by the nuclear processing of RNAP II transcribed primary transcripts (called pri-miRNAs) and their processing into pre-miRNAs involves the RNase-III endonuclease DROSHA. Subsequently, the released pre-miRNAs are exported into the cytoplasm by the Ran-GTPase Exportin-5 and are then processed into 22–24 nucleotide-long mature miRNAs by the enzyme DICER. Processed microRNAs guide the Argonaute protein (AGO2) of the RNA-induced silencing complex (RISC) to direct post-transcriptional repression of target mRNAs.[5] No fewer than 107 imprinted microRNAs and 117 imprinted snoRNAs have been identified to date (www.mirbase.org; www-snorna.biotoul.fr). Most of these are organized in large transcriptional units, each producing multiple, highly related small RNAs.[3] In the human genome, six imprinted domains comprise transcription units that generate small regulatory RNAs. Below, we review these six imprinted domains and discuss examples of microRNAs and snoRNAs that play specific roles in development and of their deregulation in cancer. Five of the six domains are structurally conserved in the mouse, which has allowed acquisition of functional data and exploration of epigenetic mechanisms.[6] As concerns the diverse roles of imprinted microRNA genes in development and disease, a comprehensive presentation of the literature is provided in **Table S1**.

The DLK1-DIO3 imprinted domain. Studies in the mouse have shown that the *Dlk1-Dio3* imprinted domain on mouse chromosome 12 plays diverse roles in development.[7] This cluster is highly conserved in humans and maps to chromosome 14q32.2. Paternal uniparental disomy (UPD) of chromosome 14 causes respiratory insufficiency, reduced thorax development and a moderate to severe mental retardation, while maternal UPD causes growth retardation, precocious puberty, obesity and mild mental retardation.[8] The 14q32.2 region expresses the non-coding transcripts MEG3 (also called GTL2), anti-RTL1 (RTL1AS), 53 microRNAs and two snoRNA clusters (the *SNORD113* and *SNORD114* clusters, coding for 9 and 31 snoRNAs, respectively) on the maternal chromosome. The paternal chromosome expresses three protein-coding genes, *DLK1*, *RTL1* and *DIO3* (**Fig. 1A**). The allelic expression of genes is controlled by the intergenic ICR of the locus, called the IG-DMR, localized at about 15 kilobases upstream of the *MEG3* gene. In the mouse, deletion of the IG-DMR imprinting control region on the maternal allele leads to loss of expression of all the maternally expressed non-coding RNAs, and to re-expression of the paternal-specific protein-coding genes (*Dlk1, Rtl1* and *Dio3*) on the maternal chromosome.[9] This is associated with late-gestational fetal mortality. Deletion of the IG-DMR on the paternal allele had no effect on the imprinted gene expression.[9] Interestingly, the microRNAs miR-127 and miR-136, expressed from the maternal chromosome, target in trans through an siRNA-like mechanism the retrotransposon-like gene 1 (*Rtl1*), a protein-coding gene expressed from the paternal chromosome that plays a role in placenta development.[10-12] Thus, these two microRNAs silence in trans the expression of another gene of the locus. Other microRNAs encoded by this cluster could be involved in the regulation of synaptic development and function (see **Table S1**) through inhibition of the presynaptic protein SV2A (miR-485),[13] or in post-synaptic regulation (miR-134)[14] of the protein Pumilio2 during activity-dependent dendritic outgrowth.[15] So far, however, gene targeting studies have not been performed to assess the involvement of this imprinted microRNA cluster in neuronal maturation and behavior. Furthermore, several of the imprinted microRNAs have been linked to stem cells and pluripotency. miR-134 was shown to recognize and downregulate the pluripotency factor SOX2 in differentiating ES cells.[16] Also, miR-380 is expressed in mouse ES cells in which it could provide constitutive cell survival by repressing p53.[17] Remarkably, the lack of expression of all the maternally-expressed ncRNA genes at the *Dlk1-Dio3* domain is frequently associated with poor developmental potential of ES and iPS cells.[18,19] Whether loss of expression of snoRNAs and/or miRNAs affects pluripotency of ES and iPS cells remains an appealing but still open question. Noteworthy, however, miR-369 was found to facilitate cellular reprogramming into induced pluripotent stem (iPS) cells.[20] Many imprinted microRNAs of this cluster (miR-136, miR-376a, miR-337, miR-410, miR-379, miR-127 and miR-431) are frequently overexpressed (5- to 100-fold compared with normal tissue levels) in murine lung cancer, the significance of which needs to be determined.[21] Concordant with the frequent overexpression of these microRNAs also in human hepatocellular carcinoma,[22,23] a recent study shows that transgenic transcriptional activation of microRNAs and snoRNAs genes from the *Dlk1-Dio3* locus induces hepatocellular carcinoma in mice.[24] Recently, Dixon-McIver and coworkers[25] detected elevated levels of multiple microRNAs of the *DLK1-DIO3* domain (miR-127, miR-370, miR-299, miR-323 and miR-154) in acute myeloid leukemia, in patients bearing t(15;17) translocations, compared with normal bone marrow biopsies. However, miR-337 and miR-134 of the imprinted domain remained unchanged in their expression levels.[25] The latter finding could indicate that perturbed expression arises at the post-transcriptional level since microRNAs of the cluster are assumed to derive from a single precursor transcript.[26,27] Further examples of aberrant expression of microRNAs from this imprinting cluster in cancer are provided in **Table S1**. A systematic analysis of the expression levels of all microRNAs from this cluster in different cancers would allow one to confirm to which extent post-transcriptional, rather than transcriptional, deregulation occurs

