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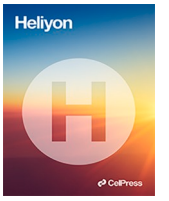

Research article

microRNAs expression profile in phyllodes tumors of the breast

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ABSTRACT

Proliferation of both stromal and epithelial components is a characteristic of fibroepithelial cancers of the breast. Certain fibroepithelial tumors of the breast, such as fibradenomas and phyllodes tumors, are challenging to distinguish and categorize. To find biomarkers for early diagnosis and improved disease management, it is crucial to deepen our understanding of the molecular pathogenesis pathways and tumor biology of PTs. It has been demonstrated that microRNAs (miRNAs) have significant roles in cancers; the expression pattern of miRNAs can help with cancer categorization and treatment. In contrast, little is understood about miRNAs in breast fibroepithelial cancers.

This study was conducted retrospectively with the goal of assessing the expression of six mature miRNAs (hsa-miR-21, hsa-miR-155, hsa-miR-182, hsa-miR-34a, hsa-miR-148a, and hsa-miR-205) in breast fibroepithelial cancers using real-time PCR and predicting these miRNAs' targets using computational techniques.

This study comprised 64 patients in total—55 with phyllodes tumors and 9 with fibroadenoma. The research was carried out at the Farhat Hached University Hospital's pathology department in Tunisia. These particular miRNAs expression levels were evaluated via qRT-PCR, and in silico techniques were utilized to predict potential miRNA targets.

Analysis of miRNA expression in fibroadenoma and phyllodes tumor tissues revealed that miR-21, miR-155 and miR-182 were upregulated in PTs compared to fibroadenoma and normal tissues. We reported that miR-34a, miR-148a and miR-205 were downregulated in both borderline and malignant PTs compared to fibroadenoma and normal tissue. In silico miRNA target prediction suggested the involvement of these molecules in a wide context of cell signaling pathways.

1. Introduction

Breast fibroepithelial lesions are biphasic neoplasms defined by the growth of both epithelial and stromal components [1]. The appearance of leaf-like stromal fronds and enhanced stromal cellularity distinguishes common benign fibroadenomas (FAs) from the considerably rarer phyllodes tumors (PTs) [1]. Phyllodes tumors, which account for fewer than 1 % of all breast tumors, are a type of confined biphasic tumor formed of multiple layers of epithelia (containing glandular epithelium and myoepithelium) surrounded by hypercellular stromal overgrowth [2]. Based on the evaluation of five histological markers, this tumor group can be classified as

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Abbreviations

aCGH	array Comparative Genomic Hybridization
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial to Mesenchymal Transition
FAs	Fibroadenomas
FFPE	Formalin-fixed paraffin-embedded
FOXO	Forkhead box protein O
miRNAs	MicroRNAs
PTs	Phyllodes Tumors
VEGF	vascular endothelial growth factor
WHO	World Health Organization

benign, borderline, or malignant [3]. These factors include stromal cellularity and overgrowth, nuclear atypia, cellular pleomorphism, mitotic activity, and tumor boundaries.

Nevertheless, the assessment of these parameters varies among observers and is subjective, with moderate reproducibility among pathologists, which makes the diagnosis of some cases challenging [4]. Therefore, expanding the current understanding of the molecular pathogenesis pathways and tumor biology of PTs constitutes an essential way to identify new biomarkers for early diagnosis and enable better disease stratification and management.

In PT, many biomarkers have been investigated [5]. Some immunohistochemistry markers have been employed, such as Ki-67, p53, CD117 (c-kit), vascular endothelial growth factor (VEGF), and epidermal growth factor receptor (EGFR). There is growing evidence that the way to cancer involves a slow accumulation of interacting epigenetic and genetic events over time. Most of the past research has focused on genetic changes in PTs, such as chromosomal abnormalities, methylation status, or somatic mutations (e.g., MED12 and TERT genes) [6].

Array comparative genomic hybridization (aCGH) was employed in some investigations. However, the aCGH data showed no connection with the expression profile in the malignant/borderline TP. As a result, alternative gene regulation mechanisms other than copy number changes may be relevant in the TP. Tan et al. recently constructed a panel of 16 genes, including MED12, RARA, TERT, KMT2D, FLNA, SETD2, TP53, RB1, NF1, PTEN, PIK3CA, EGFR, BCOR, ERBB4, MAP3K1, and IGF1R, that could assist categorize PTs [7].

