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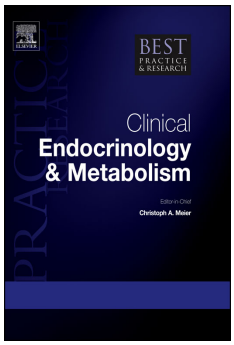

MicroRNAs in the pituitary

Erica Gentilin, Ettore degli Uberti, Maria Chiara Zatelli, MD, PhD, Prof.

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Erica Gentilin, Ettore degli Uberti, Maria Chiara Zatelli

Section of Endocrinology and Internal Medicine, Dept. of Medical Sciences, University of Ferrara, Italy

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Corresponding Author (to whom reprint requests should be addressed):

Prof. Maria Chiara Zatelli, MD, PhD

Section of Endocrinology and Internal Medicine, Dept. of Medical Sciences, University of Ferrara, Via Savonarola 9, 44100 Ferrara, Italy. Tel: +39 0532 239618; Fax: +39 0532 236514; E-mail: ztlmch@unife.it

Abstract

MicroRNAs (miRNAs) are non-coding RNAs generated from endogenous hairpin-shaped transcripts that powerfully regulate gene expression at post-transcriptional level. Each miRNA is capable to regulate the expression levels of hundreds of transcripts and each mRNA may have more than one miRNA recognition sequence. There is emerging evidence that deregulation of miRNA expression leads to the alteration of pivotal physiological functions contributing to the development of diseases and neoplasms, including pituitary adenoma. This review is aimed at providing the up-to-date knowledge concerning deregulated miRNAs of pituitary tumors and their functions. In order to take stock, pituitary tumors have been sub-divided in different classes on the basis of tumor features (histotype, dimension, aggressiveness). The overview takes full consideration of the recent advances in miRNAs role as potential therapeutics and biomarkers.

Key words: miRNA, pituitary, miRNA-based therapy, miRNA biomarkers

MicroRNAs

MicroRNAs (miRNAs) are small non coding RNAs generated from endogenous transcripts, that control gene expression at post-transcriptional level.

miRNAs are part of genome of almost all eukaryotes (1). To date, release 21 of miRBase database (2) contains 35828 miRNAs in 223 species; among them 2588 mature sequences have been identified in human genome (3). miRNA sequences have been found in intergenic DNA regions as well as inside introns of protein coding genes.

Intergenic miRNA genes are transcribed by polymerase II and III, starting from their own promotors, in a pri-miRNA, a long hairpin-shaped transcript that contains one or more miRNA sequences. pri-miRNA is processed in the nucleus by the microprocessor complex in an hairpin-shaped transcripts of 80 nt called pre-miRNA. On the other hand, intronic miRNA sequences are transcribed by polymerase II as part of pre-mRNA and excised by sliceosome generating pri-miRNAs or pre-miRNAs that are processed like intergenic miRNAs.

pre-miRNA is subsequently transported in the cytoplasm and cleaved by Dicer enzyme in a double stranded RNA of 22 nt: the mature form of miRNA. miRNA duplex is loaded in the RNA induced silencing complex (RISC) that uses a miRNA strand to recognize the 3'UTR of specific mRNA target by complementarity. ~~The degree of base-pairing determinates the fate of target mRNA: complete miRNA-mRNA match leads to mRNA degradation, while incomplete miRNA-mRNA match leads to mRNA translation inhibition.~~ The degree of base-pairing usually determines the fate of target mRNA. mRNA degradation occurs upon single extensive miRNA-mRNA perfect match and specific recruitment of RISC proteins with endonuclease activity. On the other hand, mRNA translation inhibition occurs upon multiple complementary sites with imperfect miRNA-mRNA match. Generally, mammalian miRNAs are perfectly complementary in the seed region (2-8 nucleotides in the 5' end), but not in the remaining miRNA sequence, resulting in an imperfect miRNA-mRNA match.

Each miRNA is capable to regulate the expression levels of hundreds of transcripts and each mRNA may have more than one miRNA recognition sequence. These potent gene regulators are key players of the refined process of gene expression needed for normal cellular functions. Therefore, it is easy to think that deregulation of miRNAs expression or mutation in miRNA sequences may lead to the alteration of pivotal physiological functions contributing to the development of diseases and neoplasms.

