MicroRNAs in Dopamine Agonist-Resistant Prolactinoma

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Keywords

Dopamine agonist-resistant prolactinomas · Management · MicroRNAs · Pituitary tumors · Transforming growth factor- β signaling

Abstract

Dopamine agonists (DAs) are preferred for the treatment of prolactinomas and are usually very effective. Nonetheless, 20–30% of bromocriptine- and approximately 10% of cabergoline-treated individuals exhibit resistance to DAs. In addition, the mechanism underlying this phenomenon remains elusive. In this study, we summarize the major findings regarding the role of microRNAs (miRNAs) in the pathogenesis of DA-resistant prolactinoma (DARP). Currently available evidence suggests that miRNAs are usually dysregulated in DARP and that, although controversial, the dysregulated miRNAs target the transforming growth factor (TGF)- β , dopamine 2 receptor (D2R), or estradiol (E2)/estrogen receptor (ER) signaling pathways to mediate the therapeutic effect of DAs. These findings provide new incentives for research on innovative strategies for predicting patients' responsiveness to dopamine therapies and for developing treatment approaches. Unfortunately, recent studies tended to focus exclusively on the differential miRNA expression profiles be-

tween DARP and dopamine-sensitive prolactinoma, and no definitive consensus has been reached regarding the role of these miRNAs in the modulation mechanism. Therefore, current and future efforts should be directed toward the exploration of the mechanism underlying the dysregulation of miRNAs as well as of the target proteins that are affected by the dysregulated miRNAs. Furthermore, the modulation of the expression of dysregulated miRNAs, which target the D2R, TGF- β , or E2/ER signaling pathways, might be a promising alternative to treat patients with DARP and improve their prognosis.

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Introduction

Dopamine agonists (DAs) are preferred for and are generally highly effective for treating prolactinoma. However, 20–30% of bromocriptine (BRC)- and approximately 10% of cabergoline (CAB)-treated individuals are resistant to these agents. Moreover, the underlying mechanism remains to be determined. In this review, we summarize the major findings about the role of microRNAs (miRNAs) in the pathogenesis of DA-resistant prolactinoma (DARP).

DARP

Diagnosis of DARP

Prolactinoma, which is the most prevalent neuroendocrine disease, represents approximately 40% of all pituitary lesions, which accounts for 10–15% of primary intracranial neoplasms [1]. Currently, DAs, including BRC and CAB, are regarded as first-line drugs and are usually very effective in the treatment of prolactinomas [2]. However, some patients are unresponsive to treatment with DAs, even under maximally tolerated doses, which is considered as DA resistance [3]. Unfortunately, there is no consensus regarding the concept of DARP despite the emergence of new DA drugs and enhanced clinical therapeutic opportunities [1]. The treatment goals of prolactinomas, which are active adenomas, are to normalize the serum prolactin (PRL) levels and induce tumor shrinkage [1, 4, 5]. Initially, physicians simply focused on the hormonal response to the drugs and regarded the inability to reduce PRL levels sufficiently to achieve ovulation or the failure to induce 50% reduction in hyperprolactinemia as DARP [4, 6, 7]. In 2001, Di Sarno et al. [4] advocated that normalizing the serum PRL level and inducing tumor shrinkage should be the targets for prolactinoma treatment and defined this condition as the inability to normalize serum PRL levels and shrink tumors. However, regarding tumor reduction, several supplementary criteria have also been proposed in the literature, including changes in the maximum diameter, height, surface, or volume of tumors in CT or MRI scans during treatment with DAs, and favorable responses have been reported in terms of shrinkage percentage, ranging from 25 to 80% [4, 8]. As the risk of compression depends primarily upon tumor expansion to the optic chiasm, an appropriate parameter for tumor response appears to be a reduction in tumor height of >30% or a decrease in tumor coronal surface of >50% [9]. Therefore, Molitch's [3, 10] diagnosis of DARP uses the following criteria and are widely accepted and applied: (1) failure to achieve a normal PRL level at the maximum tolerated dose or (2) failure to reduce the tumor size by at least 50%. According to this definition, he reported that hormonal resistance ranges from 11 for CAB to 24% for BRC, whereas insufficient reduction in tumor size was observed in 4 of CAB- and 36% of BRC-treated patients [5]. More recently, however, Maiter [8] reported that this simple definition is arbitrary. Considering the dose and the type of drug, as well as the period over which the drug is prescribed, he defined DARP as: (1) the failure to achieve normoprolactinemia under maximally tolerated doses of DA for at least 3–6 months and (2) the lack of a $\geq 30\%$ reduction in tumor di-

