# MiRNAs in Adrenocortical Carcinoma

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Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with a dismal prognosis and a high rate of recurrence and mortality. Therapeutic options are limited. In some cases, the distinction of ACCs from benign adrenal neoplasms with the existing widely available pathological and histopathological tools is difficult. Thus, new biomarkers have been tested. More than 10 miRNAs validated by multiple studies were found to present a diagnostic and prognostic role for ACC patients, from which miR-483-5p and miR-195 were the most frequently met biomarkers. In particular, upregulation of miR-483-5p and downregulation of miR-195 were the most commonly validated molecular alterations. Unfortunately, data on the therapeutic role of miRNA are still scarce and limited mainly at the experimental level. Thus, the role of miRNA regulation in ACC remains an area of active research.

Keywords: microRNAs ; adrenocortical carcinoma ; biomarkers ; diagnosis ; prognosis ; therapy

## 1. Introduction

Adrenocortical tumors are common and are detected in 5–7% of the general population [1] and up to 10% in the elderly [2]. Adrenocortical carcinoma (ACC) is an uncommon endocrine malignancy with an annual incidence of 1–2 cases per million [3] and with an extremely dismal prognosis with a 5-year survival rate of less than 35% [4]. Currently, the only curative therapy for localized ACC is surgery, although local recurrence is common, ranging from 19 to 34% [5]. Adjuvant treatments, including chemotherapy and radiotherapy, have shown limited therapeutic effectiveness [6]. The most widely used classification system (tumor, lymph node and metastasis (TNM)) seems to be inadequate for predicting patient outcome and survival [7].

MicroRNAs (miRNAs) are small noncoding RNAs of 21–25 nucleotides that regulate genes expression in a sequence-specific manner, inhibiting their expression by targeting the 3′-untranslated region (3′-UTR) of target messenger RNA (mRNA) [8]. MiRNAs are considered epigenetic regulators, involved mainly in the post-transcriptional regulation of gene expression [8], and they are found not only in tissues but also in body fluids [9]. More than 50% of protein-coding human genes are predicted to be modulated by miRNAs. Deregulation of miRNAs has been implicated in the pathogenesis of many human diseases, particularly cancer. The link between miRNAs and cancer was brought about by the seminal observation of Croce's group, who reported that miR-15 and miR-16, two miRNAs located in chromosome 13 (13q14), are frequently deleted in chronic lymphocytic leukemia and function as tumor suppressors [10]. Since then, miRNAs have been studied more intensively in the field of cancer, and growing evidence suggests that altered miRNA expression is involved in the pathogenesis of various types of cancers.

Recent studies have identified miRNAs that have a functional role in adrenal tumorigenesis, including benign and malignant adrenocortical tumors and pheochromocytomas [11]. The role of miRNA deregulation in ACC was first suggested in 2007, when it was discovered that a long noncoding RNA H19 gene transcript [12] was detected in the 11p15 locus, where IGF2 is also located and associated with Beckwith–Wiedemann syndrome, which leads to the development of pediatric ACC [13]. Since then, a number of studies have been performed comparing miRNA expression in ACCs with normal adrenal cortex and adrenocortical adenomas (ACAs) [14]. Given the biological heterogeneity of ACCs and the limitations of the currently used treatments, a better understanding of miRNAs function may serve as a diagnostic, prognostic and potentially therapeutic tool in the management of these patients.

## 2. miRNAs in Adrenocortical Carcinoma

More than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites, suggesting that miRNAs play an important role in the pathogenesis of cancer [15]. MiR-483-5p is one of the most investigated miRNAs in ACCs, both as a diagnostic and prognostic biomarker, and has been proven as the best single-gene malignancy marker [16]. In the study of Chabre et al. [17], miR-483-5p levels were undetectable in the blood of healthy controls, ACA and nonaggressive ACC patients, whereas high levels were detected in the serum of patients with aggressive ACC. In addition

to circulating blood miR-483-5p, its urinary counterpart was evaluated in patients with adrenal tumors [18]. However, no significant difference was detected between ACC and ACA urinary samples. The lack of significance between ACC and adrenal myelolipoma in the expression of both tissue and plasma miR-483-5p and miR-483-3p might represent a limitation in the use of these markers, though [19].

The decrease in miR-483-5p blood levels after surgery in ACC patients suggests dynamic changes in serum miRNAs in response to surgical therapy [17]. This decrease was confirmed by another study [20] but did not reach statistical significance, probably due to the differences of sampling time in relation to the date of operation, as miRNAs deriving from the adrenal tumor before being removed may still be present in the bloodstream. Treatment-induced changes were also revealed for circulating miR-483-5p after systemic therapy in ACC patients [21].

Several miRNAs that seemed to be useful as differentiators between ACCs and ACAs are also promising prognostic indicators of ACCs. The statistically significant upregulation of miR-483-5p, miR-503, miR-210 and miR-139-5p and the downregulation of miR-19 were associated with poor clinical outcome in ACCs in most of the studies. Biomarkers that could predict the biological behavior of these tumors are essential in clinical practice, as they could identify high-recurrence-risk patients that need more intensive monitoring or adjuvant therapies and identify low-recurrence-risk patients that could avoid potential morbid therapies. Indeed, high miR-210 levels were found to be associated with ACC aggressiveness and poor prognosis, affecting the OS of these patients similarly with well-established prognostic factors such as mitotic count, Ki-67 proliferation index and increased expression of SF-1 [22]. Moreover, some miRNAs have been found differentially expressed in ACC histological variants. Prominent underexpression of miR-483-5p, miR-483-3p and miR-210 levels in adrenal tissues has been observed in oncocytic compared to the classical and myxoid histotype of ACC [22]. This interesting finding was interpreted through the prism of the positive correlation of the high levels of miRNA-210 expression with parameters of hypoxia, such as necrosis and GLUT-1, and aggressive biological behavior, such as mitotic rate and Ki-67 proliferation index, which are usually low in oncocytic tumors.

