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Typical Lung Carcinoids with Metastasis

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Original Paper

Typical Lung Carcinoids with Metastasis: Potential Role of MicroRNAs in the Regulation of Adaptive Immunity Associated with Disease: a Case Study

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Key Words
Lung carcinoid • MicroRNAs • Immune system • Disease progression • Metastasis

Abstract
Background/Aims: Lung carcinoids are uncommon neuroendocrine tumours. Molecular features of lung carcinoids have been poorly defined. microRNAs (miRNAs) are potent gene expression regulators with important roles in cancer development and progression. However, little is known on the role of miRNAs in the pathogenesis of lung carcinoids. Our goals were to identify commonly deregulated miRNAs in a rare case of lung carcinoid of typical histology with metastasis, as well as map miRNA target genes in pathways potentially associated with disease development and progression. ***Methods:*** miRNA expression profiles were assessed using the TaqMan Low Density Arrays, which is a platform including 384 miRNAs. miRNA profiles were generated in the tumor and its corresponding lymph node metastasis, compared to reference normal lung tissues. Furthermore, miRNA expression was validated

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in a separate, publicly available external dataset (n=19 typical lung carcinoids; 2/19 were metastatic tumors, compared to six normal lung tissues, GSE77380). Following this analysis, computational tools were applied for data interpretation. miRTarBase was used to determine miRNA-target genes, followed by ToppGene Suite analysis to identify pathways and biological functions. In addition, the expression of genes targeted by miRNAs was validated in a second, separate external dataset (n=13 tumour samples, GSE35679). GEO2R data analysis tool was used in both validation analyses (miRNAs and genes). ***Results:*** We identified 15 commonly significantly downregulated miRNAs (fold change, FC≥2 and $p<0.05$) in the tumour and its paired metastasis, with further decreasing levels in the metastatic lesion. Downregulation of miR-126-3p and miR-146b-5p was validated in the external dataset GSE77380. In addition, *SOX2* and *TCF4* genes, targeted by miR-126-3p, were consistently overexpressed in a subset of six typical lung carcinoids from the external dataset GSE35679. Pathways analysis showed that miRNAs miR-126-3p and miR-146b-5p target genes with a role in the regulation of adaptive immune response. ***Conclusion:*** Our results contribute to the identification of miRNA expression changes in a typical lung carcinoid and its corresponding lymph node metastasis. Down-regulated levels of miR-126-3p and miR-146b-5p and target gene over-expression could play a role in the progression of this case of primary typical lung carcinoid to regional metastasis. Identified miRNAs and target genes are potential candidates for validation in a larger number of cases.

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Introduction

Lung neuroendocrine tumours comprise approximately 25% of lung cancer cases; among these, typical lung carcinoids are low-grade neuroendocrine lesions accounting for only approximately 2% of cases, with atypical carcinoids representing about 0.2% of cases [1]. Typical carcinoids are most common in younger patients (~45 years old), not usually related to smoking and infrequently associated with metastasis at diagnosis, with a 5-year overall survival of >80%. Diagnosis of typical lung carcinoids is based on histological examination and mitotic counting lower than two mitoses per 2 mm^2, without necrosis. Disease staging follows the TNM categorization, and surgery remains the standard treatment because of the high failure rates of chemo- or radiotherapy [2]. Patients eligible for surgical resection have better long-term survival, and the presence of metastatic disease is associated with shorter disease-specific survival [3]. The presence of regional lymph node metastasis is reported in approximately 9% of typical lung carcinoids [4]. Typical lung carcinoids can metastasize to distant organs, mainly the liver and bones, and atypical carcinoids may spread to multiple distant organs, including the liver, bones, brain, spleen, adrenal glands, and soft tissues [5]. Furthermore, disease recurrence has been observed even decades after primary tumour resection [6].