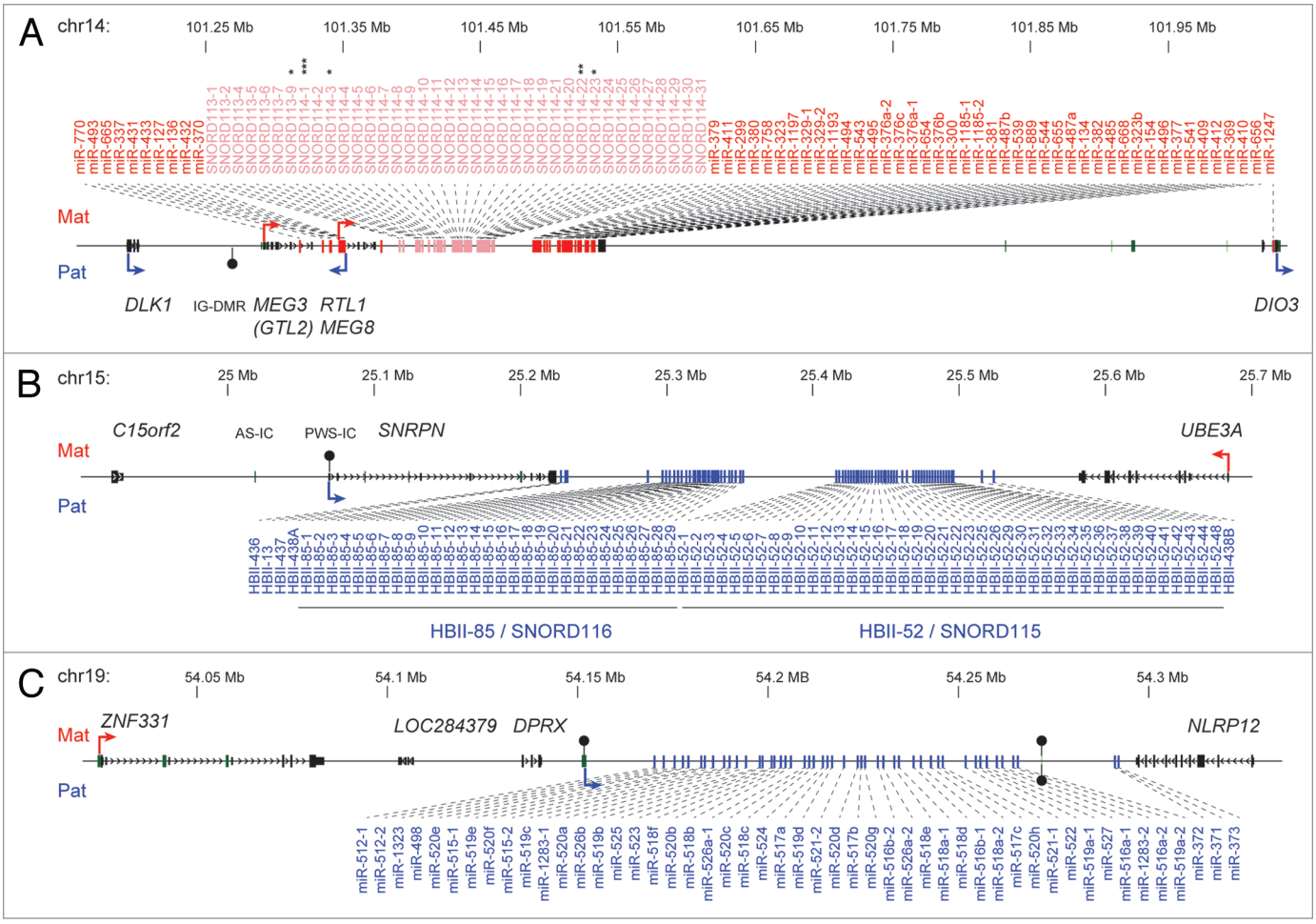

Figure 1. Human imprinted domains with clustered non-coding RNAs. (**A**) The *DLK1-DIO3* imprinted domain on chromosome 14q32.2 contains 53 microRNAs and 38 C/D-box small nucleolar RNAs (C/D snoRNAs). Eight piwi interacting RNAs (piRNAs) were identified in human testis and map uniquely to the five snoRNAs marked with an asterisk: SNORD113-1 is the precursor of piR-31650 (*); SNORD114-1 of piR-34456, piR-33510 and piR-34420 (***); SNORD114-3 of piR-34372 (*); SNORD114-22 of piR-33372 and piR-34929 (**); SNORD114-23 of piR-34291 (*).[30] (**B**) The Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS) imprinted domain on chromosome 15q11-13 contains five snoRNAs and two clusters of 29 and 47 snoRNAs, respectively. (**C**) The chromosome 19 microRNA cluster (C19MC) on chromosome 19q13 contains 46 microRNAs. Small RNAs expressed from the maternal allele are colored in red (Mat), while small RNAs expressed from the paternal allele are indicated in blue (Pat). Arrows indicate transcriptional start sites, green boxes indicate CpG islands, and black lollipops indicate differential DNA methylation (above the black line: methylation on the maternal allele; below the line: methylation on the paternal chromosome).

during tumorigenesis. Multiple C/D-box snoRNAs are also produced by the *DLK1-DIO3* imprinted domain and are arranged in two tandem arrays of 31 and 9 copies respectively. However, no specific complementarities with rRNA or snRNA have been discovered yet, questioning their function as C/D box snoRNAs.[28] Recently, levels of the 14q32.2 snoRNAs increased up to 13-fold were detected in PML-RARα-positive acute myeloid leukemia (AML) patients compared with purified myeloid progenitor cells from healthy donors and PML-RARα-negative AML patients. Furthermore, in vitro overexpression (> 50-fold) of the 14q(II-1) snoRNA gene induced cell proliferation, suggesting that these snoRNAs may indeed be involved in cancer through inhibition of the pRb pathway.[29] Intriguingly, eight Piwi-interacting RNAs (piRNAs) identified in human testis map to this snoRNA cluster (**Fig. 1A**),[30] suggesting that some of the snoRNAs of the cluster are piRNA precursors expressed during gametogenesis. The function of these piRNAs in genomic imprinting of the *DLK1-DIO3* locus is not known.

The Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS) imprinted domain. Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS) are distinct neurodevelopmental disorders mapping to the same imprinted domain on human chromosome 15q11–13. PWS is characterized by hypotonia, respiratory distress, failure to thrive in the postnatal period and by hyperphagia resulting in severe obesity. AS is characterized by ataxia, severe mental retardation, seizures and absence of speech. The PWS/AS domain is several megabases in size and comprises four protein-coding genes transcribed from the paternal chromosome (*NDN, MAGEL2, MKRN3* and *SNRPN*) and one protein-coding gene transcribed from the maternal chromosome (*UBE3A*). In addition, the paternal chromosome also generates five individual snoRNAs (HBII-436, HBII-13, HBII-438A