Furthermore, numerous research have focused on miRNAs, which play key roles in tumor growth [8]. MiRNAs are a scalably maintained type of endogenous single-stranded noncoding short RNAs of roughly 22 nucleotides in length that account for approximately 3 % of the entire human genome. MiRNAs play a significant role in tumor development by predominantly degrading target mRNAs or blocking the translation of target proteins. Unique miRNA expression profiles in serum or biopsy tissue may be detectable for differential illness diagnosis. A few studies have suggested that miRNAs can operate as differential biomarkers for both benign and malignant tumors [9].

Haug et al. used the GEO2R tool to investigate the predictive value of the mRNA-miRNA-lncRNA network in triple-negative breast cancer. They identified miRNet and mirTarBase through an upstream examination of lncRNAs and miRNAs, and they analyzed the prognostic values, determining that from 860 upregulated and 622 downregulated mRNAs, 10 essential miRNAs upstream of these major hub genes could have predictive value. Six of these elevated miRNAs were related with poor prognosis, while four downregulated miRNAs (hsa-let-7b-5p, hsa-miR-10b-3p, hsa-let-7a-5p, and hsa-miR-410-3p) were associated with excellent prognosis. Overall, two key lncRNAs (NEAT1 and MAL2) revealed the ability to distinguish between good and poor regression-free survival [10], and their expression pattern may be beneficial for cancer classification and therapy.

2. Materials and methods

2.1. Patients and tissue samples

This study examined the differential expression of microRNAs in 64 cases of fibroepithelial lesions of the breast (55 phyllodes tumors (22 benign, 17 borderline, and 12 malignant) and 9 fibroadenomas) diagnosed in the pathology laboratory at the University Hospital Farhat Hached (Sousse, Tunisia) between January 2007 and March 2018. Two pathologists (BS and MM) assessed haematoxylin and eosin-stained slides from each case to confirm the histopathological diagnosis and select the most representative paraffin blocks. The World Health Organization (WHO) classified Phyllodes tumors as benign, borderline, or malignant. For each patient, clinical data such as age, tumor size, and the occurrence of local recurrence or distant metastasis were documented. This study was approved by the ethics committee of the Farhat-Hached University Hospital (Institutional review board code: 00008924) and informed consent was obtained for all of these patients.

2.2. miRNA extraction

Total RNA (including small RNA) was extracted from FFPE tissue sections according to the manufacturer's instructions using the

miRNeasy FFPE Kit (Qiagen, Germany). Three 20 m slices of each sample were used for RNA isolation. The FFPE sections were deparaffinized in microcentrifuge tubes with xylene, followed by a declining ethanol series. After final centrifugation and removal of the supernatant, tubes were left open for 10 min to allow leftover ethanol to evaporate. Proteinase K buffer and proteinase K were added to the samples and incubated for 15 min at 56 °C and then at 80 °C. The bottom, uncolored phase was placed into a new microcentrifuge tube and incubated on ice for 3 min after incubation. Without disturbing the pellet, the supernatant was transferred to a new microcentrifuge tube. The sample was treated with DNase Booster buffer and 10l of DNase I stock. To eliminate DNA, the mixture was incubated at room temperature for 15 min. To alter binding conditions, RBC buffer was added to the solution, followed by 100 % ethanol to precipitate RNA. RNA was loaded onto RNeasy MinElute spin columns, washed, and eluted in 30 l of RNase-free water before being kept at −20 °C. A Nanodrop spectrophotometer (Genova Nano Jenway, UK) was used to measure the quality and amount of RNA.

2.3. Reverse transcription and real-time PCR

RNA of all samples was polyadenylated and reverse-transcribed to cDNA using the miScript II RT PCR kit (Qiagen, Germany) according to the manufacturer's instructions. Quality control and quantification of cDNA was performed using a Nanodrop Spectrophotometer (Genova Nano Jenway, UK). After that, miRNA quantification of hsa-miR-21, hsa-miR-155, hsa-miR-182, hsa-miR-34a, hsa-miR-148a and hsa-miR-205, was performed by real-time qPCR using SYBR Green microRNA Assays Kit and miScript Primer Assays (Table 1) in the Rotor-Gene Q (Qiagen, Germany). All reactions were due in a final volume of 20 µl with specific thermal cycling conditions: 95 °C for 10 min followed by 45 cycles of 95 °C for 20 s, 55 °C for 20 s and 72 °C for 20 s. For optimal validation of our results, all reactions were performed in triplicates. Normal breast tissues and Human U6 small nuclear RNA (Hs-RNU6–2-1 miScript Primer Assay) were used to normalize and calibrate the quantification of miRNA in fibroepithelial lesions. All samples were normalized to these controls and fold changes were calculated using the ΔΔCT method.