The histopathological features of lung carcinoids, such as well-differentiated cells, organoid growth pattern, and absence of necrosis [2], are useful for their classification; however, the molecular alterations associated with disease development and progression are poorly understood. Lung carcinoids have fewer genetic changes than other tumour types, and when these changes occur, the genes involved are more frequently related to epigenetic mechanisms, cellular metabolism, and DNA damage response [7, 8]. In addition, neuroendocrine lung tumours have a different mutational profile compared to non-small cell lung cancer [9]. These data suggest that there are different regulatory mechanisms associated with tumorigenesis and progression of lung carcinoids. However, such mechanisms are widely unknown.

microRNAs (miRNAs) are small non-coding RNAs that regulate post-transcriptional gene expression related to several cellular processes, playing an important role in disease, including benign and malignant tumours [10]. The role of miRNAs in neuroendocrine tumours has been investigated in a few studies [11–14]. Mairinger *et al.* [11] assessed miRNA expression in pulmonary neuroendocrine tumours, including typical and atypical

carcinoids, large cell neuroendocrine and small cell lung cancer. Interestingly, these authors reported decreasing expression levels of miR-29a, miR-29b, and miR-29c associated with more aggressive tumour subtypes, from lung carcinoids to large cell carcinoma and small cell lung cancer. Deng *et al.* [12] reported three miRNA target genes (*CREB5*, *PTPRB*, and *COL4A3*) as having significantly lower expression levels in carcinoid tumours compared to normal lung tissues; however the authors state that further studies are needed to elucidate the biological significance of these genes associated with lung carcinoid tumorigenesis. Rappa *et al*. [13] identified distinct miRNA expression profiles in the different subtypes of lung carcinoid tumours; they reported five miRNAs (miR-129-5p, miR-409-3p, miR-409-5p, miR-185, and miR-497) significantly overexpressed in typical compared to atypical carcinoids, and three underexpressed miRNAs (miR-409-3p, miR-409-5p, and miR-431-5p) in typical and atypical cases presenting with lymph node metastasis. Finally, Yoshimoto *et al.* [14] performed a comparative study including pulmonary carcinoids, small cell lung cancer, and gastrointestinal neuroendocrine tumours and reported specific miRNA expression profiles suggestive of a common origin for lung and gastrointestinal neuroendocrine cancers. Interestingly, miRNA expression patterns in lung carcinoids were distinct from small cell lung cancers, reflecting their differences in histogenesis.

Here, we contribute with original miRNA expression data in a rare case of typical lung carcinoid with lymph node metastasis, as well as miRNA and gene expression data validation in independent, publicly available patient datasets. Furthermore, we provide data on the analysis of pathways and biological functions of genes targeted by miRNAs. By investigating miRNA changes common to the primary tumour and the metastatic lesion from the same patient, we may be able to identify miRNAs potentially associated with disease progression.

Materials and Methods

Patient samples

A typical lung carcinoid tumour, and its paired lymph node metastasis, were obtained from surgical resection in a female patient, 38-year-old at diagnosis, non-smoker, and with no family history of cancer. Disease diagnosis confirmed a locally invasive typical carcinoid tumour (tumour size 4 x 2.5 x 2.5 cm), T2aN1M0 (pathological stage IIB), according to the American Joint Committee on Cancer staging system (AJCC, 8th edition). Formalin-fixed, paraffin-embedded (FFPE) tissues were obtained from the Pathology Department, Botucatu Clinical Hospital, FMB, UNESP, São Paulo, Brazil. Two formalin-fixed, paraffin embedded (FFPE) tissue blocks were obtained from two different areas of the primary tumour, and one FFPE sample was obtained from the lymph node metastasis. FFPE samples were cut (10 sections of 10 μm each) for needle macrodissection using the stereo microscope Leica EZ4 (Leica Microsystems, Wetzlar, Germany) before RNA extraction in order to isolate the target cell populations (tumour or normal). A lung pathologist marked the tumour or normal areas on H&E-stained tissue sections. In addition, an RNA pool of 9 histologically normal lung tissues was used as a reference to calculate the relative miRNA expression. Therefore, we generated miRNA profiles using the following samples: tumour (N=2 fragments) and lymph node metastasis (N=1) from the same patient, and histologically normal lung tissues (N=9) as a reference. Considering that distinct areas of a tumour sample may have intratumoral heterogeneity, we analysed miRNA expression in the two tumour fragments, independently, and confirmed that they were homogeneous regarding their global miRNA expression levels.

RNA extraction

RNA was extracted using the RecoverAll Total Nucleic Acid Isolation Kit for FFPE tissues (Ambion/ Thermo Fisher), according to the manufacturer's instructions.

miRNA expression analysis

TaqMan Array Human microRNA card A v.3.0 (Life Technologies/Thermo Fisher Scientific) was used to identify commonly deregulated miRNAs in tumour and metastasis based on a panel of 384 miRNAs, including controls, as previously reported [15]. Our raw, original data are fully available on Gene Expression Omnibus (GEO) under the accession number GSE138708.