and HBII-438B) and clustered snoRNAs (the SNORD116/HBII-85 and SNORD115/HBII-52 clusters) (see **Fig. 1B**). AS is caused by loss of expression on the maternal chromosome of *UBE3A*, which codes for an E3 ubiquitin-protein ligase important for proteasomal degradation of proteins playing a role in the cell cycle, signal transduction, transcription and synaptic plasticity. Conversely, PWS is caused by loss of gene expression on the paternal chromosome, but it is still not fully clear which specific genes are causally involved in this neurodevelopmental syndrome. Mice deficient for *Ndn* and *Magel2* recapitulated some of the clinical features of PWS,[31] suggesting that loss of these protein-coding genes contributes to the etiology of the disease. Importantly, microdeletion of the *HBII-85* snoRNA cluster on the paternal chromosome causes many of the features of PWS in humans, indicating that these snoRNAs likely contribute to the disease syndrome as well.[32-34] While a function in the editing and/or alternative splicing of the Serotonin Receptor-2C has been reported for the *HBII-52* snoRNA cluster,[35-37] the function of the brain-specific *HBII-85* cluster had remained elusive. Interestingly, however, mice with a targeted deletion of this snoRNA cluster (called *MBII-85* in mouse) show decreased activity and hypotonia at birth, as well as postnatal growth retardation, thus recapitulating another subset of Prader-Willi syndrome's clinical features.[38,39] Thus, loss of expression at the snoRNA *HBII-85* cluster could be particularly important in PWS as well. Further studies are required to unravel the precise RNA targets of the HBII-85 snoRNAs and how these target RNAs influence neuronal development and function. So far, deregulation of these snoRNAs has not been reported in cancer cells.

The "C19MC" imprinted domain. Human chromosome 19q13 comprises an imprinted microRNA cluster, C19MC (for chromosome 19 microRNA cluster), which evolved recently in evolution, in primates only.[40] This imprinted domain codes for 46 microRNAs (**Fig. 1C**), which all appear to be encoded within a single (or very few) primary transcript,[41] expressed from the paternal chromosome only,[42] and processed nearby its chromosomal production site by the microprocessor complex (which comprises the DiGeorge syndrome critical region gene 8 and DROSHA proteins).[43] Interestingly, the microRNAs of this cluster are imprinted in the placenta only, the only organ in which this cluster is expressed at high levels during development.[44] Although still little is known about their biological function(s),[45] some of these microRNAs have predicted targets related to embryonic and placental development, such as the *HIF1A* (*hypoxia induced factor 1A*) gene.[46] Strikingly, the clustered microRNAs are overexpressed (up to 7-fold) in male germ cell tumor cell lines,[47] and in breast cancer primary tumors and metastases.[48] Indeed, some of these imprinted microRNAs have been shown by transient transfection to promote tumor invasion and metastasis (miR-373, miR-520c and miR-517a).[22,49] Furthermore, microRNAs miR-517c and miR-520g act as putative oncogenes in primitive neuro-ectodermal brain cancer.[50] In fact, the *C19MC* locus is frequently overexpressed in cancers as a consequence of gene amplification[50] or chromosomal translocation.[51] In vitro-induced DNA demethylation increases the expression levels of the examined *C19MC* microRNAs in cells[42,52] suggesting that loss of CpG methylation could be another mechanism through which this cluster becomes overexpressed during tumorigenesis. Given that many of the *C19MC* microRNAs also have predicted targets involved in neuronal differentiation,[50] future studies into the epigenetic regulation of the cluster in neuronal tumors should be particularly interesting.

The IGF2-H19 imprinted domain. The *IGF2* (insulin-like growth factor 2) gene on human chromosome 11p15 influences cellular proliferation. This growth factor is expressed from the paternal chromosome only, while the *H19* ncRNA in the same imprinted domain is expressed from the maternal chromosome (**Fig. 2A**). In paternal UPD, the domain's ICR is methylated on both its copies, which causes *IGF2* overexpression. This is one of the causes of Beckwith-Wiedemann overgrowth syndrome (BWS), characterized by fetal overgrowth, macroglossia (enlarged tongue), anterior abdominal wall defects and other variably present symptoms, including childhood Wilms' tumor of the kidney.[53] Conversely, hypomethylation of the ICR leads to strongly reduced *IGF2* expression, responsible for the Silver-Russell syndrome (SRS) characterized by pre- and post-natal growth restriction, learning disabilities and a triangularly shaped face.[53] MicroRNA miR-675 is matured from the *H19* non-coding transcript and is thus generated from the maternal chromosome only (**Fig. 2A**). The miR-675 sequence and the exon-intron structure of its host imprinted gene (*H19*) are evolutionarily conserved in marsupials,[54] suggesting that both are functionally important. Indeed, an interesting recent study shows that miR-675 is matured from the *H19* transcript itself, at the end of gestation only, and this was shown to be involved in the control of placental growth, in part through its negative effect on the mRNA coding for the IGF1-receptor. This study further demonstrates that miR-675 levels are regulated at the posttranscriptional level by specific RNA binding proteins that recognize the spliced *H19* transcript.[55] Another recent study, on cell lines, suggests that miR-675/*H19* expression is controlled by the transcription factor SOX9,[56] and that miR-675 may target the retinoblastoma (pRb) mRNA to act as an oncogenic microRNA in colorectal cancer. miR-675 and H19 ncRNA are indeed frequently overexpressed (from 2- to 12-fold) in colon cancer cell lines and in human colorectal cancer biopsies.[57] Also the imprinted *IGF2* gene at this imprinted domain produces a microRNA, miR-483, which is matured from its second intronic sequence and is expressed in the placenta and the liver. Concordantly, increased miR-483 expression levels are highly correlated with the *IGF2* expression levels in adrenocortical tumors.[58] This microRNA is overexpressed in Wilms' tumor of the kidney as well, where it may act as an oncogene by inhibiting the pro-apoptotic protein PUMA.[59] It remains to be explored whether miR-483 levels are increased in the cases of BWS that involve IGF2 overexpression and whether this microRNA contributes to this fetal overgrowth syndrome.

The MEST domain. The mesoderm-specific transcript (*MEST*) gene on chromosome 7 is expressed from the paternal allele only, in the mesoderm lineage during embryogenesis, and in adult brain. It encodes a protein similar to the α/β hydrolase fold family of proteins and may thus control hydrolytic activities.[60] Inactivation of the paternal allele in the mouse causes