2.4. In-silico analysis of miRNAs targets

Signalling pathways target and Network analysis was performed using the two databases DIANA TarBase v8.0 and miRNET 2.0. Using bio-informatic tools facilitates the analysis and visualization of altered cellular processes and involved molecular pathways in physiopathology of fibroepithelial lesions of the breast, especially in phyllode tumors.

2.5. Statistical analysis

Expression level of miRNAs in fibroepithelial lesion tissues compared to control tissues was analyzed using the ΔΔCt method. Expression levels of miRNAs were presented as the mean of sample triplicates. Spearman's correlation test was applied for measuring the correlation between expression levels of the six miRNAs studied. All statistical tests were accomplished using SPSS version 20.0 statistical software (IBM, USA).

3. Results

In this work, we analyzed the six selected miRNAs for the first time in fibroepithelial lesions of the breast in the Tunisian population. We considered the fact that miRNAs are influenced by several factors, such as environmental and hormonal factors.

First, tumor tissues were selected, and the differential expression of miRNAs was analyzed. The expression rates of miR-21, miR-155 and miR-182 in fibro-epithelial lesions of the breast vary according to the type of lesion. In FA and benign PTs tissues, the relative expression ratio of miR-21 and miR-155 did not show any significant variation compared to normal control tissue (miR-21 expression fold change = 0.94 and 0.99, respectively, miR-155 expression fold change 0.72 and 0.43, respectively) (Fig. 1). However, the relative

Table 1
Correlation between miRNAs in fibroadenoma samples.

			miR-21	miR-155	miR-182	MiR-34a	MiR-148a	MiR-205
Rho Spearman test	**MiR-21**	Coefficient of correlation	1.000	0.200	0.033	0.117	0.650	0.250
		p	–	0.606	0.932	0.765	0.058	0.516
	MiR-155	Coefficient of correlation	.200	1.000	−.033	.633	.533	.067
		p	0.606	–	0.932	0.067	0.139	0.865
	MiR-182	Coefficient of correlation	0.033	−0.033	1.000	−0.050	0.117	0.667
		p	0.932	0.932	–	0.898	0.765	**0.050***
	MiR-34a	Coefficient of correlation	.117	.633	−.050	1.000	.683	.400
		p	.765	.067	.898	–	**0.042***	.286
	MiR-148a	Coefficient of correlation	0.650	0.533	0.117	0.683	1.000	.467
		p	0.058	0.139	0.765	**0.042***	–	.205
	MiR-205	Coefficient of correlation	0.250	0.067	0.667	0.400	0.467	1.000
		S *p*	0.516	0.865	**0.050***	0.286	0.205	–

a. Significant correlation at 0,05 (bilateral).
b. Significant correlation at 0,01 (bilateral).

expression ratio of miR-182 was slightly overexpressed with fold changes equal to 1.35 and 2.64, respectively. Interestingly, these miRNAs showed increased expression levels in borderline and malignant PT tissues, with fold changes equal to 1.99 and 7.45, respectively. In addition, the miR-21 expression fold change was 7.02 and 9.7, respectively, and the miR-155 expression fold change in malignant phyllodes tumors was 2.19.

The expression rates of miR-21, miR-155 and miR-182 in two recurrence samples of benign PTs (R1: benign and R2: malignant) showed that miR-21 was significantly overexpressed in the two recurrence samples compared to the original lesion (fold change R1 = 3.14 and R2 = 15.01). However, the expression rate of miR-155 revealed a decrease in the benign and malignant recurrence samples compared to the original lesion (fold change = 1.75 and 0.22, respectively). For miR-182, the corresponding expression rate increased in benign recurrence (fold change = 3.39) but decreased in malignant recurrence (fold change = 0.22).