Computational analyses

miRNA target genes were predicted using the updated version of miRTaRBase, in order to identify experimentally-validated miRNA-target gene interactions [16] (date of access: Sept 22, 2020). miRNA-mRNA networks were also generated using the miRNet 2.0 (https://www.mirnet.ca/miRNet/home.xhtml), a useful platform that allows integrating experimental data with existing literature datasets and scientific knowledge on interaction networks [17]. In addition, ToppGene Suite (https://toppgene.cchmc.org/) [18] (date of access: Sept 22, 2020) was used to identify statistically enriched pathways and gene ontologies (GO). A Venn diagram was generated using jvenn viewer (http://jvenn.toulouse.inra.fr/app/example.html) [19].

Validation of miRNA and target gene expression in external datasets

In order to validate miRNAs identified in our study, using independent datasets, we retrieved publicly available miRNA expression data from the Gene Expression Omnibus (GEO) [20]. We searched for publicly available datasets using the following inclusion criteria: original raw data available, on global miRNA expression generated in primary typical human lung carcinoids, including non-metastatic and metastatic cases, and with data on histologically normal lung tissues for comparison. Based on these inclusion criteria, one dataset (GSE77380) [14] was selected and included for validation. The GSE77380 dataset included two metastatic cases, and the platform of analysis was the Agilent Human_miRNA_V16.0_Microarray. miRNA expression levels were determined by comparing typical carcinoids (N=19) and normal tissues (N=6). An additional analysis was performed and included the two metastatic cases only.

In order to provide evidence of gene expression deregulation in typical lung carcinoids, target gene (mRNA) expression was validated using the GSE35679 dataset [21], which included 13 lung carcinoid samples (six typical and seven atypical tumours). We selected this dataset since the raw data was fully available for download and analysis, and the authors included only surgically resected primary tumours from treatment naïve patients. Raw miRNA or target gene expression data were analysed using the same bioinformatics tool, GEO2R [20] with its default parameters.

Statistical analysis

miRNA expression profiles were generated using ExpressionSuite software (Applied Biosystems/ Thermo Fisher Scientific), based on the Delta Delta Ct method of analysis [22]. A global normalization strategy was applied using stable endogenous controls included in the TaqMan assay. Statistical analyses used the Benjamini & Hochberg false discovery rate (FDR) method to determine corrected *p*-values [23] in all datasets: TaqMan miRNA expression analysis in tumour and metastasis *vs.* normal, and in the external validation datasets (miRNA, GSE77380 and target gene expression, GSE35679).

Results

Tissue samples exhibited histological features and biomarkers characteristic of a typical carcinoid tumour

Histological analysis of surgically resected tumour and lymph nodes showed tumour cells having nuclei with small to intermediate size and heterogeneous chromatin. The tumour stroma was vascularized. There was one mitotic cell in 10 large magnification fields. No angiolymphatic or perineural invasion or necrosis was observed. The Ki-67 proliferation index was 1%. Histopathological features of this typical carcinoid tumour are shown in Fig. 1A; neuroendocrine differentiation is shown by immunopositivity of chromogranin and synaptophysin [2] (Fig. 1B and 1C). One of the three resected lymph nodes showed cytomorphological aspects identical to the tumour. Immunohistochemical biomarker analysis of this lymph node sample confirmed epithelial histogenesis of tumour cells, showing immunopositivity to cytokeratins (AE1/AE3) (Fig. 1D). Immunopositivity for chromogranin and synaptophysin confirmed the diagnosis of a typical lung carcinoid tumour with metastasis lesions in 1/3 lymph nodes.