This review will show the up-to-date advances regarding miRNA role in normal and neoplastic pituitary and medical implications.

miRNAs: what is the role in normal pituitary?

The pituitary is a gland located in the sella turcica of sphenoid bone that controls the function of several other glands; for this reason it is often referred to as the "master gland". It is composed of an anterior lobe called adenohypophysis and a posterior lobe called neurohypophysis. The adenohypophysis is constituted by five different cytotypes that synthesize and secrete specific hormones: somatotrophs that release somatotropin (GH), thyrotrophs that release thyroid-stimulating hormone (TSH), lactotrophs that release prolactin (PRL), corticotrophs that release adrenocorticotropic hormone (ACTH) and gonadotrophs that release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The secretion of anterior pituitary hormones is controlled by hypothalamic releasing hormones. The neurohypophysis stores and secretes two hormones produced by the hypothalamus: antidiuretic hormone and oxytocin (Oxt).

miRNAs are expressed in a tissue specific manner with a precise time trend, controlling differentiation and maintaining tissue identity (4). Recently, the involvement of miRNAs also in pituitary development has been demonstrated. Indeed, miR-26b has been shown to regulate two of the main transcription factors that allow pituitary lineage differentiation (5). Moreover, several studies demonstrated the active participation of miRNAs in anterior pituitary hormonal secretion.

miR-375 negatively regulates corticotropin releasing hormone (CRH)-induced pro-opiomelanocortin (POMC) expression and ACTH secretion (6), while miR-449 controls hypothalamic-pituitary-adrenal axis in response to stress. Glucocorticoids increase miR-449 that directly targets CRH receptor mRNA, down-regulating its protein expression (7). Another important advancement is represented by the discovery that miRNAs control gonadotropin expression. Indeed, mir-361-3p negatively regulates FSH secretion (8), while miR-132 and miR-212 mediate gonadotropin releasing hormone (GnRH)-induced FSHβ expression (9) and gonadotroph motility (10). Moreover, among other factors, miR-325-3p regulates the stress-induced inhibition of LH secretion (11). miRNAs are also involved in physiological development of gonadotroph cells; miR-27 mediates GnRH-induced apoptosis in mature gonadotrophs modulating the expression of prohibitin (12). In addition, alteration in miRNA processing machine is associated with pituitary dysfunction and neurodegeneration (13), underlining the importance of miRNAs in maintenance of pituitary physiological functions. Recently, it has been also demonstrated that miRNAs regulate the secretion of posterior pituitary hormones. Indeed, miR-24 has been identified as one of the regulator of Oxt (14).

Since miRNAs are necessary for normal pituitary development and physiological functions, changes in miRNA sequences or miRNA expression may likely lead to pituitary disorders.

miRNAs in pituitary adenoma

Pituitary adenomas are mainly benign non metastasizing neoplasm that originate in adenohypophysis. These neoplasms represent the ~15 % of intracranial tumors with an overall incidence of 4 to 4.26 per 100,000 per year (15). Pituitary adenomas can be classified as micro- or macroadenomas on the basis of diameter; microadenomas are tumors smaller than 1 cm, while macroadenomas are tumors greater than 1 cm. Pituitary adenomas can be also classified as functioning tumors, that release pituitary trophic hormones causing clinical syndromes, or non functioning neoplasms (NFA), that do not secrete pituitary hormones. Pituitary adenoma