Relative expression analysis of miR-34a, miR-148a and miR-205 in breast fibroepithelial samples showed that they were overexpressed in fibroadenomas and benign PTs tissues compared to breast normal tissue (fold change miR-34a = 4.5 and 2.92, respectively; fold change miR-148a = 1.32 and 1.22, respectively; fold change miR-205 = 1.97 and 2.92, respectively) (Fig. 2). In the case of borderline and malignant PT tissues, we noticed downregulation compared to the control tissue (fold change miR-34a = 0.58 and 0.46, respectively; fold change miR-148a = 0.49 and 0.94, respectively; fold change miR-205 = 0.91 and 0.52, respectively). The expression rate of these miRNAs in two recurrent samples showed that miR-34a was underexpressed in the benign recurrence sample R1 (fold change = 0.57) but overexpressed in the malignant recurrence sample R2 (fold change = 4.43) compared to the original lesion. In contrast, the expression rate of miR-148a increased in sample R1 (fold change = 1.85) but decreased in R2 (fold change = 0.31). For miR-205, the expression rate of miR-205 decreased in both recurrence samples compared to the original lesion (fold change 0.67 and 0.26, respectively).

Correlation analysis has shown that there are relations with a positive coefficient between several miRNAs in fibroadenoma and phyllodes tumor samples. In the case of fibroadenomas, we found significant correlations between two groups of miRNAs (Table 1). The first group consisted of miR-148a and miR-34a (Spearman's Rho: 0.042; $p < 0.05$). The second group consisted of miR-205 and miR-182 (Spearman's Rho: 0.05; $p < 0.05$). In the case of PTs, we noticed a significant correlation between the 4 groups of miRNAs (Table 2). miR-148a and miR-182 were characterized by the highest correlation value (Spearman's Rho: 0.508; $p < 0.01$), followed by the correlation between miR-148a and miR-34a (Spearman's Rho: 0.462; $p < 0.01$), the correlation between miR-148a and miR-155 (Spearman's Rho: 0.348, $p < 0.01$) and finally the correlation between miR-155 and miR-182 (Spearman's Rho: 0.267; $p < 0.05$).

Using the two databases DIANA-TarBase v.8 and miRNET 2.0, we detected miRNA target genes as well as different signaling pathways shared by these miRNAs. TarBase allowed us to illustrate different signaling pathways involving the six miRNAs studied as well as target genes for each miRNA analyzed on a diagram called a "heatmap". Among the signaling pathways involved, we found signaling pathways FOXO (hsa 04068), p53 (hsa 04115), TGF-Beta (hsa 04350), and pathways involved in misregulation of transcription process (hsa05202), of the cell cycle (hsa 04110), and the adhesion of cellular junction (hsa 04520) (Fig. 3). For each signaling pathway, we determined which miRNAs are mostly involved, and we presented their target genes. The creation of a network linking the analyzed miRNAs with their targets was achieved using the miRNET 2.0 database. This base enabled us to visualize a broad spectrum of molecular targets corresponding to the analyzed miRNAs (Fig. 4).

4. Discussion

To date, miRs have been known as important players in breast cancer angiogenesis and therapeutic targets [11]. The role of miRNAs in breast PTs has not been well investigated. In the case of PTs, studies based on miRNA profiling have reported that their

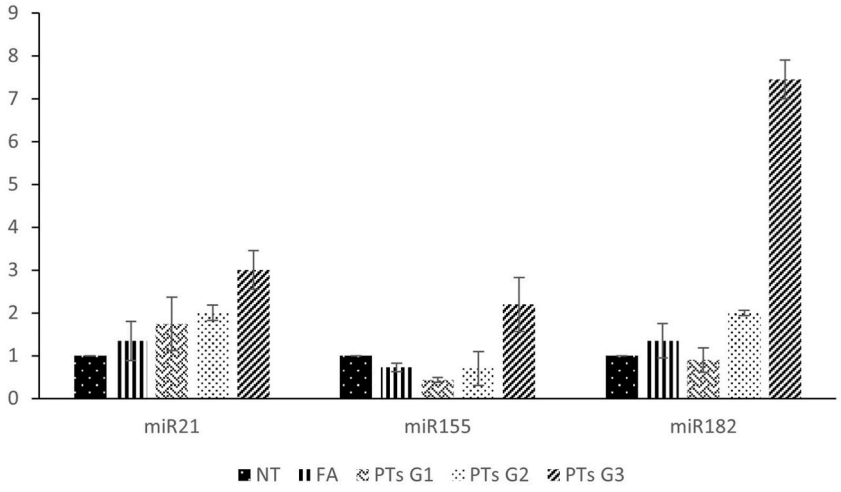

Fig. 1. Relative expression of onco miRNAs: miR-21, miR-155 and miR-182 in normal tissues of the breast (NT), fibroadenomas (FA) and phyllodes tumors grade 1 (PTs G1), grade 2 (PTs G2) and grade 3 (PTs G3).