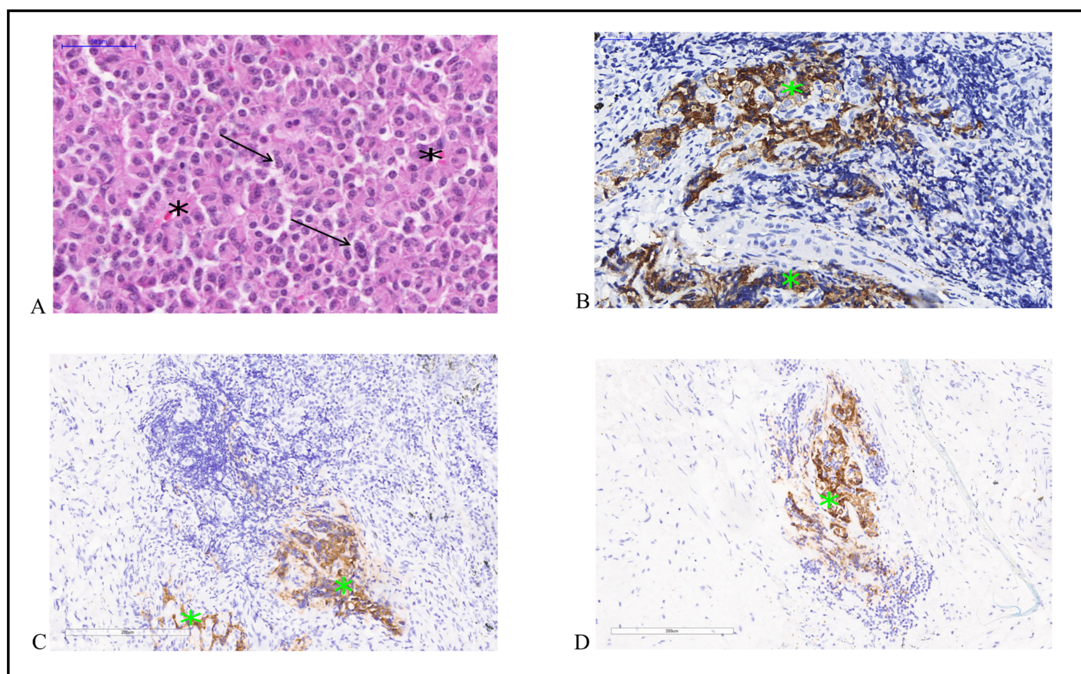

Fig. 1. (A) Haematoxylin and Eosin (H&E) stained section of the low grade, typical carcinoid tumour of the lung. Chromatin in cell nuclei show evident "salt and pepper" patterning as indicated by black arrows. Tumour shows characteristic organoid tumour growth with vascularization (indicated by black stars *) and absence of necrosis and lack of mitotic activity, 400X magnification. (B) Immunohistochemical stained section showing positivity for synaptophysin, a specific biomarker expressed in the typical subtype of carcinoid tumours, 400X magnification. (C) Immunohistochemical stained section showing positivity for chromogranin A, 200X magnification. (D) Immunohistochemical stained section showing positivity for cytokeratins (AE1/AE3), 200X magnification. The green stars* indicate positive immune staining areas.

A subset of miRNAs is commonly deregulated in tumour and its corresponding lymph node metastasis

28 miRNAs were significantly deregulated (21 downregulated and 7 upregulated) (FC≥2 and p<0.05) in the two fragments of the tumour and 40 (38 down and 2 upregulated) in the metastatic sample. Global miRNA expression profiles were highly correlated in the two tumour areas (r=0.97, Pearson correlation) demonstrating that the two tumour fragments were homogeneous regarding their miRNA expression levels.

We were able to identify 17 commonly deregulated miRNAs (FC≥2 and p<0.05), with most miRNAs (15) being downregulated, one miRNA (miR-411-5p) was upregulated and one miRNA (miR-191-5p) showed opposite expression levels (upregulated in tumour and downregulated in metastasis) (Supplementary Table 1, Fig. 2 – for all supplementary material see www.cellphysiolbiochem.com). Of the downregulated miRNAs, 12 exhibited a notable further decrease in expression in the metastasis compared to the primary tumour (Table 1).

miRNA target genes are known to regulate pathways of adaptive immune response

miRTaRBase target prediction analysis [16] was performed using the commonly deregulated miRNAs in tumour and metastasis. Prediction analysis showed 485 unique gene targets with experimental evidence, provided by different methods including luciferase reporter assay, immunoblotting and others (Supplementary Table 2). Genes were further mapped on 15 significantly (p<0.01) enriched pathways including at least 500 genes in each annotation. Among the pathways identified, 59 genes were involved in adaptive immune response and 61 genes encoding extracellular matrix and extracellular matrix-associated