pathogenesis is not fully elucidated. The most accredited theory considers that a genetic alteration causes the neoplastic transformation of a pituitary cell predisposing to hyperplasia development and, subsequently to the acquisition of promoting/facilitating alterations, to clonal neoplasm formation (16). Several efforts have been made in order to identify the genes involved in pituitary neoplastic transformation. The number of genes linked to pituitary pathogenesis is currently increasing year by year demonstrating the great interest in this field. The alteration of cell cycle regulators is one of the more frequent modification occurring in~80% of pituitary adenomas (17). Indeed, protein expression alteration of cyclins, cyclin-dependent kinase inhibitors, pRb (17) and protein kinase C delta (PRKCD) has been reported (18). Moreover, an increasing number of oncogenes (GNAS, PI3KCA, PTTG), tumor suppressor genes (GADD45γ, AIP, MEN1, PRKAR1A, Reprimo), structural proteins (Magmas) and epigenetic modifications of several genes (FGFR2, MEGE-A, MEG3) have been related to pituitary adenoma development and progression (19, 20, 21). Recently, the fundamental role of miRNAs in pituitary tumorigenesis has been defined (19). Early indication that miRNAs are involved in pituitary adenoma development was uncovered by the observation that pituitary tumors frequently show a deletion at chromosome 13 where mir-15a and mir-16-1 localize (22). The study of Bottoni and colleagues demonstrated that these miRNAs are down-regulated in pituitary adenomas versus normal pituitary and that their expression is inversely correlated with tumor diameter (22). This evidence has opened the door to further studies concerning miRNA in the pituitary.

It is well known that each pituitary adenoma histotype displays specific biological features. Previous evidence demonstrated that miRNA profiles can distinguish pituitary adenoma from normal pituitary and can predict pituitary adenoma histotype (23). In order to highlight the current knowledge on the influence of miRNAs in pituitary neoplastic transformation, this review will highlight the scientific advances in this field for each pituitary adenoma sub-type.

miRNAs in ACTH-secreting pituitary adenoma

The present literature reports that miRNAs are important emerging elements in the pathogenesis of ACTH-secreting pituitary adenoma. It has been observed that several miRNAs are expressed at low levels (let-7a, miR141, miR-143, miR-145, miR-15a, miR-150, miR-16, miR-21) while others are expressed at high levels (miR-122, miR-26a, miR-493) as compared to normal pituitary (23, 24, 25, 26, 27). The knowledge concerning the biological effects of miRNA expression alteration is still scarce. An inverse association between let-7 and high mobility group A2 (HGMA2) protein expression has been reported correlating to clinical and pathological features of pituitary tumors (25). Moreover, corticotroph pituitary adenomas displaying lower miR-141 levels show a higher remission rate after surgery (24). Nevertheless, among these miRNAs, only miR-26 family has been characterized in the settings of corticotroph adenoma cells. miR-26a directly targets PRKCD, a known regulator of many physiological processes, like transcription, proliferation, apoptosis and differentiation (28). An anti-miR-26a molecule, that reduces miR-26a expression, is capable to restrain cell cycle progression, reducing cyclin E and cyclin A, in a PRKCD-dependent manner (27). Moreover, the simultaneous reduction in miR-26b and increase in miR-128 levels inhibits the capability of the mouse ACTH-secreting pituitary adenoma cell line, the AtT20 cells, to invade and form new colonies by regulating PTEN/AKT pathway (29). These results demonstrate that miR-26 exerts a pivotal role in controlling cell growth and cell cycle progression of ACTH-secreting pituitary adenoma cells.

ACTH-secreting pituitary adenoma tissues also show an under-expression of 9 miRNAs belonging to the DLK1/MEG3 locus that was already associated to pituitary adenoma pathogenesis. The silencing of this miRNAs cluster may represent an important step of corticotroph neoplastic transformation (30).

miRNAs in LH/FSH-secreting pituitary adenoma

The knowledge of miRNAs role in LH/FSH-secreting pituitary tumors is still lacking, probably because the pathogenesis of these neoplasms is less known as compared to other pituitary

adenomas. It has been reported that miR-374b, miR-548b-3p, miR-603, miR-570 and miR-663 are down-regulated in gonadotroph pituitary adenomas as compared to normal pituitary tissues (31). miR-10b and miR-122, among many other deregulated miRNAs found by Liang S. and colleagues, are the most up-regulated miRNAs (32).

miRNAs in PRL-secreting pituitary adenoma

It has been reported that some miRNAs are down-regulated (miR-125b, miR-130a, miR-15a, miR-16-1, miR-199b-3p and miR-200b, miR-320, miR-34b, miR-374) while others are up-regulated (miR-23b, miR-342-3p, miR-432, miR-493, miR-493*, miR-664*) as compared to normal pituitary (22, 31, 33). miR-493* and miR-432 expression positively correlates with PRL serum levels while miR-342-3p expression positively correlates with invasiveness (33).