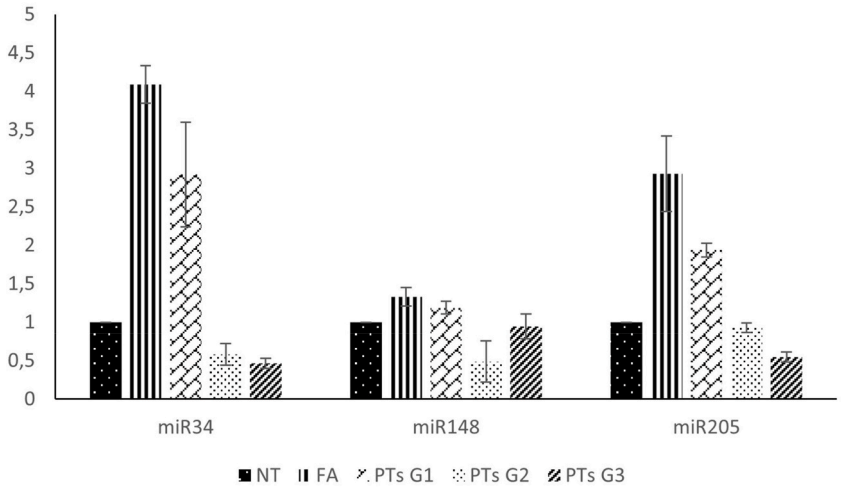

Fig. 2. Relative expression of tumors suppressor miRNAs: miR-34a, miR-148a and miR-205 in normal tissues of the breast (NT), fibroadenomas (FA) and phyllodes tumors grade 1 (PTs G1), grade 2 (PTs G2) and grade 3 (PTs G3).

Table 2
Correlation between miRNAs in phyllodes tumor samples.

			MiR-21	MiR-155	MiR-182	MiR-34a	MiR-148a	MiR-205
Rho Spearman test	**MiR-21**	Coefficient of correlation	1.000	−0.103	−0.232	0.298	0.042	0.220
		p	–	0.455	0.088	**0.027***	0.758	0.106
	MiR-155	Coefficient of correlation	−0.103	1.000	0.267	−0.014	0.384	−0.144
		p	0.455	–	**0.049***	0.918	**0.004****	0.293
	MiR-182	Coefficient of correlation	−0.232	0.267	1.000	−0.240	0.508	0.249
		p	0.088	**0.049***	–	0.078	**0.000****	0.067
	MiR-34a	Coefficient of correlation	0.298	−0.014	−0.240	1.000	0.462	0.053
		p	**0.027***	0.918	0.078	–	**0.000****	0.701
	MiR-148a	Coefficient of correlation	0.042	0.384	0.508	0.462	1.000	0.128
		p	0.758	**0.004****	**0.000****	**0.000****	–	0.350
	MiR-205	Coefficient of correlation	0.220	−0.144	0.249	0.053	0.128	1.000
		p	0.106	0.293	0.067	0.701	0.350	–

a. Significant correlation at 0,05 (bilateral).
b. Significant correlation at 0,01 (bilateral).

expression can segregate between the two types of PTs (benign and malignant) and may provide a more objective method for diagnosis [12].

In the present study, using real-time PCR, we compared the expression levels of six mature miRNAs (hsa-miR-21, hsa-miR-155, hsa-miR-182, hsa-miR-34a, hsa-miR-148a and hsa-miR-205) in both fibroadenoma and PT tissues. The choice of these miRNAs was determined according to the literature review and to their potential involvement in breast cancer in general and in mammary phyllodes tumors in particular. Relative quantification has reported that miRNA expression levels vary significantly according to the type of lesion. Among the differentially expressed miRNAs, overexpression of miR-21, miR-155 and miR-182 in malignant PTs compared to normal tissues was observed. Conversely, miR-34a, miR-148a and miR-205 were downregulated in malignant tissues compared to fibroadenoma, benign PTs and normal tissues. The results from our in silico study indicate that several signaling pathways and miRNA target genes are involved in the PT tumorigenesis process. In accordance with these findings, some studies have shown that miRNA dysregulation in tumoral tissues is widely associated with oncogenic pathway stimulation.