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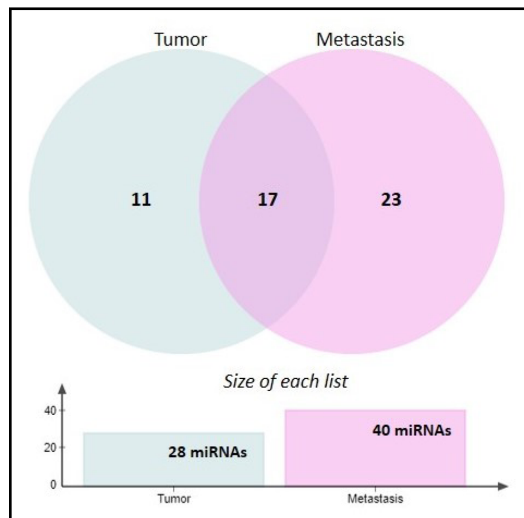

Fig. 2. Venn diagram depicting the number of deregulated miRNAs commonly deregulated and exclusive to tumour and metastasis.

Table 1. Commonly deregulated miRNAs in typical carcinoid tumour and metastasis. FC: fold change (log_{10} values). Corrected p-values were determined by the Benjamini Hochberg method

miRNA ID	Tumour		Metastasis	
	FC	p value of FC	FC	p value of FC
let-7b-5p	-1,174	0,001	-2,699	0,001
let-7e-5p	-0,450	0,016	-1,444	0,002
miR-126-3p	-1,222	0,019	-1,328	0,018
miR-146b-5p	-1,201	0,004	-0,444	0,036
miR-16-5p	-0,416	0,038	-0,793	0,015
miR-186-5p	-0,602	0,049	-1,215	0,005
miR-24-3p	-0,423	0,029	-1,131	0,004
miR-26a-5p	-1,420	0,001	-1,854	0,002
miR-26b-5p	-0,757	0,032	-1,237	0,018
miR-29a-3p	-0,807	0,002	-0,955	0,005
miR-30c-5p	-1,745	0,001	-1,745	0,003
miR-320a	-0,503	0,021	-1,119	0,006
miR-342-3p	-0,435	0,015	-0,680	0,011
miR-494-3p	-1,523	0,011	-1,260	0,008
miR-660-5p	-0,678	0,022	-1,523	0,006
miR-411-5p	1,820	0,015	1,254	0,031

proteins, which have known roles related to invasion and metastasis (Supplementary Table 3). Interaction networks identified were significantly ($p<0.05$) enriched by deregulated miRNAs and genes that play roles in adaptive immune response (Supplementary Fig. 1).

Downregulation of miR-126-3p and miR-146b-5p was validated in an independent, external miRNA expression dataset

When we compared our miRNA data with the GSE77380 dataset [14], we found that two miRNAs identified in our results were validated as significantly under-expressed: miR-126-3p and miR-146b-5p (Table 2).

Table 2. Validated miRNAs in the external dataset GSE77380 [14]. FC: fold change (log_{10} values). *Corrected p-values were determined by the Benjamini Hochberg method

miRNA ID	log FC	*p value of FC
miR-126-3p	-4.14	1.10e-04
miR-146b-5p	-5.33	6.16e-03

Overexpression of SOX2 and TCF4 genes, targeted by miR-126-3p, was verified in typical carcinoid tumours from an independent, external gene expression dataset

The GSE35679 dataset [21] was used for validation of deregulated gene expression in lung typical (n=6) *versus* atypical (n=7) carcinoids. This dataset did not include histologically normal samples for comparative analysis. Results showed a total of 28 deregulated genes targeted by the deregulated miRNAs identified in the TaqMan array analysis. Supplementary Table 4 shows the expression of the 28 genes in typical *vs*. atypical tumours, and corresponding miRNAs. The expression of all genes and miRNAs was inversely correlated (miRNAs downregulated/genes upregulated, and vice-versa).