ameter. According to these criteria, the authors found that the incidence of resistant prolactinomas was approximately 20–30% for BRC, and that the hormonal resistance to CAB was <10% for microprolactinomas and 15–20% for macroprolactinomas [10–12]. Therefore, the prevalence of DA resistance often varies widely among clinical studies because of the different definitions of DA resistance and the types of DA drugs used in specific studies.

Management of DARP

The management of DARP has been reviewed in detail previously [8, 13, 14] and is not the focus of this review. In brief, a patient diagnosed with resistance to BRC should first switch to another DA, such as CAB, and undergo treatment for 3–6 months with the maximal dose (3.5 mg/week) [5, 15]. If the patient meets the requirements for a diagnosis of DARP discussed earlier and resistance to CAB has been confirmed, the physician should generally follow a sequence involving surgical debulking, radiotherapy, or administration of experimental medicines such as temozolomide [5, 8].

Potential Mechanisms of DA Resistance

The mechanism underlying DA resistance in prolactinoma remains elusive, although much effort has been dedicated to this subject. As shown in Figure 1, several potential mechanisms of DA resistance have been proposed, which are as follows: (1) decreased expression of dopamine 2 receptor (D2R) and small cellular downstream signaling molecules of dopamine receptors [6, 16–21]; (2) alterations in other receptors that modulate the expression of the dopamine receptor, such as the nerve growth factor receptor [19], estrogen receptor (ER) [19, 22], and mTOR signaling [23]; (3) impairment of the transforming growth factor (TGF)- β signaling pathway [24–27]; and (4) dysregulation of miRNAs [27–30]. In this review, we focus on recent advances in the study of miRNAs, their potential involvement in the resistance to DA treatment, and future perspectives.

miRNAs in DARP

miRNAs in Prolactinoma

The first miRNA was discovered nearly 40 years ago in the nematode *Caenorhabditis elegans* with the identification of the developmental regulator *lin 4*. Currently, over 2,000 miRNAs have been identified in humans, and the dysregulated expression of miRNAs is believed to be associated with numerous pathological conditions, such as

Fig. 1. Potential mechanisms involved in the DARP. DARP, dopamine agonist-resistant prolactinoma; D2R, dopamine 2 receptor; TGF, transforming growth factor; BRC, bromocriptine; CAB, cabergoline; miRNA, microRNA; ER, estrogen receptor; PRL, prolactin.

pituitary adenomas [31], cancer progression [32], and chemotherapy resistance [33]. Current evidence suggests that miRNAs play a vital role in pituitary development and pituitary hormone secretion [34–38]; moreover, dysregulated miRNA expression has been confirmed to be involved in pituitary disorders [31, 39, 40].

The first investigation of miRNAs in patients with pituitary adenoma, which was conducted by Bottoni et al. [41], shed light on the role of miRNAs in pituitary adenoma and demonstrated that the expressions of miR-15a and miR-16-1 were downregulated in pituitary adenomas compared with those in normal pituitary tissue. Subsequent studies focused on differential miRNA expression between prolactinomas and the pituitary gland and their potential role in the tumorigenesis of prolactinomas. Table 1 summarizes the dysregulated miRNAs identified to date [42–47]. Collectively, these reports suggest that dysregulated miRNAs play a significant role in the development of prolactinomas via the mediation of the expression of target genes.