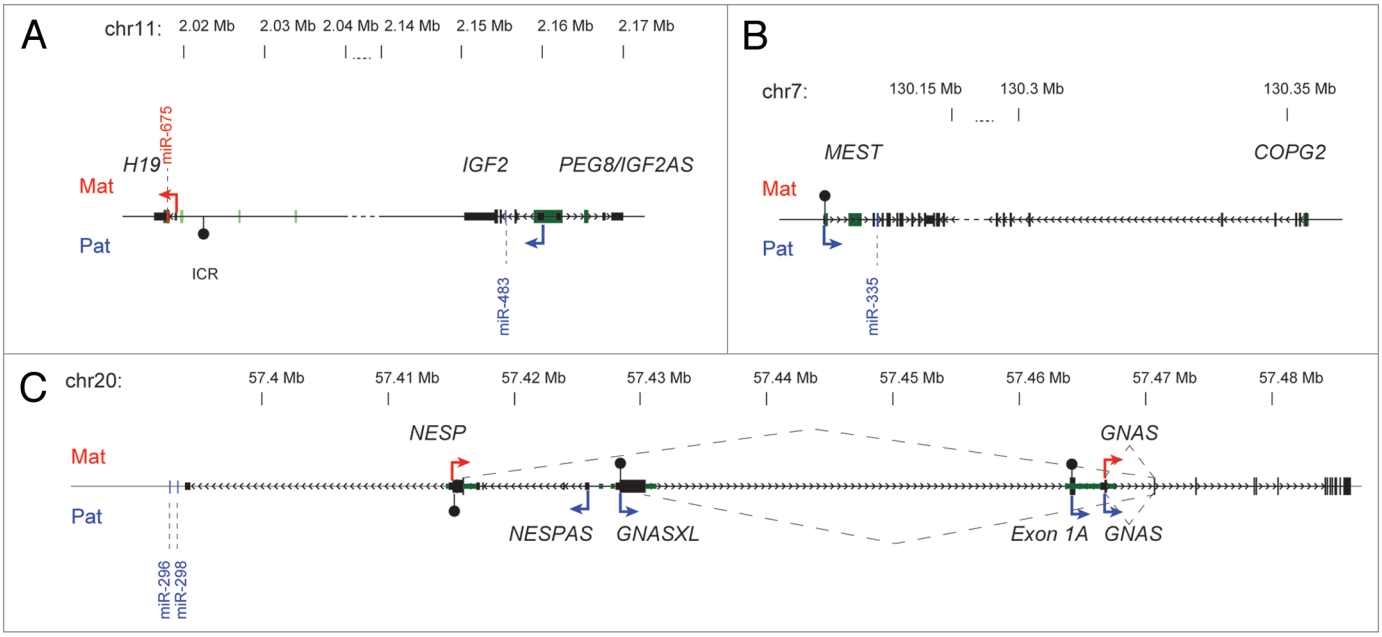

Figure 2. Human imprinted genes comprising isolated microRNAs. (**A**) The *H19/IGF2* imprinted domain on chromosome 11 contains 2 microRNAs, miR-675 matured from the H19 transcript expressed from the maternal chromosome, and miR-483 matured from the IGF2 transcript expressed from the paternal chromosome. The ICR (imprinted control region) is methylated on the paternally inherited chromosome. (**B**) The *MEST* imprinted domain on chromosome 7 contains the miR-335 precursor sequence in the second intron of the *MEST* gene expressed from the paternal chromosome. The ICR comprises the *MEST* promoter and is methylated on the maternal chromosome. (**C**) The *GNAS* imprinted domain on chromosome 20 contains two microRNAs (miR-296, miR-298) matured from the 3' UTR of the *NESPAS* transcript expressed from the paternal chromosome. Dotted lines depict splicing events during transcription of the locus.

embryonic growth retardation.[61] Interestingly, *Mest*-deficient females also show abnormal maternal behavior and impaired placentophagia,[61] indicating that *Mest* is involved in adult brain functions as well. Loss of imprinting of the *MEST* domain has been reported in some patients that presented Silver-Russell Syndrome (SRS)-like symptoms,[62] but how this links in with the frequent epigenetic perturbation of the *IGF2-H19* domain in classical SRS remains to be determined. The second intron of the *MEST* gene produces a microRNA, miR-335 (**Fig. 2B**). This microRNA appears to be a tumor suppressor, possibly through targeting of the progenitor cell transcription factor SOX4, since its overexpression in breast cancer cells prevents metastasis formation following their injection into immune-suppressed mice.[63] Indeed, *MEST* is frequently inactivated in breast cancer, either by deletion or aberrant DNA methylation at its promoter.[64] Functionally, miR-335 has been shown to decrease cell viability and to increase apoptosis upon overexpression in cultured cells.[65] Although an increasing number of target mRNAs are being discovered and validated in cancer, by luciferase assays and/or western-blotting, the miR-335 targets during embryonic development have remained elusive.

The GNAS imprinted domain. The *GNAS* domain (**Fig. 2C**) on human chromosome 20q13.11 encodes the stimulatory G-protein subunit Gsα. *GNAS* transcription is predominantly bi-allelic with selective imprinting and alternative promoter usage in different tissues. Maternally-transmitted inactivating mutations and DNA methylation defects at the differentially methylated regions (DMRs) of this imprinted locus cause pseudoparathyroidism (PHP1a), a heterogeneous group of disorders whose common feature is end-organ resistance to parathyroid hormone leading to hypocalcemia, hyperphosphatemia and obesity.[66] Two microRNAs (miR-296 and miR-298), expressed in the embryo, kidney and nervous system,[67] are matured from the long non-coding antisense transcript *NESPAS* expressed from the paternal chromosome. NESPAS regulates in cis the imprinted expression of the *GNAS* cluster and is the precursor transcript for miR-296 and miR-298, which were shown to regulate *Ikbke* and *Tmed9* gene expression in trans.[68] miR-296 also targets the coding sequence of *Nanog* mRNA and inhibits its translation upon ES cell differentiation,[16] and targets the 3' UTR of p21/WAF1 during carcinogenesis, and may thus contribute to human cell immortalization by downregulating the p53-p21(WAF1) pathway.[69] miR-296 levels are upregulated about 3-fold in brain tumors where its overexpression could contribute to angiogenesis, by translational inhibition of the hepatocyte growth factor-regulated tyrosine kinase substrate involved in the degradation of the growth factor receptors VEGFR2 and PDGFRβ.[70] The other microRNA of the imprinted locus, miR-298, is strongly predicted to inhibit the translation of β-amyloid precursor protein converting enzyme (BACE1) in neuronal cells.[71] This protein is responsible for the proteolytic cleavage of amyloid precursor protein (APP) leading to the cerebral deposition of amyloid β peptide characteristic of Alzheimer disease.[71] These observations evoke the intriguing possibility that loss of expression of the imprinted miR-298, normally expressed in the brain, could contribute to Alzheimer disease.

Do Small Regulatory RNAs Contribute to Genomic Imprinting?