miR-16-1 levels are negatively correlated with arginyl-tRNA synthetase (RARS) expression and directly correlated with the secretion of tRNA-interacting factor p43. The latter not only is capable to modulate RARS activity, but also represents a proinflammatory cytokine with important anticancer functions. These results indicate that miR-16-1 controls some molecular components implicated in lactotroph tumor growth (22).

miR-300, miR-329, miR-381 and miR-655 are expressed at low levels in pituitary adenoma than normal pituitary. Ectopic expression of these miRNAs, targeting pituitary tumor transforming 1 (PTTG1) mRNA, reduces pituitary cell viability and proliferation both in vivo and in vitro, decreases cell motility and increases programmed cell death in two rat pituitary adenoma cell lines, the MMQ and GH3 cells, secreting PRL and GH/PRL, respectively (34). In addition, miR-200c, directly targets phosphatase and tensin homolog (PTEN) mRNA controlling apoptosis in MMQ cells (35).

PRL-secreting pituitary adenomas, as well as ACTH-secreting pituitary adenomas and NFA, show a down-regulation of some miRNAs belonging to the DLK1/MEG3 locus. The silencing of this miRNAs cluster seems to represent an important step in neoplastic transformation process of specific pituitary cytotypes (30).

miRNAs in GH-secreting pituitary adenoma

It is known that some miRNAs are over-expressed in GH-secreting pituitary adenomas, while other miRNAs are considerably down-regulated as compared to normal pituitary (22, 29, 31, 36, 37). Among these miRNAs, miR-326, miR-432, and miR-570 directly target HMGA2, miR-34b and miR-548c-3p target both HMGA1 and HMGA2 and miR-326 and miR-603 target E2F1 (31). The reduced levels of these miRNAs may clarify high levels of HMGA and E2F1 proteins usually observed in GH-secreting pituitary adenomas. The over-expression of HMGA and E2F1 targeting miRNAs, as well as miR-130b reduces somatotroph cell proliferation and restrain cell cycle progression (31, 37).

Long-acting somatostatin analogs are usually used as medical therapy for somatotroph pituitary adenomas. Some patients show resistance to pharmacological treatment, which may be due to the reduction in the expression of somatostatin receptor subtype 2 (SSTR2). The latter has been identified as a target of miR-185 whose over-expression inhibits GH3 cell growth and activate late apoptosis (38). These results demonstrate that miR-185 is involved in somatotroph drug resistance and pathogenesis.

It is also known that sporadic GH-secreting pituitary adenomas display low levels of aryl hydrocarbon receptor-interacting protein (AIP) (39). Recently, it has been demonstrated that somatotroph adenoma displays high miR-34a and miR-103 levels as compared to normal pituitary tissue and that these miRNAs are capable to reduce AIP protein expression in an vitro model (39, 40), suggesting that both AIP and these miRNAs may be linked to pituitary adenoma tumorigenesis. Palumbo T. and colleagues found that simultaneous treatment with a miR-26 inhibitor and a miR-128 mimic blocks both tumorigenicity and invasiveness of GH3 and the pituitary somatotrophic MtT/S cell line. They identified PTEN as a direct target of miR-26b and polycomb complex protein (BMI1) as a direct target of miR-128. These two miRNAs, regulating PTEN/AKT pathway, not only control cell tumorigenicity and invasiveness in vitro, but also cell growth in vivo (29). Moreover, miR-16-1 levels are negatively correlated with RARS expression and directly correlated

with the secretion of tRNA-interacting factor p43, not only in PRL-, but also in GH-secreting pituitary adenomas (22).

