MicroRNAs-based network: A novel therapeutic agent in pituitary adenoma

Xiuhua Shi a,1, Bangbao Tao b,1, Hua He b,1, Qingfang Sun a,e, Changyan Fan a, Liuguang Bian a, Weiguo Zhao a, Yi-cheng Lu b,*

a Department of Neurosurgery, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, 197 Rui Jin Second Road, Shanghai 200025, PR China
b Department of Neurosurgery, Changzheng Hospital, Second Affiliated Hospital of Second Military Medical University, 415 Feng Yang Road, Shanghai 200003, PR China

ABSTRACT

Pituitary adenomas are common benign intracranial neoplasms representing about 10–25% of all intracranial neoplasm. Significant morbidity can occur along with pituitary adenomas due to hormonal dysfunction and mass effects. The pathogenesis of pituitary adenoma is unclear, however, etiologic factors include genetic events, hormonal stimulation, and growth factors [1], all of which promote cell proliferation and transformation in the tumor. However, genetic events play the most important role in tumorigenesis. MicroRNAs (miRNAs), a class of non-coding RNAs, not only have function in pituitary cell proliferation and apoptosis but also in neoplastic transformation. It has been shown that miRNAs are differentially expressed in pituitary adenoma when compared with the normal pituitary gland; moreover, miRNAs have been identified as a predictive signature of pituitary adenoma and can be used to predict the histotype. The expression of miRNAs can be used not only to differentiate microadenomas from macroadenomas, but to also distinguish samples of treated patients from samples of non-treated patients. Therefore, we hypothesized that a miRNA-based network may be involved in pituitary tumorigenesis and it can potentially serve as useful diagnostic markers to improve the classification of pituitary adenomas. Here, we reviewed the therapeutic potential that different types of miRNAs may play in tumorigenesis. Moreover, miRNAs may emerge as potential therapeutic targets. We speculated the mechanism of miR-21 is involved in tumorigenesis, leading to improvements in therapies and prevention of metastasis.

© 2011 Elsevier Ltd. All rights reserved.

Background introduction

miRNAs are a family of 21–25-nucleotide small RNAs, which are a class of non-coding RNAs that function as endogenous triggers of the RNA interference pathway. They are generated by a series of cleavage from long polymerase II-transcribed RNA [2] and can recognize RNA interference pathway. They are generated by a series of cleavages of RNA polymerase II transcripts, which results in a melting effect on neighboring regions, thereby altering the secondary structure and enabling or inhibiting the binding of other miRNAs. Therefore, it will be interesting to test these hypotheses [5].

Recently, Asa et al. identified a much higher (approximately 16.7% of the general population [6]) incidence of pituitary tumors using molecular biology than previously published. Although pituitary dysfunction is uncommon, pituitary adenomas seriously affect quality of life. Early studies have proven that the adenomas of adenohypophysis are monoclonal neoplasms [7]. Interactions between etiologic factors containing genetic events, hormonal stimulation, and growth factors can promote cell proliferation and transformation in the pathogenesis of pituitary adenoma [1].

Zhang et al. reported that more than 50% of miRNAs genes are located in cancer-associated genomic regions or in fragile sites, suggesting that miRNAs may play a more important role in the pathogenesis of a limited range of human cancers than previously thought [8]. Notably, recent reports have suggested that miRNAs play roles in human cancers [9–11]. Similarly, miRNAs roles have been proven in plants, mammals, and other species. Additionally, miRNA databases reveal that approximately 10% of human miRNAs are homologous with cloned miRNAs from other species (mainly mouse) [12]. The most recent released miRNA Targets Base [13] (15 October

* Corresponding authors. Tel.: +86 21 64333548 (Q. Sun); fax: +86 21 64333548 (Q. Sun), Luyi_cheng@hotmail.com (Y.-c. Lu).
E-mail addresses: rjns123@yahoo.com, Sunqingfang44@hotmail.com (Q. Sun), Luyi_cheng@hotmail.com (Y.-c. Lu).
1 The authors equally contributed to the work.
2010 of miRTarBase) lists 3576 curated MTIs between 657 miRNAs and 2297 target genes. In the miRTarBase, hsa-miR-122 is recorded as having 45 target genes, validated experimentally by luciferase reporter assays or Western blot. Computational algorithms predict that 976 miRNAs may exist in the human genome [14]. Lewis et al. identified orthologous genes from human, mouse, and rat, which show conserved predicted sites for the same miRNA [15].