As a master-oncomiR, miR-21 is consistently upregulated in many tumor types, including breast cancers [13]. Overexpression of this miRNA in tumoral tissues is frequently associated with a poor outcome and disease progression [14]. In accordance with our study, Gong et al. showed that miR-21 overexpression induces the differentiation of myofibroblasts by promoting the malignant evolution of phyllodes tumors. This study suggests that the involvement of miR-21 in this process is mediated by the regulation of Smad7 and PTEN gene expression [5,15]. Other studies have suggested that overexpression of miR-21 in breast tumor tissue is often associated with advanced clinical stage [14,16]; it is also known to be upregulated in other types of cancer, such as colorectal [17], ovarian [18] and breast [19] cancers. Another study reported that miRNA expression analysis of human glioblastoma showed an upregulation of miR-21 compared to controls gliobastoma [20]. Furthermore, our in silico study showed that miR-21 was stimulated in a variety of cancer-related pathways, including transcriptional misregulation, cell cycle misregulation, the p53 signaling pathway and the Forkhead box protein O (FOXO) signaling pathway. According to our results, Lei et al. reported that miR-21 was reported to induce cell proliferation and suppress chemosensitivity in glioblastoma cells via downregulation of the FOXO signaling pathway [21] and showed

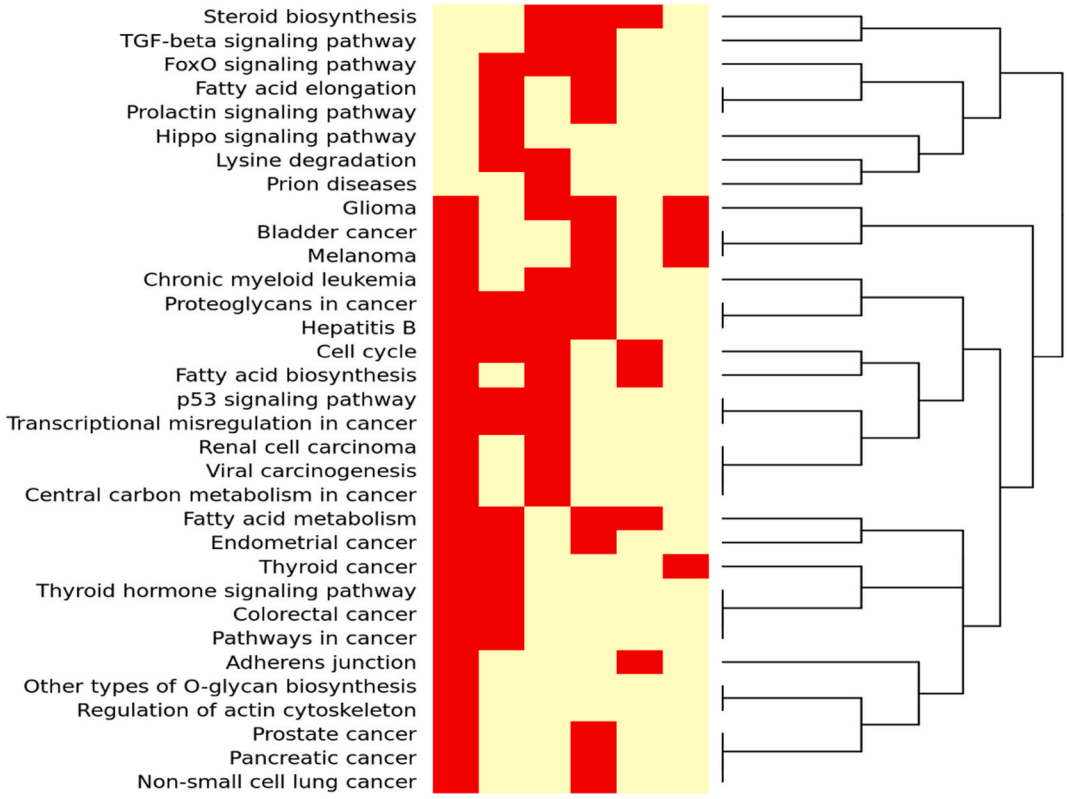

Fig. 3. Heatmap of different pathways and associated pathologies involving with studied miRNAs.

that hsa-miR-21 was associated with the p53 signaling pathway, and hsa-miR-326 was associated with the FOXO signaling pathway [22].