Recently, the focus of research in this field has shifted to the expression profile of miRNAs after pharmacological treatment: Wang et al. [48] examined 15 patients with prolactinoma and found that there were 80 upregulated miRNAs and 71 downregulated miRNAs in treated versus untreated patients. The expressions of miR-206, miR-516b, and miR-550 were confirmed to be significantly upregulated, whereas that of miR-671-5p was significantly downregulated in treated versus untreated prolactinomas. A subsequent bioinformatics analysis demonstrated that mitogen-activated protein kinase and survival (mTOR and TGF- β) signaling pathways, which are involved in the pathogenesis of DARP, were abundant among the significantly enriched pathways.

miRNAs in DARP

Current evidence suggests that some miRNAs and their target genes have been identified in DARP after the investigation of differential miRNA expression in pa-

Table 1. Summary of miRNAs involved in prolactinoma and DARP

miR-RNAs	Expression	Model/cell line	Potential target genes or signaling pathway	Biological effect	References
miR-320	↓	Patients/GH3/HP75/MEG01	/	/	D'Angelo et al. [43]
miR-34b	↓	Patients/GH3/HP75/MEG01	HMGA1 and HMGA2	Affects G1-S transition	
miR-374	↓	Patients/GH3/HP75/MEG01	/	/	Chen et al. [42]
miR-342-3p, miR-432, miR-23b, miR-493, miR-493*, miR-664*	↑	Patients	/	/	
miR-199b-3p, miR-125b, miR-200b, miR-130a	↓	Patients	/	/	
miR-15, miR-16, miR-26a, let-7a, miR-196a2	↓	Patients/GH3/MEG01	HMGA1 and HMGA2	Negatively regulates pituitary cell proliferation	Palmieri et al. [44]
miR-410	↑	Patients/HEK-293	CCNB1	Inhibits tumor cell proliferation	Mussnich et al. [45]
miR-183	↓	Patients/HeLa/ZR-75-1	KIAA0101	Inhibits tumor cell proliferation	Roche et al. [46]
miR-340*	↓	Patients	NEK2	Linked to the aggressiveness of prolactinomas	
miR-98	↓	Patients	AURKB, CCNB2, CENPK, UBE2T, E2F2		
miR-744	↓	Patients	TGF-beta 1	Inhibits tumor cell invasion	Lei et al. [47]
miR-137	↓	Patients/MMQ/GH3	MtIF/Wnt β-catenin pathway	Mediates therapy resistant to DA of tumor	Jian et al. [30]
miR-145-5p	↓	Patients/MMQ	TPT1	DA of tumor	
miR-93-5p	↑	Patients/MMQ/GH3	ATG7	Mediates therapy resistant to DA of tumor	Wu et al. [29]
miR-93	↑	Patients/MMQ	p21	Mediates therapy resistant to DA of tumor	Wu et al. [28]
miR-17, miR-22*, miR-126*, miR-142-3p, miR-144*, miR-486-5p, miR-451, miR-92a	↑	Patients	/	/	
miR-30a, miR-382, miR-136	↓	Patients	/	/	
miR-93-5p	↑	Patients/MMQ/HS27	Smad7 and TGF-β1/Smad3	Mediates therapy resistant to DA of tumor	Hu et al. [27]
miR-1229	↑	Patients/MMQ	FOXO1	Mediates therapy resistance to DA of tumor	Xiao et al. [49]
miR-142-5p, miR-148-3p, miR-100-5p, miR-582-3p, miR-29c-3p	↓	Patients	/	Might be related to DA therapy resistance	
miR-125a-5p, miR-1-3p, miR-133a-3p, miR-224-5p, miR-485-5p, miR-331-3p, miR-664a-5p, miR-98-5p	↑	Patients	/	Might be related to DA therapy resistance	

DARP, dopamine agonist-resistant prolactinoma; miRNA, microRNA; DA, dopamine agonist.