However, these results should be considered with great caution because the analysis of miRNAs expression, as well as its correlation with prognosis, differed among studies, either due to the different methodology used for molecular and/or statistical analysis. Several studies [23][24][17][25][26] used Kaplan–Meier curves and the log-rank test to associate miRNA (low vs. high) levels with worse prognosis. Only three studies [22][20][27] performed, in addition to the log-rank test, univariate and multivariate Cox proportional hazard regression analysis, including, however, different prognostic parameters in their multivariate model. In particular, Duregon et al. [22] included myxoid or classical ACC histotype (mitotic count ≥11, Ki-67 proliferation index ≥20) SF-1 protein expression and miR-210 and found that only mitotic count remains a significant prognostic factor. Salvianti et al. [20] included age, sex and miR483-5p and found that miR483-5p was associated with recurrence-free survival. Finally, Oreglia et al. [27] included tumor size, Ki67, ENSAT stage and miR-483-5p and found that miR483-5p was associated only with recurrence-free survival but not with OS. In addition, one study [20] performed only Spearman correlation to analyze the correlation of miRNAs with distant metastases and disease progression.

Another point of issue is the different cut-offs used for the expression of miRNA levels among the different studies. Receiver operating characteristic (ROC) analyses were performed to determine cut-off values in three studies [20][17][27], and only two studies [23][25] used the dichotomized relative to the median value to determine cut-off values. Three studies [24][22][17] did not mention the cut-off value they used, whereas Agosta et al. [26] used the same cut-off values with Chabre et al. [17] study. Moreover, there was heterogeneity in the compared groups included in the ROC analyses. In particular, Chabre et al. [17] compared ACC patients with aggressive tumors defined as recurring tumors or tumors that were already metastatic at diagnosis with patients with nonaggressive ACC tumors. Oreglia et al. [27] divided patients with ACC into two groups: patients who showed a recurrence within 3 years (group R < 3 years) and patients who showed no recurrence during the first 3 years of follow-up. Salvianti et al. [20] divided ACC patients based on low (stage 1/2) versus high (stage 3/4) disease stages. Furthermore, the studied population concerning ACC patients was heterogenous among studies. For example, Duregon et al. [22] included also other than the classical histological types of ACC (oncocytic and myxoid), whereas Oreglia et al. [27] performed analyses only on postsurgical blood samples of patients with ACC.

Finally, all studies used data of miRNA expression deriving from RT-PCR but one [23], which used data from microarrays analysis. In the study of Ozata et al. [24], only three out of six miRNAs were found to present a statistically significant prognostic role, and the microarray-based results were also validated by RT-PCR.

Across several studies, differences in the expression between tissue and blood miRNA levels were observed, suggesting that the predictive role of blood miRNAs may be independent of tissue specimens. A potential explanation for this finding

could be that released miRNAs do not reflect completely the cellular profile, as some miRNAs are retained or released selectively in the blood circulation [28].

Other components in the miRNA biogenesis pathway also seem to be useful as diagnostic and prognostic markers in adrenocortical tumors. Particularly, TARBP2, DICER and DROSHA miRNA-target genes are significantly overexpressed in ACCs when compared with adenomas and normal adrenal tissue samples [29]. A weak DICER1 protein expression is associated with reduced disease-free and OS serving as a predictor of recurrence in ACCs [30]. Furthermore, the top five upregulated target genes in ACCs, YWHAZ, GATA6, LDLR, BZW1 and IGFBP5, and five downregulated target genes, such as TXNIP, MAPKAPK5, PMAIP1, RAD51 and MICA, interact with several miRNAs [31].

Thus, identifying the relationships between miRNA signatures and ACCs could help better understand the underlying mechanisms and help develop new therapeutic strategies. Overexpression of miRNAs can be triggered by using synthetic miRNA mimics. Conversely, overexpressed miRNAs can be silenced by antagomiRs to restore miRNA balance in cancer networks [32]. For example, inhibition of miR-21 and miR-17-92 was associated with reduced tumor growth, invasion, angiogenesis and metastasis [33]. Indeed, the therapeutic potential of the miR-122 antagonist, miravirsen, in the treatment of hepatitis C was evident from a multicentric phase II trial [34]. Despite the great potential of miRNAs as novel therapeutic targets in the management of ACCs, there are a variety of technical challenges limiting the practical application of miRNA therapy in clinical practice, e.g., the availability of targeted delivery vesicles. Liposome delivery was the first delivery vehicle in clinical trials for miRNA [11]. Liposomal delivery of chemotherapeutics has already been studied in xenograft models of adrenocortical tumors. A significant reduction in tumor size was detected in an ACC xenograft model after a single treatment with anti-IGF1 receptor (IGF1-R) immunoliposomes (SSLD-1H7) [35]. Liposomally encapsulated miRNAs, in combination with cytostatic agents or alone, may represent a novel treatment option for ACC in the future.

## 3. Conclusions

Despite significant advances in the understanding of the molecular landscape of ACC, major efforts are still needed to improve diagnosis, surveillance and treatment of patients with ACC. MiRNAs detected both in adrenal tissue and in human body fluids can be envisaged as potential noninvasive biomarkers of malignancy and/or disease recurrence. Altering the expression of the miRNAs might eventually expand the rather limited therapeutic repertoire in the management of adrenal tumors. The role of miRNA regulation in ACC remains an area of active research with the potential to further enhance our understanding of its tumor biology and the molecular pathways involved.

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