miRNAs in pituitary non-functioning pituitary adenoma

A large study concerning miRNAs in NFA tissues was made by Butz and colleagues (41). They compared the expression levels of 670 miRNAs of 10 NFA with 10 normal pituitary tissues. They found that 92 miRNAs are down-regulated, while 70 are up-regulated. They identified miR-124*, miR-515-5p and miR-872 as expressed only in NFA samples and miR-198, miR-299-5p, miR-497*, miR-548c-3p and miR-622 as displayed only in normal tissues. Prediction analysis indicated that a specific sub-set of these miRNAs may be associated with the down-regulation of some molecular components of transforming growth factor beta (TGFβ) signaling pathway (Smad3, Smad6, Smad9, MEG and DLK1). Moreover, it has been demonstrated that 3 of the 80 genes belonging to the imprinted DLK1/MEG3 locus are scanty or not expressed as compared to normal pituitary tissues (30). Recently, 13 miRNAs mapping in this locus were found significantly down-regulated as compared to normal pituitary, suggesting that this chromosomal region is a potential hot-spot for molecular alterations involved in NFA development. One of these miRNAs, miR-134, restrains cell cycle progression in G2/M phase in folliculostellate cells originated from a human NFA, underlying an onco-suppressor activity of the DLK1/MEG3 locus in these tumors (30).

Wee1 protein, a mitotic inhibitor that is capable to block cell cycle in G2 phase, is usually expressed at low levels in NFA as compared to normal pituitary. Three miRNAs (miR-128, miR-155 and miR-516a-3p) have been reported as over-expressed in NFA and Wee1 has been identified as their target. The over-expression of miR-128, miR-155 and miR-516a-3p reduces Wee1 expression and human ovarian cancer HeLa cell viability (42), suggesting that these miRNAs are involved in pituitary tumorigenesis .

miRNAs and tumor size

In 2005 the first evidence that micro- and macroadenomas have different miRNAs expression profile had been reported. Indeed, Bottoni and colleagues demonstrated that miR-15a

and miR-16-1 levels inversely correlate with tumor diameter of GH- and PRL-secreting pituitary adenomas (22). Later, the same authors demonstrated that mir-140, mir-099b, mir-099, mir-030c, mir-030b and mir-138-2 are differentially expressed in micro- vs. macro-NFA (23). Henceforward, other miRNAs have been identified as differentially expressed in micro- and macro adenomas. miR-184, miR-524-5p, miR-629 and miR -766 are up-regulated, while miR-124, miR-222, miR-32, miR-744 and miR-765 are down-regulated in macro- as compared to micro-GH-secreting pituitary adenomas (36). In addition, Butz and colleagues found a negative correlation between the levels of 18 miRNAs (miR-424, miR-503, miR-450b-5p, miR-542-3p, miR-592, miR-629, miR-214, miR-224, miR-581, miR-510, miR-221*, miR-504, miR-215, miR-143, miR-181a, miR-103, miR-186 and miR-708) and tumor size of NFA (41).

All together these results indicate that miRNAs signature is associated with pituitary tumor growth.

miRNAs in aggressive pituitary adenomas

miR-183 has been identified as one of the principal miRNA that drives the progression of PRL-secreting pituitary tumors. This miRNA, that is under-expressed in aggressive tumors as compared to non-aggressive tumors, targets PCNA-associated factor (KIAA0101) allowing the lactotroph cells to acquire aggressive and malignant features (43). In addition, it has been found that miR-132 and miR-15a/16 are under-expressed in invasive pituitary tumors as compared to non-invasive ones. The increase in miR-132 and miR-15a/16 levels reduces cell proliferation, migration, invasion and epithelial to mesenchymal transition process via Sox5 transcription factor. Accordingly, the latter is over-expressed in invasive pituitary tumors as compared to non-invasive ones (44). In addition, it has been found that miR-34a negatively regulates AIP levels and is related to low AIP expression displayed by GH-secreting pituitary adenomas that are invasive and resistant to treatment (39).