Recent studies in the mouse have shown that small RNAs of the piRNA class are involved in the acquisition of imprinted DNA methylation at the rodent-specific imprinted locus *Rasgrf1*[72] Imprinted DNA methylation at this paternal ICR is acquired during spermatogenesis and requires the piRNA pathway proteins MitoPLD (also called Zucchini) and MIWI2/PIWIL4.[72] So far, however, *Rasgrf1* is the only imprinted locus found to be controlled by the piRNA pathway in the mouse, and this locus is not imprinted in humans. In addition to piRNAs, other types of small RNAs are expressed in developing germ cells as well, including miRNAs and siRNAs.[73,74] To test whether the latter could be somehow involved in the regulation of DNA methylation imprints, conditional knockouts in primordial germ cells were generated in the mouse with targeted alterations at the *Dicer*[63] and *Argonaute-2* [64] genes. However, these studies did not provide any evidence for perturbation of imprinting.[75,76] Although this finding argues against the involvement of microRNAs or siRNAs in the regulation of DNA methylation imprints at ICRs, it should be interesting to reinvestigate this question now that all DMRs at imprinted gene loci are known.[1]

Could small RNAs be involved in the posttranscriptional control of imprinted gene expression, rather than in the regulation of DNA methylation at imprinted domains? We mentioned above the two microRNAs generated by the *Rtl1*-antisense transcript that control the expression of the *Rtl1* gene on the opposite parental chromosome at the imprinted *Dlk1-Dio3* locus.[10,11] Whether other microRNAs could have a similar role in the regulation of imprinted gene expression as well, is not yet known. However, imprinted genes appear to be co-regulated to a certain extent[77,78] and part of their remarkable interdependence might well involve imprinted microRNAs, as demonstrated by the *Rtl1/Rtl1as* locus.

Remains the question of how imprinted small RNAs have evolved, particularly those that reside in large multi-copy clusters. At the human *C19MC* domain, this process occurred during primate evolution. Intriguingly, the imprinted expression at this locus seems to have arisen together with the acquisition of the microRNA repeat itself.[3] Similarly, a large imprinted locus comprising multiple pre-miRNA genes evolved in the rodent lineage only. This *Sfmbt2* imprinted domain, on mouse chromosome 2, shows allelic expression specifically in the early embryo and the extra-embryonic lineages.[79] Interestingly, in other animal groups, the *Sfmbt2* gene does not comprise microRNAs and is not imprinted either. These observations led us to propose that, at some imprinted gene loci, the parental allele-specific expression may have arisen as a consequence of RNA insertion and expansion.[3] The initial step, presumably, consisted of insertion of a retrotransposed gene, followed by an increase in copy number and diversification of small RNA genes, which would not have been counter-selected at the loci involved. This, in turn, could have contributed to the imprinted expression at the host locus, involving allelic acquisition of DNA methylation at key regulatory sequences.[80] Alternatively, imprinted gene loci could be relatively tolerant to transposon insertion and expansion, possibly because of their unusual regulation in germ cells, explaining why the densest clusters of snoRNAs and micoRNAs in mammals are found at imprinted gene loci. It would be interesting to explore further to which extent imprinted small RNAs themselves have contributed to mammalian evolution by modulating biological functions. Whereas some retrotransposed imprinted genes have been shown to play important roles in distinct biological processes, particularly in placentation,[12,81] our knowledge about the biological roles of imprinted microRNAs and snoRNAs is still too limited for evolutionary predictions to be made. Given the rapidly growing interest in small non-coding RNAs, however, many novel insights will undoubtedly emerge during the years to come.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Supplemental Materials

Supplemental materials may be found here:
www.landesbioscience.com/journals/epigenetics/article/22884

References

1. Kelsey G, Feil R. New insights into establishment and maintenance of DNA methylation imprints in mammals. Phil Trans R Soc B 2012; 368:20110336.
2. Barlow DP. Genomic imprinting: a mammalian epigenetic discovery model. Annu Rev Genet 2011; 45:379-403; PMID:21942369; http://dx.doi.org/10.1146/annurev-genet-110410-132459.
3. Labialle S, Cavaillé J. Do repeated arrays of regulatory small-RNA genes elicit genomic imprinting? Concurrent emergence of large clusters of small noncoding RNAs and genomic imprinting at four evolutionarily distinct eutherian chromosomal loci. Bioessays 2011; 33:565-73; PMID:21618561; http://dx.doi.org/10.1002/bies.201100032.
4. Kiss T. Biogenesis of small nuclear RNPs. J Cell Sci 2004; 117:5949-51; PMID:15564372; http://dx.doi.org/10.1242/jcs.01487.
5. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136:215-33; PMID:19167326; http://dx.doi.org/10.1016/j.cell.2009.01.002.
6. Williamson CMBA, Thomas S, Beechey CV, Hancock J, Cattanach BM, Peters J. World Wide Web Site - Mouse Imprinting Data and References. MRC Harwell, Oxfordshire 2012; http://www.har.mrc.ac.uk/research/genomic_imprinting/.
7. da Rocha ST, Edwards CA, Ito M, Ogata T, Ferguson-Smith AC. Genomic imprinting at the mammalian Dlk1-Dio3 domain. Trends Genet 2008; 24:306-16; PMID:18471925; http://dx.doi.org/10.1016/j.tig.2008.03.011.
8. Kagami M, Sekita Y, Nishimura G, Irie M, Kato F, Okada M, et al. Deletions and epimutations affecting the human 14q32.2 imprinted region in individuals with paternal and maternal upd(14)-like phenotypes. Nat Genet 2008; 40:237-42; PMID:18176563; http://dx.doi.org/10.1038/ng.2007.56.
9. Lin SP, Youngson N, Takada S, Seitz H, Reik W, Paulsen M, et al. Asymmetric regulation of imprinting on the maternal and paternal chromosomes at the Dlk1-Gtl2 imprinted cluster on mouse chromosome 12. Nat Genet 2003; 35:97-102; PMID:12937418; http://dx.doi.org/10.1038/ng1233.
10. Seitz H, Youngson N, Lin SP, Dalbert S, Paulsen M, Bachellerie JP, et al. Imprinted microRNA genes transcribed antisense to a reciprocally imprinted retrotransposon-like gene. Nat Genet 2003; 34:261-2; PMID:12796779; http://dx.doi.org/10.1038/ng1171.
11. Davis E, Caiment F, Tordoir X, Cavaille J, Ferguson-Smith A, Cockett N, et al. RNAi-mediated allelic trans-interaction at the imprinted Rtl1/Peg11 locus. Current biology: CB 2005; 15:743-9.
12. Sekita Y, Wagatsuma H, Nakamura K, Ono R, Kagami M, Wakisaka N, et al. Role of retrotransposon-derived imprinted gene, Rtl1, in the feto-maternal interface of mouse placenta. Nat Genet 2008; 40:243-8; PMID:18176565; http://dx.doi.org/10.1038/ng.2007.51.
13. Cohen JE, Lee PR, Chen S, Li W, Fields RD. MicroRNA regulation of homeostatic synaptic plasticity. Proc Natl Acad Sci U S A 2011; 108:11650-5; PMID:21697510; http://dx.doi.org/10.1073/pnas.1017576108.