Recently, it has been shown that miRNAs might be a new class of genes involved in human tumorigenesis [5,12,16,17]. Bottini et al. previously reported downregulation of miR-15a and miR-16a in pituitary adenoma [16]. In later studies, this group identified 30 miRNAs that were differentially expressed by comparing normal pituitary gland with pituitary adenoma by microarray and Real-Time PCR analysis of the entire mRNAome in 32 pituitary adenomas and six normal pituitary samples. Furthermore, 24 miRNAs have been identified as predictive signatures of pituitary adenoma and 29 miRNAs can be used to predict histotypes of pituitary adenoma. The expression of miRNAs could differentiate microadenomas from macroadenomas and distinguish samples of treated patients from samples of non-treated patient [16,17]. Current research suggests that the expression of miRNAs can change dynamically, influencing pituitary gene expression with high plasticity.

Hypothesis

Pituitary adenomas display a specific miRNA signature depending not only on their characteristics, such as size, hypotype, remission, and previous medical treatments, but also on the function in the ongoing neoplastic process [18]. Moore et al. have reported that miR-21 is overexpressed in glioma and glioma cell lines [19]. Sayed et al. have shown that miR-21 can be used as a biomarker of oncogenic miRNAs for the detection of leukemia, pancreatic, lung, and liver cancers. miR-21 and miR-155 are overexpressed in tumors or tumor cell lines [20,21]. In addition, miR-155 is also overexpressed in pituitary adenomas, while the expression of miR-21 is downregulated; therefore, we speculated that miR-21 maybe involved in the tumorigenesis of pituitary adenoma.

It has been shown that validated targets of miR-21 in glioma include: ephosphatase and tensin homologue (PTEN), reversion-inducing-cysteine-rich protein with kazal motifs (RECK), and tissue inhibitor of metalloprotease 3 (TIMP3) [19]. A common characteristic of these targets is their tumor suppressor activities. The inactivation of PTEN leads to deregulating PI3K/Akt signaling and uncontrolled growth of tumor cells. Overexpression of miR-21 activates the PI3K/Akt pro-survival pathway, leading to the phosphorylation of effector proteins, consequently improving cell survival [22]. In addition, RECK and TIMP3 are unregulated upon inhibition of the expression of miR-21 and downregulated with the overexpression of miR-21, and have been shown to play a key role in tumor invasion and metastasis [19]. Furthermore, this study confirmed direct regulation of RECK but not TIMP3 by miR-21 via luciferase assay, suggesting that TIMP3 may be an indirect target of miR-21 or that it serves as a downstream effector [23]. In addition to the roles of miR-21 in the regulation of RECK and TIMP3, miR-21 has been shown to directly inhibit Fas ligand (FasL), a key activator of the extrinsic apoptotic pathway, in cardiac myocytes [20] but not in glioma. Furthermore, miR-21 can also activate mitogen-activated protein kinase (MAPK) pathway by inhibiting Spry1, which has been identified as a direct target of miR-21 in murine cardiac fibroblasts [24], leading to increased phosphorylation of extra cellular-signal-regulated kinase (ERK) and consequently, protecting from apoptosis in vitro and in a mouse model [24]. Therefore, we speculated that miR-21 is an oncogenic miRNA that could be used to detect pituitary adenomas. Moreover, based

![miR-21 as a potential therapeutic agent](image)

Fig. 1. A flow chart showing miR-21 as a potential therapeutic target for pituitary adenoma. We hypothesized that targets for miR-21 were PTEN (downregulated), RECK, TIMP3 (upregulated), and Fasl (upregulated), which may play a key role in pituitary adenoma-associated tumorigenesis via PI3K/Akt, MMPs, MAPK and ERK signaling pathway influencing tumor cell growth, apoptosis, invasion, and metastasis. PTEN: phosphatase and tensin homologue; RECK: reversion-inducing-cysteine-rich protein with kazal motifs; TIMP1: tissue inhibitor of metalloprotease 1; Fasl: Fas ligand; MMPs: matrix Metalloproteinase; MAPK: mitogen-activated protein kinase; ERK: extracellular signal regulated kinase.
on the fact that miR-21 can regulate cell growth of pituitary tumors and affects invasion and metastasis of pituitary adenomas by regulating its target genes (Fig. 1), we predicted that miRNAs can be used as a potential agent to treat pituitary adenomas.