Fig. 2. Dysregulation of miRNA may be involved in DARP by impairing signaling of TGF receptors and D2R. DARP, dopamine agonist-resistant prolactinoma; miRNA, microRNA; TGF, transforming growth factor; D2R, dopamine 2 receptor; BRC, bromocriptine; CAB, cabergoline; PRL, prolactin.

tients with DARP versus those with DA-sensitive prolactinoma. A study conducted by Wu's team [28] explored the expression of miRNAs in DARP and reported that 41 miRNAs were differentially expressed between the 2 groups. Among them, 9 miRNAs were confirmed to be upregulated and 3 were downregulated, and these results were validated using qRT-PCR. Further studies revealed that miR-93 could modulate the expression of p21, leading to an increased response of MMQ cells to dopamine treatment [28]. Wu et al. [29] also demonstrated that miR-93 was upregulated in DARP and mediated DA resistance through autophagy downregulation by targeting ATG7. Another study showed that miR-145-5p was an important regulator of BRC resistance by controlling TPT1 expression because the upregulation of miR-145-

5p expression led to a reduction in the IC_{50} of the drug and an increase in TPT1 expression [30]. Moreover, Xiao et al. [49] reported that miR-1299 expression was upregulated in DARP and inhibited FOXO1 expression; the authors concluded that miR-1299 and FOXO1 are potential therapeutic targets for drug-resistant prolactinomas. These miRNAs are also summarized in Table 1.

The Potential Role of miRNAs in DARP

miRNAs Might Mediate the TGF Signaling Pathway in DARP

Accumulating evidence supports the contention that the TGF- β 1 signaling pathway is involved in the patho-

genesis of DARP [24–27]. As a multifunctional cytokine, TGF- β 1 is secreted to the extracellular matrix by pituitary lactotrophs, where it inhibits the proliferation of and PRL secretion by lactotroph cells, mediated largely via TGF- β type II receptors (TGF- β RII), in a similar manner to that described for dopamine. Recently, a series of 12 patients who underwent surgery because of resistance to BRC exhibited a significant reduction of TGF- β 1 and the downstream intracellular effectors Smad2 and Smad3 compared with normal human anterior pituitaries [24]. Therefore, it is crucial to explore the potential mechanism of impairment of TGF- β signaling pathways in DARP [24, 25]. Recently, dysregulated miRNAs have drawn much attention because miRNAs can regulate the expression of their target genes. Posttranscriptional regulation of the TGF- β signaling pathway by miRNAs, including miR-93 [27], miR-142-3p [50], and miR-30a [51], may have effects in DARP (Fig. 2). In 2014, Wu et al. [28] investigated the miRNA expression profile of patients with resistance to BRC compared with those sensitive to BRC. Forty-one miRNAs were differentially expressed between the 2 groups, according to Solexa sequencing. Using stem-loop qRT-PCR to verify the differential expression of these miRNAs, the authors showed that the expressions of miR-93, miR-17, miR-22*, miR-126*, miR-142-3p, miR-144*, miR-486-5p, miR-451, and miR-92a were upregulated, whereas those of miR-30a, miR-382, and miR-136 were downregulated in BRC-resistant prolactinomas. Finally, the authors selected miR-93 as a candidate miRNA for biological function analysis. A further study performed using MMQ cells, which are a cell type isolated from the 7315a rat prolactinoma that are responsive to dopamine [52], indicated that miR-93 (which targets the 3'-UTR of p21) silencing resulted in increased sensitivity of the cells to DA treatment by increasing p21 expression in the cells [28]. Interestingly, p21, which is mediated by miR-93, played a significant role in G1 arrest and was a potent cyclin-dependent kinase inhibitor that was required for TGF- β -mediated cell cycle arrest in melanoma [53]. More recently, Hu et al. [27] demonstrated that miR-93-5p targeted Smad7 to regulate the TGF- β 1/Smad3 pathway and mediated fibrosis in drug-resistant prolactinoma. Using high-throughput sequencing for the analysis of miRNAs, they found that miR-93-5p expression was significantly upregulated in prolactinoma samples with a high degree of fibrosis compared with that in samples without fibrosis. Furthermore, they demonstrated that miR-93-5p expression was negatively correlated with Smad7 expression and positively correlated with TGF- β 1 expression in clinical prolactinoma samples. Lu-

ciferase reporter assays supported the contention that miR-93-5p could downregulate the *Smad7* gene and activate TGF- β 1/Smad3-signaling-mediated fibrosis in a feed-forward loop. Hence, miR-93-5p could enhance the drug resistance of prolactinoma cells by regulating TGF- β 1/Smad3-dependent fibrosis [27]. In addition to miR-93, miR-142-3p expression was also confirmed to be upregulated in patients with DARP [28] and was found to mediate TGF- β receptor expression in non-small cell lung cancer [50]. Whether miRNA-142-3p is involved in resistance to DA therapy and the mechanism underlying this phenomenon require further investigation.