14. Numakawa T, Richards M, Adachi N, Kishi S, Kunugi H, Hashido K. MicroRNA function and neurotrophin BDNF. Neurochem Int 2011; 59:551-8; PMID:21723895; http://dx.doi.org/10.1016/j.neuint.2011.06.009.
15. Fiore R, Khudayberdiev S, Christensen M, Siegel G, Flavell SW, Kim TK, et al. Mef2-mediated transcription of the miR379-410 cluster regulates activity-dependent dendritogenesis by fine-tuning Pumilio2 protein levels. EMBO J 2009; 28:697-710; PMID:19197241; http://dx.doi.org/10.1038/emboj.2009.10.
16. Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 2008; 455:1124-8; PMID:18806776; http://dx.doi.org/10.1038/nature07299.
17. Swarbrick A, Woods SL, Shaw A, Balakrishnan A, Phua Y, Nguyen A, et al. miR-380-5p represses p53 to control cellular survival and is associated with poor outcome in MYCN-amplified neuroblastoma. Nat Med 2010; 16:1134-40; PMID:20871609; http://dx.doi.org/10.1038/nm.2227.
18. Stadtfeld M, Apostolou E, Akutsu H, Fukuda A, Follett P, Natesan S, et al. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. Nature 2010; 465:175-81; PMID:20418860; http://dx.doi.org/10.1038/nature09017.
19. Liu L, Luo GZ, Yang W, Zhao X, Zheng Q, Lv Z, et al. Activation of the imprinted Dlk1-Dio3 region correlates with pluripotency levels of mouse stem cells. J Biol Chem 2010; 285:19483-90; PMID:20382743; http://dx.doi.org/10.1074/jbc.M110.131995.
20. Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y, et al. Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell 2011; 8:633-8; PMID:21620789; http://dx.doi.org/10.1016/j.stem.2011.05.001.
21. Liu X, Sempere LF, Ouyang H, Memoli VA, Andrew AS, Luo Y, et al. MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. J Clin Invest 2010; 120:1298-309; PMID:20237410; http://dx.doi.org/10.1172/JCI39566.
22. Toffanin S, Hoshida Y, Lachenmayer A, Villanueva A, Cabellos L, Minguez B, et al. MicroRNA-based classification of hepatocellular carcinoma and oncogenic role of miR-517a. Gastroenterology 2011; 140:1618-28 e16.
23. Luk JM, Burchard J, Zhang C, Liu AM, Wong KF, Shek FH, et al. DLK1-DIO3 genomic imprinted microRNA cluster at 14q32.2 defines a stemlike subtype of hepatocellular carcinoma associated with poor survival. J Biol Chem 2011; 286:30706-13; PMID:21737452; http://dx.doi.org/10.1074/jbc.M111.229831.
24. Wang PR, Xu M, Toffanin S, Li Y, Llovet JM, Russell DW. Induction of hepatocellular carcinoma by in vivo gene targeting. Proc Natl Acad Sci U S A 2012; 109:11264-9; PMID:22733778; http://dx.doi.org/10.1073/pnas.1117032109.
25. Dixon-McIver A, East P, Mein CA, Cazier JB, Molloy G, Chaplin T, et al. Distinctive patterns of microRNA expression associated with karyotype in acute myeloid leukaemia. PLoS One 2008; 3:e2141; PMID:18478077; http://dx.doi.org/10.1371/journal.pone.0002141.
26. Seitz H, Royo H, Bortolin ML, Lin SP, Ferguson-Smith AC, Cavaillé J. A large imprinted microRNA gene cluster at the mouse Dlk1-Gtl2 domain. Genome Res 2004; 14:1741-8; PMID:15310658; http://dx.doi.org/10.1101/gr.2743304.
27. Tierling S, Dalbert S, Schoppenhorst S, Tsai CE, Oliger S, Ferguson-Smith AC, et al. High-resolution map and imprinting analysis of the Gtl2-Dnchc1 domain on mouse chromosome 12. Genomics 2006; 87:225-35; PMID:16309881; http://dx.doi.org/10.1016/j.ygeno.2005.09.018.
28. Cavaillé J, Seitz H, Paulsen M, Ferguson-Smith AC, Bachellerie JP. Identification of tandemly-repeated C/D snoRNA genes at the imprinted human 14q32 domain reminiscent of those at the Prader-Willi/Angelman syndrome region. Hum Mol Genet 2002; 11:1527-38; PMID:12045206; http://dx.doi.org/10.1093/hmg/11.13.1527.
29. Valleron W, Laprevotte E, Gautier EF, Quelen C, Demur C, Delabesse E, et al. Specific small nucleolar RNA expression profiles in acute leukemia. Leukemia 2012; 26:2052-60; PMID:22522792; http://dx.doi.org/10.1038/leu.2012.111.
30. Girard A, Sachidanandam R, Hannon GJ, Carmell MA. A germline-specific class of small RNAs binds mammalian Piwi proteins. Nature 2006; 442:199-202; PMID:16751776.
31. Kernohan KD, Bérubé NG. Genetic and epigenetic dysregulation of imprinted genes in the brain. Epigenomics 2010; 2:743-63; PMID:22122080; http://dx.doi.org/10.2217/epi.10.61.
32. Sahoo T, del Gaudio D, German JR, Shinawi M, Peters SU, Person RE, et al. Prader-Willi phenotype caused by paternal deficiency for the HBII-85 C/D box small nucleolar RNA cluster. Nat Genet 2008; 40:719-21; PMID:18500341; http://dx.doi.org/10.1038/ng.158.
33. Duker AL, Ballif BC, Bawle EV, Person RE, Mahadevan S, Alliman S, et al. Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116 C/D box snoRNA cluster in Prader-Willi syndrome. Eur J Hum Genet 2010; 18:1196-201; PMID:20588305; http://dx.doi.org/10.1038/ejhg.2010.102.
34. de Smith AJ, Purmann C, Walters RG, Ellis RJ, Holder SE, Van Haelst MM, et al. A deletion of the HBII-85 class of small nucleolar RNAs (snoRNAs) is associated with hyperphagia, obesity and hypogonadism. Hum Mol Genet 2009; 18:3257-65; PMID:19498035; http://dx.doi.org/10.1093/hmg/ddp263.
35. Kishore S, Stamm S. The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. Science 2006; 311:230-2; PMID:16357227; http://dx.doi.org/10.1126/science.1118265.