**miRNA related with pituitary adenoma**

Characterization of adenoma size, hypotype, remission and previous medical treatments has been researched. For example, miR-15a and miR-16 inversely correlated with tumor diameter related with the secretion of the anaplastic cytokine p43 [16], miR-140 overexpressed in macro- than micro-adenoma [17]. The interaction of let-7/high-mobility group A2 (HMG2A) in number of human pituitary tumors is believed to correlate with the high grade, size and proliferation marker of pituitary adenomas (pa) [18]. The downregulated miR-141 in ACTH-secreting adenomas can influence the ratio of remission after surgery [25], miR-148 is overexpressed in treated NFAs when compared to the untreated NFA [9]. Furthermore, some differentially expressed miRNAs are associated with tumor size, lanreotide treatment, and responsiveness to SSA [26].

**Functions of miRNAs in pituitary adenoma**

Cimmino et al. have reported that both miR-15a and miR-16 potentiate the normal apoptotic response by targeting the anti-apoptotic gene BCL-2 [27], direct evidence for a role of miRNAs in tumorigenesis has been demonstrated in a mouse model of Burkitt’s lymphoma [9], and also suggest roles of miR-15a and miR-16-1 as tumor suppressor genes in pituitary adenoma [16]. The most likely candidate for cancer susceptibility gene (located at chromosome 13q14) is the miRNA cluster miR-15a and miR-16, containing miR-15a and miR-16-1 [28]. As mentioned above, miR-15a and miR-16-1 act as regulators of the anti-apoptotic BCL2 oncprotein in the leukemia cell line MEG-01, expression of miR-15a and/or miR-16-1 leads to downregulation of the expression of BCL2 and apoptosis [27]. Furthermore, such abnormal expression is found not only in malignant cells but also in premalignant stages, such as expression of miR-143 and miR-145 is reduced in pituitary adenomas [29]. Finally, a target gene of miR-16-1 is RARS gene, which is inversely related with miR-16-1 and is a part of the aminoacyl-tRNA synthetase complex (ARS), where it is associated with the aminoacyl tRNA synthetase interacting multifunctional protein (AIMP1), a secretary protein representing the precursor of the inflammatory cytokine endothelial monocye activating polypeptide II (EMAPII) released under apoptotic conditions. Furthermore, EMAPII also acts as a potent anticaner agent [16,17]. Furthermore, the expression of let-7 is reduced in pituitary adenomas, possibly causing upregulation of the human RAS family of oncoproteins [17].

By using the algorithms described in “Materials and Methods” section, the analysis of predicted target genes was performed for the most differentially expressed miRNAs in pituitary adenomas versus normal pituitary, including miR-024-1, miR-212, miR-026a, and miR-098 [17]. Cluster analysis, based on differentially expressed miRNAs, generated a hierarchy showing a clear distinction between pharmacologically treated and non-treated, non-functioning pituitary adenomas (NFAs) [17]. Four histotypes of pituitary adenomas (NFAs) [17]. Between microadenomas of the pituitary gland and pituitary macroadenomas the pituitary gland miR-140 is upregulated in macroadenomas. Cheng et al. have described that the expression of miR-140 reduces cell growth in the lung carcinoma cell line A549 [32] suggesting that increased expression of this miRNA in NFAs could be involved in the control of tumor growth. Results of cluster analysis suggest that the size of these samples might be more similar to that of micro-adenomas [17].

Cheng et al. have reported that inhibition of the expression of miRNAs such as miR-150, miR-152, miR-191, and miR-192 (upregulated in pituitary adenomas) suppresses cell growth [32], suggesting that the overexpression of these miRNAs might result in increased pituitary cell proliferation. This also indicates that altered expression of miRNAs is important in the development of pituitary tumors [32].