These results indicate that pituitary adenoma cells acquire an aggressive behavior, at least in part, by miRNAs deregulation.

miRNA and current available pituitary adenoma therapy

In 2007, miRNAs expression profile was associated with response to therapy. mir-134, mir-148, mir-155, mir-029b and mir-200a were identified as differentially expressed between pharmacologically treated and untreated NFA (23, 45). Similar results were underlined also in other hystotypes. Tissues deriving from GH-secreting pituitary adenoma patients treated with Lanreotide display a different miRNAs expression profile as compared to Lanreotide-untreated patients. The former display 8 up-regulated miRNAs (miR-183, miR-193a-5p, miR-222, miR-516b, miR-524-5p, miR-601, miR-629 and miR-99b) and 5 down-regulated miRNAs (miR-124, miR-32, miR-574-5p, miR-744 and miR-96) as compared to Lanreotide-untreated patients (36). Moreover, Fan and colleagues, investigating 20 GH-secreting pituitary adenomas, found that miR-155, miR-185, miR-297, miR-519d, miR-766, and miR-934 are more expressed in Lanreotide-non responder group as compared to responder group. miR-185 controls the expression of SSTR2, that was validated as its target. Indeed, Lanreotide-non responder group displays lower SSTR2 levels than responders (38). These results suggest that GH-secreting pituitary adenomas response to Lanreotide may be attributed to not only SSTR2 expression, but also to miR-185 levels. It has also been demonstrated that miR-34 expression is inversely correlated with long-acting octreotide response of GH-secreting pituitary adenomas, an effect likely mediated by AIP, a miR-34 target (39).

It has also been reported several differentially expressed miRNAs in bromocriptine-resistant prolactinomas as compare to bromocriptine-sensitive prolactinomas (46, 47). Among these miRNAs, miR-93 is capable to increase the response of MMQ cells to dopamine treatment modulating the expression of its target, p21 (47).

All together these results demonstrate that miRNAs are associated with tumor response to therapy by modulating the expression of known players of pituitary pathogenesis.

miRNAs in pituitary carcinoma

Pituitary carcinoma is an aggressive neoplasm that originates from adenohypophysial cells, likely developing from a benign pituitary adenoma. It accounts for 0.1% of all pituitary neoplasms and, despite the availability of multidisciplinary medical approaches, it is usually related to a grim prognosis. In the presence of systemic metastases, these patients survive <12 months after diagnosis (48). The acquisition of molecular alterations increasing invasive and metastatic potential of pituitary cells represents a key step of pituitary carcinoma pathogenesis. It has been observed an expression deregulation of genes known to be involved in cell cycle control that are already associated to some aggressive forms of pituitary adenoma, but not to benign pituitary tumors. Indeed, pituitary carcinomas scarcely express the cell cycle regulators p53, pRB and nm23, as well as many other genes (HER- 2/neu, cyclooxygenase-2, EGFR) (48). Recently, miRNAs have been called upon to clarify, at least in part, the complex mechanism of carcinoma development. It is known that miR-122 and miR-493 are over-expressed in ACTH-secreting pituitary carcinoma as compared to normal pituitary and ACTH-secreting pituitary adenomas (26). miR-20 and miR-17-5p are over-expressed in non-functioning metastatic pituitary carcinoma as compared to adenomas. Consistently, their putative targets, PTEN and TIMP metallopeptidase inhibitor 2, are under-expressed (49).

These results indicate that miRNAs are involved in pituitary tumor progression and suggest that miRNAs profile may differentiate pituitary carcinoma from adenoma.

Useful tools to identify miRNA targets and their possible role

There are several bioinformatic tools that can be used to predict miRNA targets (miRanda, Targetscan, PicTar, RNAhybrid, DIANA-microT) (50). The results of these softwares give back a list of putative miRNA targets, that need to be be validated. To date, literature is full of manuscripts reporting putative miRNA targets without any experimental validation, promoting data misinterpretation. In order to minimizing this issue, in 2006, a database recording published experimentally supported miRNA targets was developed. Recently, it has been released a new

version of this database called DIANA-TarBAse, indexing more than half a million experimentally validated miRNA targets (51). Deregulated miRNAs in pituitary tumors with experimentally validated targets are reported in Tab.1.

It is worth mentioning a new web-server called DIANA-miRPath (52). It provides the pathways in which a specific miRNA is involved, using miRNA targets predicted by DIANA-microT or validated miRNA targets recorded in DIANA-TarBase.