36. Cavaillé J, Buiting K, Kiefmann M, Lalande M, Brannan CI, Horsthemke B, et al. Identification of brain-specific and imprinted small nucleolar RNA genes exhibiting an unusual genomic organization. Proc Natl Acad Sci U S A 2000; 97:14311-6; PMID:11106375; http://dx.doi.org/10.1073/pnas.250426397.
37. Vitali P, Basyuk E, Le Meur E, Bertrand E, Muscatelli F, Cavaillé J, et al. ADAR2-mediated editing of RNA substrates in the nucleolus is inhibited by C/D small nucleolar RNAs. J Cell Biol 2005; 169:745-53; PMID:15939761; http://dx.doi.org/10.1083/jcb.200411129.
38. Ding F, Li HH, Zhang S, Solomon NM, Camper SA, Cohen P, et al. SnoRNA Snord116 (Pwcr1/MBII-85) deletion causes growth deficiency and hyperphagia in mice. PLoS One 2008; 3:e1709.
39. Skryabin BV, Gubar LV, Seeger B, Pfeiffer J, Handel S, Robeck T, et al. Deletion of the MBII-85 snoRNA gene cluster in mice results in postnatal growth retardation. PLoS Genet 2007; 3:e235; PMID:18166085; http://dx.doi.org/10.1371/journal.pgen.0030235.
40. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, et al. Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 2005; 37:766-70; PMID:15965474; http://dx.doi.org/10.1038/ng1590.
41. Bortolin-Cavaillé ML, Dance M, Weber M, Cavaillé J. C19MC microRNAs are processed from introns of large Pol-II, non-protein-coding transcripts. Nucleic Acids Res 2009; 37:3464-73; PMID:19339516; http://dx.doi.org/10.1093/nar/gkp205.
42. Noguer-Dance M, Abu-Amero S, Al-Khtib M, Lefèvre A, Coullin P, Moore GE, et al. The primate-specific microRNA gene cluster (C19MC) is imprinted in the placenta. Hum Mol Genet 2010; 19:3566-82; PMID:20610438; http://dx.doi.org/10.1093/hmg/ddq272.
43. Bellemer C, Bortolin-Cavaillé ML, Schmidt U, Jensen SM, Kjems J, Bertrand E, et al. Microprocessor dynamics and interactions at endogenous imprinted C19MC microRNA genes. J Cell Sci 2012; 125:2709-20; PMID:22393237; http://dx.doi.org/10.1242/jcs.100354.
44. Kotlabova K, Doucha J, Hromadnikova I. Placental-specific microRNA in maternal circulation--identification of appropriate pregnancy-associated microRNAs with diagnostic potential. J Reprod Immunol 2011; 89:185-91; PMID:21513988; http://dx.doi.org/10.1016/j.jri.2011.02.006.
45. Flor I, Bullerdiek J. The dark side of a success story: microRNAs of the C19MC cluster in human tumours. J Pathol 2012; 227:270-4; PMID:22374805; http://dx.doi.org/10.1002/path.4014.
46. Patel J, Landers K, Mortimer RH, Richard K. Regulation of hypoxia inducible factors (HIF) in hypoxia and normoxia during placental development. Placenta 2010; 31:951-7; PMID:20869770; http://dx.doi.org/10.1016/j.placenta.2010.08.008.
47. Port M, Glaesener S, Ruf C, Riecke A, Bokemeyer C, Meineke V, et al. Micro-RNA expression in cisplatin resistant germ cell tumor cell lines. Mol Cancer 2011; 10:52; PMID:21575166; http://dx.doi.org/10.1186/1476-4598-10-52.
48. O'Day E, Lal A. MicroRNAs and their target gene networks in breast cancer. Breast Cancer Res 2010; 12:201; PMID:20346098; http://dx.doi.org/10.1186/bcr2484.
49. Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S, et al. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol 2008; 10:202-10; PMID:18193036; http://dx.doi.org/10.1038/ncb1681.
50. Li M, Lee KF, Lu Y, Clarke I, Shih D, Eberhart C, et al. Frequent amplification of a chr19q13.41 microRNA polycistron in aggressive primitive neuroectodermal brain tumors. Cancer Cell 2009; 16:533-46; PMID:19962671; http://dx.doi.org/10.1016/j.ccr.2009.10.025.
51. Rippe V, Dittberner L, Lorenz VN, Drieschner N, Nimzyk R, Sendt W, et al. The two stem cell microRNA gene clusters C19MC and miR-371-3 are activated by specific chromosomal rearrangements in a subgroup of thyroid adenomas. PLoS One 2010; 5:e9485; PMID:20209130; http://dx.doi.org/10.1371/journal.pone.0009485.
52. Tsai KW, Kao HW, Chen HC, Chen SJ, Lin WC. Epigenetic control of the expression of a primate-specific microRNA cluster in human cancer cells. Epigenetics 2009; 4:587-92; PMID:19923923; http://dx.doi.org/10.4161/epi.4.8.10230.
53. Lim DH, Maher ER. Human imprinting syndromes. Epigenomics 2009; 1:347-69; PMID:22122706; http://dx.doi.org/10.2217/epi.09.24.
54. Smits G, Mungall AJ, Griffiths-Jones S, Smith P, Beury D, Matthews L, et al.; SAVOIR Consortium. Conservation of the H19 noncoding RNA and H19-IGF2 imprinting mechanism in therians. Nat Genet 2008; 40:971-6; PMID:18587395; http://dx.doi.org/10.1038/ng.168.
55. Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G, et al. The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. Nat Cell Biol 2012; 14:659-65; PMID:22684254; http://dx.doi.org/10.1038/ncb2521.
56. Dudek KA, Lafont JE, Martinez-Sanchez A, Murphy CL. Type II collagen expression is regulated by tissue-specific miR-675 in human articular chondrocytes. J Biol Chem 2010; 285:24381-7; PMID:20529846; http://dx.doi.org/10.1074/jbc.M110.111328.
57. Tsang WP, Ng EK, Ng SS, Jin H, Yu J, Sung JJ, et al. Oncofetal H19-derived miR-675 regulates tumor suppressor RB in human colorectal cancer. Carcinogenesis 2010; 31:350-8; PMID:19926638; http://dx.doi.org/10.1093/carcin/bgp181.