The expression of miR-155 and miR-182 was similarly reported to be increased in several cancers [23,24], such as breast cancer. The overexpression of miR-155 induces the proliferation and migration of breast cancer cells by downregulating the expression of SOCS1 and upregulating the expression of MMP16 [25]. miR-182 is also known to be upregulated in tumor tissues. A study conducted on human melanoma tissues reported that miR-182 was involved in cell apoptotic inhibition through targeting the FOXO-3 gene [26]. MiR-182-5p increased cell proliferation by suppressing FOXO3a, resulting in the activation of the AKT/FOXO3a pathway [27]. Our in silico findings suggested that miR-155 and miR-182 are involved in cancer-related pathways, such as the FOXO signaling pathway, TGF-β signaling pathways and cell junction adherence misregulation.

The obtained results revealed an increase in oncogenic miRNAs (oncomiRs) in PTs, which encouraged us to study the expression of tumor suppressor miRNAs, also called anti-oncogenic miRNAs. Among the best-known tumor suppressor miRNAs, we chose miR-34a, which was consistently downregulated in many tumor types [28]. miR-34a is known to be directly controlled by the suppressor gene p53, which is known to regulate several cellular processes, such as the cell cycle, cell proliferation and apoptosis [29]. A previous study by Okada et al. suggested the presence of a retroactivation loop between p53 and miR-34a, which is mediated in part by the negative control of miR-34a on the MDM4 gene, a negative regulator of p53 [30,31]. In addition, other studies have shown that miR-34a contains a consensus p53-binding site and that a reduction in miR-34 function attenuates p53-mediated cell death [30]. Furthermore, miR-34a is known to regulate the epithelial to mesenchymal transition (EMT) process, which is frequently deregulated in tumor progression [32]. The involvement of miR-34a in EMT reactivation is mediated by mutual control between proteins involved in this process and miR-34a; for example, the direct binding of ZEB-1 protein to miR-34a promoter regions is widely associated with EMT reactivation [33].

However, other miRNAs were implicated in the control of the EMT process, such as miR-148a. In this context, Zhang et al. demonstrated that miR-148a was involved in inhibiting the mechanism of EMT, and Yan et al. also reported the involvement of miR-148a in decreasing the invasive capacity of cancer cells through the inhibition of EMT [34,35]. Its role was also reported by different cancer studies [36]. In breast cancer tissues, it was reported that miR-148a reduces angiogenesis by targeting the ERBB3 gene [37] or by inhibiting the BCL-2 gene [38]. The downregulation of miR-148a has been demonstrated in many cancer types, including gastric, colorectal, pancreatic, liver, esophageal, breast, lung, and urogenital tract cancers [36]; this is in agreement with our results from the borderline and malignant PTs samples. However, Kim et al. reported an overexpression of miR-148a in a case of glioblastoma. This study confirmed that miR-148a was involved in this process and proved that the regulation of apoptosis is related to miR-148a control by targeting the MIG6 and BIM genes that regulate the expression of the EGFR gene [39]; this is consistent with our results regarding