Although a lack of knowledge about miRNA target genes hampers the full understanding of the biological functions of miRNAs, the predicted target genes of several miRNAs have been reported in pituitary adenomas [17]. Among of the known putative targets, miR-24 and miR-98 are downregulated in pituitary adenoma. We found that a transcription factor called Caudal-type home box protein 2 (CDX-2) as a putative target of miR-24 was overexpressed in 81% of intestinal neuroendocrine carcinomas [33]. Moreover, vascular endothelial growth factor receptor 1 (VEGFR-1), which is correlated with development of cancers [34], is upregulated in NFAs by a cAMP-mediated mechanism. Furthermore, treatment with somatostatin analogues completely abrogates the growth-promoting effects of VEGF on human NFAs primary cultured cells expressing VEGF-R1 [35]. Human proto-oncogene proviral insertion site in moloney murine leukemia virus kinase (Pim-1), an oncogene, is involved in the control of cell growth, differentiation, and apoptosis, and its overexpression is a potential biomarker in prostate carcinoma [36]. In addition, the guanine nucleotide exchange factor (Vav-1), a proto-oncogene, has been implicated in the regulation of numerous pathways downstream of receptor tyrosine kinases participating in tumorigenesis.

miR-98 is a RIO kinase 3 which belongs to a family of kinases required for proper cell-cycle progression and chromosome maintenance [37]. MiR-98 also targets the HMG2A gene [38], and its overexpression is associated with HMG2A protein in PRL- or GH-secreting pituitary adenomas of transgenic mice [17]. Putative targets for downregulated miRNAs, such as miR-24 and miR-98, involve cytoskeleton organization and vesicle trafficking of CK2 interacting protein 1 [39] and Syntaxin [40]. All these findings confirm that many putative targets for downregulated miR-24 and miR-98 as encode proteins are associated with cell proliferation or with potential oncogenic functions of the pituitary gland.

The expressions of miR-26a and miR-212 are strongly upregulated in adenomas. The predicted target for miR-26a is home box
protein Hox-A5 and its expression is reduced or absent in inactive angiogenic endothelial cells in breast tumors or in proliferating infantile hemangiomas [41]. Recently, miR-26a has been shown to suppress cell growth and induces p53-dependent apoptosis, and its expression is higher in differentiated colon epithelial cells when compared to undifferentiated ones [17]. Moreover, pleomorphic adenoma gene 1 (PLAG1), a target of miR-26a [42] and a member of the PLAG family of tumor genes, is highly expressed in normal anterior pituitary gland. PLAG1 is downregulated in most pituitary adenomas [17], suggesting that this gene family might be involved in the development of pituitary adenomas.

In pituitary adenomas, miR-212 regulates cell death or dominance-containing protein (DEDD), as well as other proteins participating in apoptosis, which are involved in apoptotic signaling [17]. Functions of miRNAs as oncogenes or tumor suppressor genes in pituitary adenomas were summarized in Table 1. Thus, similar to the use of antisense miRNAs and RNAi, which are widely used as tools for studying gene functions and in some cases for gene therapy, regulation of cancer formation could be achieved by changing expressions of miRNAs and/or injection of miRNAs [5].

The hypothesis that upregulated miRNAs acting as oncogenes is also supported by in vivo models. For example, recent published methods for the inhibition of miRNAs in vivo using complementary oligonucleotides should encourage the application of gene ‘knockdown’ approaches for the efficient analysis of miRNAs function in cultured cells or intact animals [43,44]. Costinean et al. previously established a miR-155 transgenic mouse model that displays pre-B-cell expansion followed by B cell malignancy [45], and the miR-17-92 cluster that acts together with MYC to accelerate tumor development in a mouse B-cell lymphoma model [9].

**Antagomirs therapeutic role of miRNAs**

The presence of miRNAs has been verified in many diverse species. In plants, miRNAs have been used for gene therapy [46]. Krutzfeldt et al. have demonstrated that four miRNAs are successfully inhibited by injection of antagonoms (modified antisense RNAs) in adult mice [47]. Moreover, Dickins et al. have found that miR-30-based shRNAs (called shRNA-mirs) suppress gene expression when driven by Pol II promoters. They also observed that tumor growth is controlled by tightly regulating Trp53 knock-down using tetracycline-based systems, and gene knock-down by the expression of shRNA-mirs may be similar to overexpression of protein-coding cDNAs [48]. Three daily intravenous injections lead to robust suppression of miRNA in most tissues and last for weeks. These data support the idea of “antagomirs”, or therapeutic inhibitors of miRNAs [47].

**Summary and perspective**

For almost three decades, the alteration of protein-coding oncogenes and/or tumor-suppressor genes has been thought to cause tumorigenesis. Recent work has demonstrated that modified antisense RNAs can inhibit miRNAs function in the adult mouse [47]. None of the predicted or validated target genes, except for BCL2, has been analyzed in normal and pathologic pituitary gland so far, leaving this research field open [17].