miRNAs Might Mediate D2R Transcription in DARP

The potential structure of DA treatment aiming at decreasing serum PRL levels and shrinking tumor sizes was discussed by Liu et al. [54]. They hypothesized that treatment with BRC and CAB induces pituitary adenoma cell death possibly through 3 mechanisms: apoptosis, autophagic cell death, and paraptosis [54]. This report supports the hypothesis that the therapeutic effect of DA is mediated by D2R or dopamine transporters present on the surface of lactotroph cells. Therefore, most researchers have focused on the structure, ligand affinity, isoforms, and density of D2R, as well as the intracellular transduction of the D2R signaling pathway in resistant prolactinomas [6, 17]. Current evidence suggests that D2R expression is downregulated in DARP [17, 19, 20]. This decreased D2R expression not only directly decreases the therapeutic effect of DA but also results in the decrease of both TGF- β 1 secretion and TGF- β RII expression in lactotroph cells [55], which in turn lessens the sensitivity of the response to DA.

D2R exhibits 2 alternatively spliced isoforms: the long (D2L) and short (D2S) forms. D2S is characterized by the absence of 29 amino acid residues within the third intracellular loop [56] compared with D2L. Both isoforms have been shown to exhibit comparable affinity to the ligand and might activate distinct intracellular signal transduction pathways to maintain lactotroph homeostasis in physiological conditions [57, 58]. In fact, the physiological function of the 2 isoforms remains elusive. D2S might inhibit PRL synthesis and lactotroph cell proliferation, whereas D2L might facilitate PRL secretion [59]. Increasing evidence indicates that the D2S/D2L ratio might play a part in the responsiveness of prolactinoma to DA medication [20, 21, 60]. Caccavelli et al. [17] found that the D2S/D2L ratio was lower in resistant patients than in responsive patients. Wu et al. [20] also demonstrated that lower D2S expression and higher D2L expression were

related to the resistance of prolactinomas to DA medication. A subsequent study by them showed that forced D2S expression via adenovirus-mediated D2S transfer could sensitize GH3 cells to BRC therapy in vitro and in vivo. They speculated that adenovirus-mediated D2S transfer combined with BRC treatment may serve as a novel approach for the treatment of DARP [60]. However, other research studies demonstrated that the D2L expression level is associated with responsiveness to DA treatment in prolactinomas. Passos et al. [19] reported that 2 patients with drug-resistant prolactinomas exhibited high D2R expression. They speculated that there could be a predominance of the D2L over the D2S receptor subtype. Shimazu et al. [21] reported that the reduction of the D2L mRNA level is correlated with resistance to DAs in prolactinoma.

D2R polymorphism has also been evaluated, and the NcoI-T allele was found to be significantly associated with biological and radiological resistance to DAs [61]. However, another study found no correlation between D2R polymorphism and CAB responsiveness in patients with prolactinomas [62].

The modulation and mechanisms underlying decreased D2R expression have also attracted attention. Many miRNAs, including miR-9 [37], miR-326 [63], and miR-200a [64], target D2R expression. In 2017, Gangisetty et al. [37] reported that upregulated expression of miR-9 produced by the pituitary of Fischer 344 rats under fetal alcohol exposure could repress D2S expression by targeting its 3'-UTR, resulting in an increase in PRL production and secretion. However, the role of dysregulated miRNAs after exposure to DAs in mediating D2R expression in DARP should be examined further.