Circulating miRNAs: the new frontiers in pituitary research

Recently, extracellular miRNAs have been identified in many biological fluids, including blood, urine, saliva, tears, breast milk, cerebrospinal and peritoneal fluids (53). miRNAs can be secreted by several cell types as part of microvesicles or complexed with argonauta proteins or high-density lipoproteins (54). Circulating miRNAs are capable to modulate other cell behaviour as a paracrine action. Gastric cancer cells are able to secrete tumor-suppressor miRNAs to preserve their oncogenic processes (55), highlighting the existence of a miRNA-based cell communication. Extracellular miRNAs, secreted by a tissue with a specific disease, may represent potential biomarkers of a pathologic process. To date, there are no studies regarding circulating miRNAs in pituitary tumors, but it has been reported that extracellular miRNAs are differentially expressed in GH treated subjects as compared to both individuals with high levels of GH and normal controls (56). In the light of this, circulating miRNAs represent a promising field for future research in pituitary diseases.

RNAs as therapeutic agents

The chance that miRNAs may represent an innovative therapeutic agent is one of the main exciting ideas that swirls around in our head. An hypothesis that still persists today surrounded by a series of concerns. Many technologies to manipulate miRNA functions in vivo have been developed. Three

approaches are usually employed to reduce miRNAs function: generation of genetic knockout animals, miRNA sponges and antimiR oligonucleotides. miRNAs knockout is the first method used to identify the role of specific miRNAs in C. elegans, mouse and Drosophila (57, 58, 59). On the other hand, the approach based on miRNA sponge consists in transfection or viral delivery of a transgene that contains multiple miRNAs target sites allowing the inhibition of an entire family of miRNAs. This approach has confirmed to be a helpful method to explore miRNA role in several models (60). The third approach consists in the employment of chemically modified oligonucleotides, called antimiRs, that bind to a specific miRNA preventing its action. Literature is studded of studies demonstrating the feasibility of oligonucleotides "in vivo" delivery and antimiR efficacy (61). MiRNA-Masking Antisense oligonucleotides Technology (miR-Mask) is another method to prevent miRNA action. miRMASKs are chemically modified antisense oligonucleotides that bind to the miRNA site in the 3'UTR of a miRNA target. miR-Mask, as suggested by its name, masks miRNA-binding site preventing target down-regulation. This approach has been efficaciously employed to protect TGF-β signaling pathways from miR-430 effects in zebrafish (62).

There are also approaches to increase miRNAs function: generation of systemic or organ-specific transgenic animals (63, 64), miRNA mimics and vector-based miRNA up-regulation (65, 66).

To date, the efficacy of a miRNA-based therapy has been demonstrated in neoplasms different from pituitary adenomas. It has been hypothesized that the silencing of the oncogenic miR-21 may represent a therapeutic strategy in pituitary adenomas (67). Unfortunately, it is only a theory that should be verified.

Much progress has been made in pituitary adenomas therapy employing epi-drugs. It is known that epigenetic modifications are involved in gene expression regulation; as for coding-genes, also miRNAs expression can be turned off by hypermethylation of CpG islands and hystone modifications.

Epigenetic therapy is aimed at overcoming the alteration of gene expression by modulating the action of epigenetic machine components. Recently, it has been demonstrated that epi-drugs allow the re-expression of silenced miRNAs, targeting HMGA mRNAs, in an "in vitro" model of GH-secreting pituitary adenoma cells (68), suggesting their promising role as miRNA-modulators in pituitary adenomas.

The capability of miRNAs to regulate multiple genes makes them suitable to be targets of innovative therapeutic strategy. While, this ability represents an advantage to block more than one signaling pathways, on the one hand, it may increase the number of unwanted side effects making their usage extremely difficult.

Summary

miRNAs are key regulators of gene expression and, as such, carry out important physiological processes in many tissues including pituitary. For over 30 years, the onset of tumors has been related only to the alterations in protein coding genes. Today, it is known that also miRNAs are involved in pituitary neoplastic processes. The scientific community reached a great success identifying ~~thousands~~ several ~~of~~ miRNAs with altered expression in pituitary tumors. At this juncture we need to take stock. Since pituitary tumors display a different behaviour on the basis of the histotype, it would be worth to categorize deregulated miRNAs belonging to a specific class of tumor. miRNAs have shown themselves to be possible pituitary tumor biomarkers. Indeed, their expression is histotype-specific and correlates with tumor size, clinicopathological features and treatment response. Despite there is reliable evidence to show miRNA participation in pituitary neoplastic process, the mechanisms in which they are involved are little understood. The validation of miRNAs targets should be the next step to go deeply inside their role. These advancements will give us the opportunity to manipulate miRNAs functions for therapeutic use. To this aim, several issues should be addressed: the possibility to successfully deliver a miRNAs-based therapy to pituitary, its efficacy and side-effects. The identification of miRNAs targets will be the first step to answer these questions. miRNAs are promising diagnostic, prognostic and therapeutic tools, but the