However, some obstacles need to be overcome. First, specific miRNAs in a specific hypotype of pituitary adenoma should be identified. Only after specific miRNAs are identified and their mechanisms are elucidated in pituitary adenoma, we can manipulate these miRNAs for therapeutic purposes. The delivery of these miRNAs into targeted tissues and maintenance of continuous activity is another obstacle. Last, whether RNAi can be used to achieve these goals [5] is also an issue.

Pituitary adenoma is a complicated disease to classify and treat. Researchers have studied the mechanisms and treatment of pituitary adenoma for several decades; however, therapy for pituitary adenoma is not fully effective. Furthermore, agreement regarding microRNA-based network as a new therapeutic has not been reached. This strategy gives us a new point to investigate a

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Deregulation</th>
<th>Function</th>
<th>Validated targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-15a and miR-16a</td>
<td>Downregulated</td>
<td>Induces apoptosis and inhibits tumorigenesis [16]</td>
<td>BCL-2, WT1, RAB9B, MAGE83, WNT3A, CCND1, MCL1[17,38]</td>
</tr>
<tr>
<td>miR-98</td>
<td>Downregulated</td>
<td>Involved in cell growth and proliferation or with potential oncogenic functions [17]</td>
<td>CK2 interacting protein[41] and syntax in 17</td>
</tr>
<tr>
<td>miR-24</td>
<td>Downregulated (in PA and lung carcinoma cells; upregulated in NFAs)</td>
<td>Involved in cell growth and proliferation or with potential oncogenic functions [17]</td>
<td>CDX-2 [33], VEGFR-1 [34], Pim-1, Vav-1 [17, CK2 interacting protein[37] and syntax in 17</td>
</tr>
<tr>
<td>miR-26b</td>
<td>Downregulated (in GH and PRL-secreting adenomas compared with NFA)</td>
<td>Induces apoptosis and inhibits tumorigenesis [10]</td>
<td>TGF-dependent gene[17,31]</td>
</tr>
<tr>
<td>miR-132</td>
<td>Downregulated</td>
<td>Induces apoptosis and inhibits tumorigenesis [16]</td>
<td>Unreported</td>
</tr>
<tr>
<td>miR-143 and miR-145</td>
<td>Downregulated</td>
<td>Induces apoptosis and inhibits tumorigenesis [16,17]</td>
<td>Unreported</td>
</tr>
<tr>
<td>miR-23a, miR-23b, miR-24-2</td>
<td>Upregulated in GH-secreting and PRL-adenomas, downregulated in ACTH-adenoma, NFA</td>
<td>Growth &amp; localization of hematopoietic progenic cells; Neuronal development [2]</td>
<td>RAS, MYC and HMGA2[17,18]</td>
</tr>
<tr>
<td>miR-30a, miR-30b, miR-30c, and miR-30d</td>
<td>Upregulated in ACTH-secreting adenomas, downregulated in PRL-secreting adenoma</td>
<td>Early determination of corticortroph lineage during pituitary cytodifferentiation [17]</td>
<td>DEDD [17]</td>
</tr>
<tr>
<td>miR-140</td>
<td>Upregulated in macro-adenoma compared to micro-adenoma</td>
<td>Downregulate of the TGF-beta signaling through Smad 3 [48]</td>
<td>Could be ascribed to the early determination of corticotroph lineage during pituitary cytodifferentiation [30]</td>
</tr>
<tr>
<td>miR-150, miR-152</td>
<td>Upregulated</td>
<td>Unreported</td>
<td>Involved in the control of tumor growth [32]</td>
</tr>
<tr>
<td>miR-191, miR-192</td>
<td>Upregulated</td>
<td>Unreported</td>
<td>Involved in cell growth and apoptosis [32]</td>
</tr>
<tr>
<td>miR-148</td>
<td>Upregulated in treated NFAs</td>
<td>Different expressions and effects in two reports [32,17]</td>
<td>Involved in induction of apoptosis [17]</td>
</tr>
</tbody>
</table>
potential regulation of development and disease. Finally, this opens a new avenue of investigation into miRNAs as effectors of diverse cellular processes, new biomarkers for diagnoses and treatment of pituitary adenomas.

Conflict of interest statement
None declared.

Acknowledgments
This study was sponsored by National Natural Science Foundation of China (No: 30930094) and National Natural Science Foundation of Shanghai (Z082R1413).

References