miRNAs Might Mediate the E2/ER Signaling Pathway in DARP

Estradiol (E2) has been acknowledged to play a vital role in the tumorigenesis of pituitary adenoma in rats [65, 66], and it appears to increase the risk factors for prolactinomas in humans [67–69]. The interaction of E2 with the TGF- β system and D2R has been also described [69]. When exposed to E2, TGF- β 1 and TGF- β RII expression is downregulated in lactotroph cells [70, 71]. Moreover, E2 reduces both the levels of dopamine and D2R activity [72–74] and increases the D2L/D2S ratio [74–76] in lactotroph cells. Based on the evidence described above, it is reasonable to presume that the E2 signaling pathway is involved in the responsiveness of prolactinomas to DAs. The biological effects of estrogen are mainly mediated by ER α ; therefore, the association between ER α expression

and responsiveness to DA therapy has been studied intensively. Wu et al. [20] found that the median ER α mRNA expression in resistant patients was 1.5-fold higher than that in responsive patients. Passos et al. [19] and Cristina et al. [77] obtained similar results, demonstrating that ER α expression was higher in resistant patients, although ER α expression was not significantly different between the responsive and resistant groups. However, Delgrange et al. [78] reported that patients who were resistant to medical treatment were mainly male; PRL tumors in men are characterized by low ER α expression, which was related to resistance to treatment and overall poor prognosis. Therefore, no consensus has been reached on this topic, and further investigation regarding gender disparities in the response to DA treatment is necessary. Finally, a better understanding of the mechanisms underlying such phenomena will enable the development of optimal therapeutics.

The correlation between miRNAs and estrogen action has also been reviewed [79, 80]. Many of the dysregulated miRNAs identified in resistant prolactinomas (Table 1) are reported to potentially modulate E2 and ER α expression in different conditions. Under hypoxia, increased miR-98 expression was suspected to contribute to a reduction in E2 production through the downregulation of CYP19A1 mRNA and protein expressions in H295R cells [81]. miR-224 was found to promote E2 secretion from granulosa cells, partially by increasing CYP19A1 mRNA levels [82, 83]. Moreover, Hossain et al. [84] demonstrated that miR-17-5p repressed ER-mediated signal transduction pathways by targeting AIB1 in breast cancer cells, and miR-142-3p was proven to inhibit ER expression in ER-positive breast cancer [85]. Spizzo et al. [86] found that miR-145 could downregulate ER α protein expression through direct interaction with 2 complementary sites within its coding sequence in breast cancer cells. However, to date, there is no direct evidence that dysregulated miRNAs modulate E2 and ER α expression in DARP; thus, further investigation is required to clarify whether the dysregulated miRNA expression in DARP impairs the responsiveness to DA therapy via the E2/ER signaling pathway.

Conclusion

To date, the management of DARP remains challenging in clinical scenarios. It is reasonable to explore potential mechanisms and alternative medical treatments to improve the prognosis of patients with DARP. As one of

its characteristics, the dysregulation of miRNA expression, although debatable, might contribute to the regulation of D2R, TGF- β , and estrogen signaling pathways. These findings provide new incentives for research on innovative strategies for predicting patients' responsiveness to dopamine therapies and developing treatment approaches. However, recent studies tended to focus solely on the differential expression profiles of miRNAs between DARP and dopamine-sensitive prolactinomas, and no definitive consensus has been reached regarding the role of these miRNAs or the mechanism of modulation. Therefore, research efforts should be directed to explore the mechanism underlying the dysregulation of miRNAs as well as their target proteins. The identification and monitoring of the expression profiles of miRNAs might be useful to predict pharmacological responses and assist in the classification of prolactinomas (DA sensitive and resistant) before treatment initiation. Furthermore, modulation of the expression of dysregulated miRNAs, which target the D2R, TGF- β , or estrogen signaling pathways, might be a promising alternative for the management of patients and for improving the prognosis of DARP.

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Conflict of Interest Statement

There are no conflicts of interest.

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Author Contributions

Conceptualization and methodology: X.W. and T.L.; investigation and original draft preparation: X.W., Z.Y., Z.T., Z.C., Y.Q., L.L., Y.X., J.C., and T.L.; review and editing: X.W., Z.T., Y.X., J.C., and T.L.; supervision: X.W., Y.X., J.C., and T.L.

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