Table 3. Expression levels of SOX2 and TCF4 in a separate subset of typical (n=6) vs. atypical (n=7) lung carcinoids. Results were derived from GEO2R [20] analysis of the dataset GSE35679 [21]. Note that SOX2 over-expression was detected by two different probes in the Affymetrix array platform (U133 Plus 2.0) used for gene expression analysis. *Corrected p-values: Benjamini Hochberg method

Affymetrix Probe ID	Target genes	*p-value	log FC
213721_at	SOX2	0.0013814	2.417
228038_at	SOX2	0.0007607	4.65
228837_at	TCF4	0.0018855	1.802

We were thus able to validate over-expression of *SOX2* and *TCF4* genes, directly targeted by miR-126-3p, in typical lung carcinoids (Table 3). *TCF4* was identified as included in 2/15 (13%) pathways and in 44/437 (10%) of gene ontology annotations. *SOX2* appeared in a larger dataset of significant ($p<0.01$) results, being identified in 4/15 (26%) of significant ($p<0.01$)

pathways and in 110/437 (25%) of gene ontology annotations (GO). Supplementary Table 3 shows the enriched pathways and GO annotations. miR-126-3p (miRNA-mRNA network) is shown in Fig. 3. A putative model of miRNA-modulated pathways associated with carcinoid progression is illustrated in Fig. 4.

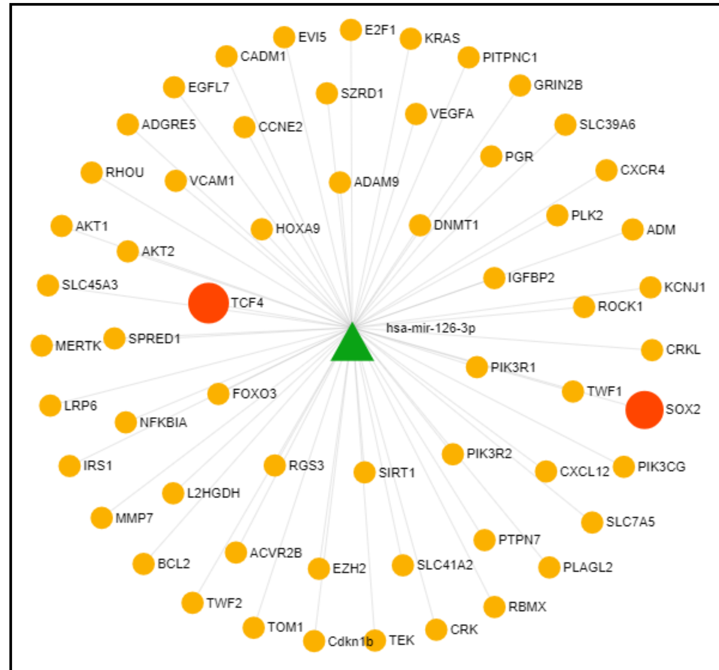

Fig. 3. miRNA-mRNA interaction network showing miR-126-3p regulated targets, highlighting SOX2 and TCF4 genes, which had increased expression validated in typical lung carcinoids (external dataset GSE35679). Genes showed in this network are expressed in lung tissue. Network was built using miRNet web-based interaction tool [17].