current knowledge can be only compared to a snapshot. Even though there are several unanswered questions, none of these are unanswerable. Concerning miRNAs knowledge, it really can be said "use it or lose it".

Research agenda

- Identify deregulated miRNAs in the setting of a specific class of pituitary tumor (micro-, macroadenomas, NFA, GH-, PRL-, LH/FSH-, ACTH and TSH-secreting pituitary tumors)
- Identify the targets of deregulated miRNA in pituitary tumors
- Explore the pathways in which deregulated miRNAs are involved and define a dynamic miRNA/target network
- Investigate the functions of circulating miRNAs in pituitary tumorigenesis and their role as biomarkers of pituitary neoplasms
- Verify the feasibility of miRNAs-based therapy in "in vivo" models of pituitary tumors

Conflict of interest statement

We disclose the lack of any financial and personal relationships with other people or organizations that could inappropriately influence the content of this article.

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Tab.1 miRNAs and their targets in pituitary tumors

miRNA	Expression in pituitary adenoma	Tumor type	Validated target	Cell model for target validation	Processes under miRNA control in pituitary	Ref.
miR-107	over-expressed	GH-, NFA	AIP	GH3	cell proliferation	40
miR-128	down-regulated	GH-	BMI1	GH3 MtT/S	cell proliferation, invasiveness	29
miR-128a	over-expressed	NFA	Wee1	HELA	-	42
miR-130b	down-regulated	GH-, FSH/LH-, NFA	CCNA2	HEK-293	GH3 cell proliferation and cell cycle	37
miR-155	over-expressed	NFA	Wee1	HELA	-	42
miR-185	down-regulated	GH-	SSTR2	GH3	cell proliferation, apoptosis	38
miR-200c	over-expressed	PRL-	PTEN	MMQ	apoptosis	35
miR-26a	over-expressed	ACTH-	PRKCD	AtT20/D16v-F2	cell viability, cell cycle	27
miR-26b	over-expressed	GH-	PTEN	GH3 MtT/S	cell proliferation, invasiveness	29
miR-300	down-regulated	PRL- GH-	PTTG1	GH3 MMQ	cell proliferation, cell viability, motility, apoptosis	34
miR-326	down-regulated	GH-	HMGA2, E2F1	MEG-01	GH3 cell proliferation and cell cycle	31
miR-329	down-regulated	PRL- GH-	PTTG1	GH3 MMQ	cell proliferation, cell viability, motility, apoptosis	34
miR-34a	over-expressed in low AIP protein somatotropinomas	GH-	AIP	GH3	invasiveness	39
miR-34b	down-regulated	GH-	HMGA1, HMGA2	MEG-01	GH3 cell proliferation and cell cycle	31
miR-570	down-regulated	GH-	HMGA2	MEG-01	GH3 cell proliferation and cell cycle	31
miR-381	down-regulated	PRL- GH-	PTTG1	GH3 MMQ	cell proliferation, cell viability, motility, apoptosis	34
miR-432	down-regulated	GH-	HMGA2	MEG-01	GH3 cell proliferation and cell cycle	31
miR-516a-3p	over-expressed	NFA	Wee1	HELA	-	42
miR-548c-3p	down-regulated	GH-	HMGA1, HMGA2	MEG-01	GH3 cell proliferation and cell cycle	31
miR-603	down-regulated	GH-	E2F1	MEG-01	GH3 cell proliferation and cell cycle	31
miR-655	down-regulated	PRL- GH-	PTTG1	GH3 MMQ	cell proliferation, cell viability, motility, apoptosis	34