58. Patterson EE, Holloway AK, Weng J, Fojo T, Kebebew E. MicroRNA profiling of adrenocortical tumors reveals miR-483 as a marker of malignancy. Cancer 2011; 117:1630-9; PMID:21472710; http://dx.doi.org/10.1002/cncr.25724.
59. Veronese A, Lupini L, Consiglio J, Visone R, Ferracin M, Fornari F, et al. Oncogenic role of miR-483-3p at the IGF2/483 locus. Cancer Res 2010; 70:3140-9; PMID:20388800; http://dx.doi.org/10.1158/0008-5472.CAN-09-4456.
60. Kaneko-Ishino T, Kuroiwa Y, Miyoshi N, Kohda T, Suzuki R, Yokoyama M, et al. Peg1/Mest imprinted gene on chromosome 6 identified by cDNA subtraction hybridization. Nat Genet 1995; 11:52-9; PMID:7550314; http://dx.doi.org/10.1038/ng0995-52.
61. Lefebvre L, Viville S, Barton SC, Ishino F, Keverne EB, Surani MA. Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. Nat Genet 1998; 20:163-9; PMID:9771709; http://dx.doi.org/10.1038/2464.
62. Eggermann T, Spengler S, Begemann M, Binder G, Buiting K, Albrecht B, et al. Deletion of the paternal allele of the imprinted MEST/PEG1 region in a patient with Silver-Russell syndrome features. Clin Genet 2012; 81:298-300; PMID:22211632; http://dx.doi.org/10.1111/j.1399-0004.2011.01719.x.
63. Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD, et al. Endogenous human microRNAs that suppress breast cancer metastasis. Nature 2008; 451:147-52; PMID:18185580; http://dx.doi.org/10.1038/nature06487.
64. Png KJ, Yoshida M, Zhang XH, Shu W, Lee H, Rimner A, et al. MicroRNA-335 inhibits tumor reinitiation and is silenced through genetic and epigenetic mechanisms in human breast cancer. Genes Dev 2011; 25:226-31; PMID:21289068; http://dx.doi.org/10.1101/gad.1974211.
65. Heyn H, Engelmann M, Schreek S, Ahrens P, Lehmann U, Kreipe H, et al. MicroRNA miR-335 is crucial for the BRCA1 regulatory cascade in breast cancer development. Int J Cancer 2011; 129:2797-806; PMID:21618216; http://dx.doi.org/10.1002/ijc.25962.
66. Kelsey G. Imprinting on chromosome 20: tissue-specific imprinting and imprinting mutations in the GNAS locus. Am J Med Genet C Semin Med Genet 2010; 154C:377-86; PMID:20803660; http://dx.doi.org/10.1002/ajmg.c.30271.
67. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007; 129:1401-14; PMID:17604727; http://dx.doi.org/10.1016/j.cell.2007.04.040.
68. Robson JE, Eaton SA, Underhill P, Williams D, Peters J. MicroRNAs 296 and 298 are imprinted and part of the GNAS/Gnas cluster and miR-296 targets IKBKE and Tmed9. RNA 2012; 18:135-44; PMID:22114321; http://dx.doi.org/10.1261/rna.029561.111.
69. Yoon AR, Gao R, Kaul Z, Choi IK, Ryu J, Noble JR, et al. MicroRNA-296 is enriched in cancer cells and downregulates p21WAF1 mRNA expression via interaction with its 3' untranslated region. Nucleic Acids Res 2011; 39:8078-91; PMID:21724611; http://dx.doi.org/10.1093/nar/gkr492.
70. Würdinger T, Tannous BA, Saydam O, Skog J, Grau S, Soutschek J, et al. miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. Cancer Cell 2008; 14:382-93; PMID:18977327; http://dx.doi.org/10.1016/j.ccr.2008.10.005.
71. Boissonneault V, Plante I, Rivest S, Provost P. MicroRNA-298 and microRNA-328 regulate expression of mouse beta-amyloid precursor protein-converting enzyme 1. J Biol Chem 2009; 284:1971-81; PMID:18986979; http://dx.doi.org/10.1074/jbc.M807530200.
72. Watanabe T, Tomizawa S, Mitsuya K, Totoki Y, Yamamoto Y, Kuramochi-Miyagawa S, et al. Role for piRNAs and noncoding RNA in de novo DNA methylation of the imprinted mouse Rasgrf1 locus. Science 2011; 332:848-52; PMID:21566194; http://dx.doi.org/10.1126/science.1203919.
73. Kuramochi-Miyagawa S, Watanabe T, Gotoh K, Totoki Y, Toyoda A, Ikawa M, et al. DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. Genes Dev 2008; 22:908-17; PMID:18381894; http://dx.doi.org/10.1101/gad.1640708.
74. Watanabe T, Totoki Y, Toyoda A, Kaneda M, Kuramochi-Miyagawa S, Obata Y, et al. Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. Nature 2008; 453:539-43; PMID:18404146; http://dx.doi.org/10.1038/nature06908.
75. Hayashi K, Chuva de Sousa Lopes SM, Kaneda M, Tang F, Hajkova P, Lao K, et al. MicroRNA biogenesis is required for mouse primordial germ cell development and spermatogenesis. PLoS One 2008; 3:e1738.
76. Morita S, Horii T, Kimura M, Goto Y, Ochiya T, Hatada I. One Argonaute family member, Eif2c2 (Ago2), is essential for development and appears not to be involved in DNA methylation. Genomics 2007; 89:687-96; PMID:17418524; http://dx.doi.org/10.1016/j.ygeno.2007.01.004.
77. Zacharek SJ, Fillmore CM, Lau AN, Gludish DW, Chou A, Ho JW, et al. Lung stem cell self-renewal relies on BMI1-dependent control of expression at imprinted loci. Cell Stem Cell 2011; 9:272-81; PMID:21885022; http://dx.doi.org/10.1016/j.stem.2011.07.007.
78. Varrault A, Gueydan C, Delalbre A, Bellmann A, Houssami S, Aknin C, et al. Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. Dev Cell 2006; 11:711-22; PMID:17084362; http://dx.doi.org/10.1016/j.devcel.2006.09.003.
79. Wang Q, Chow J, Hong J, Smith AF, Moreno C, Seaby P, et al. Recent acquisition of imprinting at the rodent Sfmbt2 locus correlates with insertion of a large block of miRNAs. BMC Genomics 2011; 12:204; PMID:21510876; http://dx.doi.org/10.1186/1471-2164-12-204.
80. Cowley M, Oakey RJ. Retrotransposition and genomic imprinting. Brief Funct Genomics 2010; 9:340-6; PMID:20591835; http://dx.doi.org/10.1093/bfgp/elq015.
81. Ono R, Nakamura K, Inoue K, Naruse M, Usami T, Wakisaka-Saito N, et al. Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. Nat Genet 2006; 38:101-6; PMID:16341224; http://dx.doi.org/10.1038/ng1699.