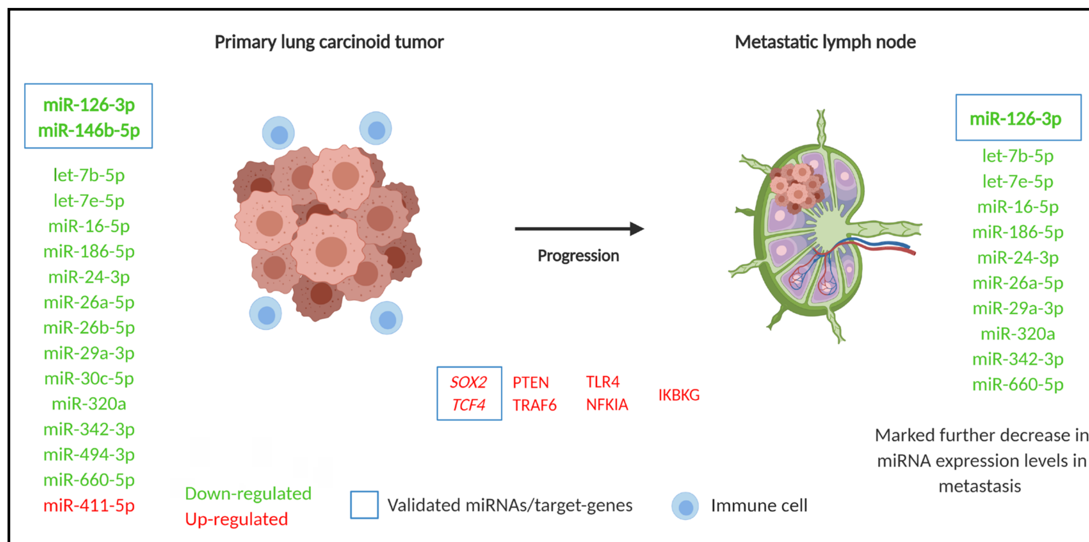

Fig. 4. The 16 commonly altered miRNAs in primary tumour and lymph node metastasis regulate the expression of genes involved in immune response, cellular proliferation, and extracellular matrix remodelling, related to invasion and metastatic potential. A subset of 12 miRNAs was further downregulated in metastasis, suggesting a potential role for deregulated miRNA-mRNA networks in disease progression. This figure was generated using BioRender.com.

Discussion

Lung typical carcinoids are not commonly observed, and the presence of lymph node metastasis is reported in about 9% of cases [4]. The case presented here matches the classification criteria for typical carcinoid tumours, with less than 2 mitoses per 2 mm^2 and the absence of necrosis [2]. Interestingly, digestive neuroendocrine cancers are highly vascularized, with well-differentiated tumours having a higher microvessel density compared to poorly differentiated cases [24]. This characteristic of neuroendocrine cancers, associated with the acquisition of molecular changes, may explain disease progression with metastatic spread observed in some typical, well-differentiated lung carcinoid tumours.

Lung carcinoids have a low frequency of mutations [7]. The underlying molecular mechanisms of carcinoid tumorigenesis may include deregulation of miRNAs, which are potent gene expression modulators. Here, we were able to identify miRNAs as potential drivers of disease progression, by analysing tumour and metastasis from the same patient.

Importantly, we identified that a subset of miRNAs was commonly downregulated in tumour and lymph node metastasis with marked further decreased expression levels in metastasis compared to the primary tumour. Our results are comparable to previous studies that also identified miRNA underexpression in typical and atypical lung carcinoids with lymph node metastasis [13, 14]. Altogether, these data suggest that miRNAs may have a tumour suppressive role with downregulated levels associated with disease progression. Our main findings agree with current evidence showing that metastatic progression accumulates further changes compared to the primary tumour (28 miRNAs deregulated in tumour and 40 miRNAs deregulated in metastasis, with 17 in common being 16 with decreased levels in paired lymph node metastasis). Our data support existing evidence on metastatic dissemination upon immune system modulation by identifying candidate miRNAs that play important regulatory roles in immunoregulatory responses including the adaptive immunity [25].

The 16 commonly deregulated miRNAs in tumour and regional metastasis directly target carcinogenesis-associated genes, a finding that is consistent with their tumour suppressive effect. Five out of 16 identified miRNAs (let-7e-5p, miR-186-5p, miR-24-3p, miR-29a-3p, and miR-411-5p) have been previously reported in a comparison between typical *vs*. atypical lung carcinoids [13].

Although our study included the analysis of only one patient, we validated our miRNA and gene expression results in previously published datasets. To the best of our knowledge, among published studies reporting large-scale microRNA data on lung carcinoids [11–14], two studies included metastatic typical lung carcinoid cases [13, 14]. We were able to obtain raw data from Yoshimoto et al. (GSE77380) [14], and validated downregulation of miR-126-3p and miR-146b-5p in typical metastatic carcinoids. Interestingly, our miRNA profiling results were more consistent with the metastatic cases, rather than the entire patient set including non-metastatic cases. These results corroborate the putative role of identified miRNAs in carcinoid tumour metastatic phenotype. To date, miR-126-3p underexpression has been identified in lung adenocarcinoma tissues, and able to predict pathological stage, tumour size, and the presence of lymph node metastasis [26]. The study by Rapa et al. [27] reported high expression of *ACVR2B* correlated with aggressive carcinoid tumour features. Interestingly, this gene is a predicted target of miR-126-3p.

In NSCLC, lower miR-146b-5p expression was associated with decreased survival and negatively correlated with *TRAF6* (TNF receptor-associated factor-6). In addition, miR-146b-5p overexpression in NSCLC cells triggered cell cycle arrest at the G1 phase, and reduced cell proliferation, migration and invasion [28].