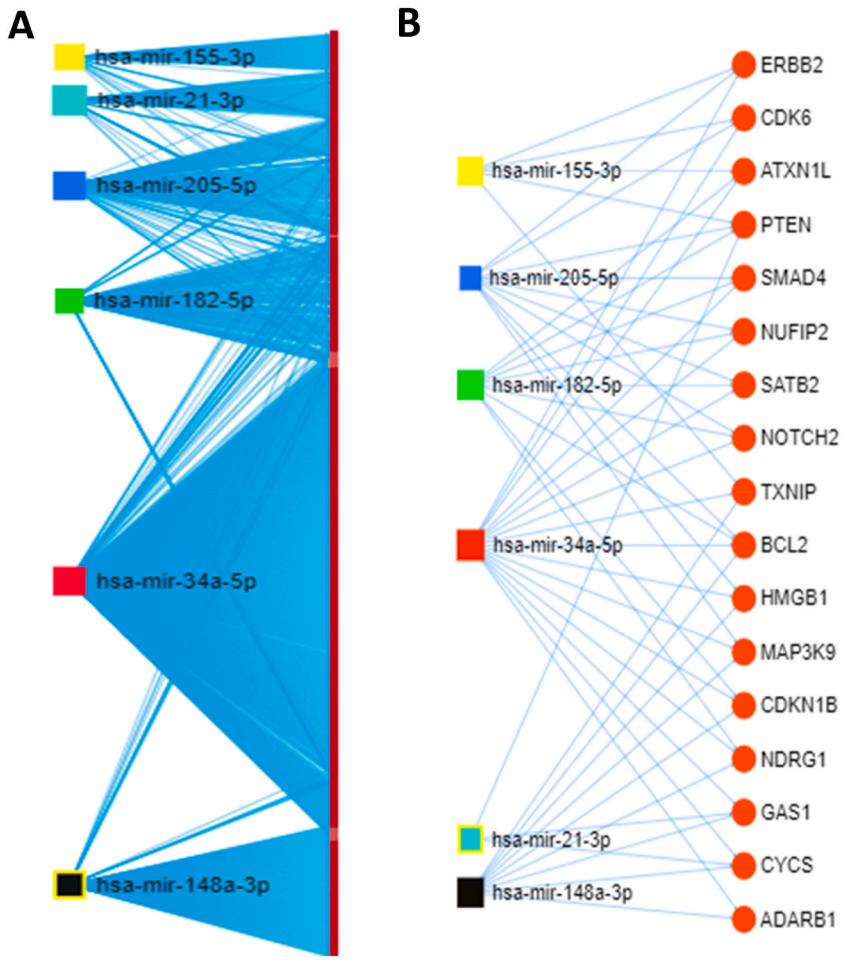

Fig. 4. miRNAs network, A: all target genes, B: relevant target genes in correlation at least with 2 miRNAs.

FA breast tissue and benign PTs tissues compared to normal tissues.

miR-205 acts as an oncogene or a tumor suppressor according to the type of cancer, and it is involved in tumorigenesis as a tumor suppressor by inhibiting proliferation and invasion or as a proto-oncogene by facilitating tumor initiation and proliferation [40]. In our study, the expression rate of miR-205 decreased in borderline and malignant GST relative to normal tissue. Several previous studies support our findings and suggest a tumor suppressor role for miR-205 [40,41]. The study by Hailong and colleagues suggests that this miRNA is significantly underexpressed in breast cancer tissues and is involved in inhibiting proliferation, tumor growth and invasion through its interaction with ErbB3 and VEGF-A factors [42]. In contrast, Piovana and colleagues reported an overexpression of miR-205 in breast tumor tissue. Restoration of miRNA expression has been shown to significantly reduce tumor proliferation and progression, and the anti-oncogenic activity of miR-205 is mediated primarily by its interaction with p53 [43]. However, Chu and colleagues demonstrated that miR-205 is overexpressed in ovarian cancer cases; this study suggested that miR-205 improves the proliferative capacity of ovarian cancer cells by regulating the expression of the PTEN and SMAD4 genes [44].

miR-34a, −148a, and −205 have been shown to control several cancer-related processes. Our in silico study has shown that miR-34a, miR-148a, and miR-205 are usually associated with multiple cancer-related pathways, such as cell cycle disorders, transcription disorders, the p53 signaling pathway, the FOXO signaling pathway, and the TGF-β signaling pathway.

5. Conclusion

MiRNAs and proteins play critical roles in the progression of breast cancer. Expression dysregulation of miRNA in tumoral tissues, including phyllodes tumors of the breast, may be responsible for altering the tumor phenotype. Our quantitative analysis of miRNAs revealed that the expression of oncogenic and tumor suppressor miRNAs varied between different PT groups. Indeed, we have shown that miR-21 and miR-182 have a profile corresponding to significant oncogenic activity in PTs. Borderline and malignant PTs exhibited the lowest levels of expression of tumor suppressor miRNAs (miR-34a, miR-148a and miR-205) relative to FA and normal tissues. Our results suggest that these miRNAs can be exploited as diagnostic biomarkers in the case of PTs. However, future research should focus

on the clinical therapeutic strategies of these miRNAs. In addition, studying the role of different noncoding RNAs, especially miRNAs, in drug resistance and immune checkpoint inhibitors resistance can be beneficial in selecting more effective treatments for breast cancer [45].

Ethics statement

The current investigation was conducted in conformity with the Helsinki Declaration's ethical criteria. It was authorized by the Medical and Ethical Committee of Farhat Hached University Hospital in Sousse, Tunisia (Institutional review board code: 00008924). The samples for this investigation were gathered in the context of regular laboratory diagnosis, and all patients provided informed consent.

Data availability statement

The authors are unable or have chosen not to specify which data has been used.

CRediT authorship contribution statement

Mohamed Ridha Hachana: Conceptualization. **Mouna Maatouk:** Writing – review & editing, Writing – original draft. **Zayneb Lassouad:** Writing – original draft, Investigation. **Badreddine Sriha:** Data curation. **Moncef Mokni:** Supervision, Project administration.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mohamed Ridha Hachana reports financial support was provided by Farhat Hached University Hospital of Sousse. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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