Our study showed an enrichment of miRNA target genes with roles in adaptive immune response, including *PTEN*, *TRAF6, TLR4*, *IKBKG*, *NFKBIA*, *SOX2* and *TCF4*. Reduced PTEN expression has been shown able to increase the production of immunosuppressive cytokines, and to reduce T cell recruitment to the tumour microenvironment to lessen cell death mediated by T cells [29]. TRAF6 mediates TNF receptor superfamily and Interleukin 1

receptor signalling, playing a central role in NF-kB activation [30]. *PTEN*, *TRAF6* and *NFKB* among other immune-related genes were included in the enriched pathways identified in our analysis.

Notably, both *SOX2* and *TCF4* play roles associated with adaptive immunity. Transcription factor 4 (*TCF4*) is expressed and controls differentiation of plasmacytoid dendritic cells, which are an important component of adaptive immunity [31, 32]. *SOX2* encodes a transcription factor important to pluripotency of embryonic stem cells; it has an important role in lung development [33–35] and has high expression levels in lung cancer including neuroendocrine tumours [36]. SOX2 is a tumour-associated antigen in lung squamous cell carcinoma and adenocarcinoma with a potential role as a biomarker to stratify patients who may benefit from anti-PD-1 checkpoint inhibitors [37]. Importantly, Pyfferoen et al. reported a tumour-infiltrating dendritic cell (TIDC) miRNA signature with negative prognostic impact in NSCLC. Notably, among the identified miRNAs, miR-126-3p was downregulated in TIDCs [38] suggesting a regulatory role of this miRNA in the tumour microenvironment.

A limitation of our study is the inclusion of samples from one patient only. Regarding to biological sample source, fresh frozen tissues in general show a better quality, and if possible may not be replaced by FFPE tissues. Although, FFPE tissues are a valuable sample source and a realistic substitute when fresh frozen tissues are unavailable, being useful for molecular analysis in the research and clinical settings [39]. Here, miRNA profiles were generated using FFPE samples. Notwithstanding, the platform of choice (TaqMan® arrays) contains probes optimized and suitable for robust and reproducible amplification of miRNAs using FFPE-derived RNA. An additional limitation is the lack of gene expression validation in the same patient samples, due to the unavailability of tissues or RNA for additional analysis. However, we were able to verify increased mRNA levels of *SOX2* and *TCF4*, which are targeted by the downregulated miR-126-3p, in typical carcinoids from an external dataset GSE35679 [21]. Interestingly, both *TCF4* and *SOX2* were included in enriched pathways and gene ontology annotations associated with immune response. Altogether, our results contribute to a better understanding of metastatic typical lung carcinoid biology.

miRNAs miR-126-3p and miR-146b-5p are commonly underexpressed in a typical lung carcinoid tumour and its lymph node metastasis. The identified miRNAs and target genes are useful to conduct future validation studies, necessary to implicate these molecules in the tumorigenesis and progression of these rare tumours.

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Author Contributions

Ana L. Seneda: study design, data generation and interpretation, manuscript writing. Rainer M. Lopez Lapa: bioinformatics data analysis and interpretation, manuscript writing. Tainara F. Felix: data generation, manuscript writing. Iael W. Minutentag: data analysis and interpretation, manuscript writing. Carolina F. Campos: RNA extraction, data generation and manuscript writing. Rogério A. de Oliveira: statistical analysis and statistical review, data analysis, manuscript writing. Cristiano C. Oliveira: histopathological analysis, data interpretation, manuscript writing. Érica N. Hasimoto: collection of samples and clinical data, data interpretation, manuscript writing. Daniele C. Cataneo: collection of samples and clinical data, data interpretation, manuscript writing. Antonio J. M. Cataneo: collection of samples and clinical data, data interpretation, manuscript writing. Julio De Faveri: histopathological analysis, data interpretation, manuscript writing. Sandra A. Drigo: study design, data interpretation, manuscript writing. Luis A. J. Mur: study design, data interpretation, manuscript writing. Patricia P. Reis: study design, data interpretation, study supervision, funding, manuscript writing.

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Statement of Ethics

This study was performed in accordance with the Declaration of Helsinki and approved by the Faculty of Medicine Research Ethics Board, under REB# CAAE 63732616.0.0000.5411. Patient informed consent was obtained before sample collection, data generation and analysis.

Disclosure Statement

The authors have no conflicts of interest to declare.

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