Liquid biopsies for NETs – Circulating tumour cells, DNA and microRNAs

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Synopsis

Effective management of Neuroendocrine Tumours (NETs) depends on early diagnosis, personalised risk stratification and proper monitoring of patients' response to therapy. However, these goals are difficult to achieve because of the lack of sensitive and specific biomarkers. During cancer progression, tumours shed circulating tumour cells (CTCs), circulating tumour DNA (ctDNA) and microRNAs (miRNAs) into the bloodstream. The analysis of these novel biomarkers offers the prospect of a liquid biopsy from a patient's blood to predict and monitor therapeutic responses, assess the emergence of drug resistance, and quantify minimal residual disease. Moreover, compared with single-site biopsies, CTCs, ctDNA and miRNA have the potential to inform intratumour heterogeneity and tumour evolution in a reproducible and less invasive way. This chapter summarizes the current state-of-the-art on the potential role of CTCs, ctDNA and miRNA as prognostic and predictive biomarkers in NETs.

Key points:

- Although the significant improvements in NET treatment over the last years, novel biomarkers are still needed to help clinicians in the management of NETs.
- “Liquid biopsies” are faster, more economical and less invasive than tissue biopsies and have the potential to monitor therapeutic response and to predict recurrences through serial sampling.
- Circulating tumour cells, circulating tumour DNA and microRNAs have recently emerged as prognostic and predictive novel biomarkers in NETs.
- Larger and prospective studies are required to fully understand the role of these biomarkers in neuroendocrine tumourigenesis and to incorporate them into routine clinical practice.

Introduction

Neuroendocrine tumours(NETs) are a heterogeneous group of malignancies with a variable prognosis and behavior, that to date has been mainly defined by tissue-based characteristics such as Ki67 index, grade and morphology. Although there has been significant improvements in NET treatment over the last years[1], challenges still exist with regards to patient stratification and in monitoring treatment. Novel biomarkers are therefore needed to aid in clinical-decision making and ultimately improve patient outcomes[2]. Biomarkers can be divided into three main subgroups: diagnostic if they help to determine the presence and type of cancer, prognostic if they provide information on the patient's overall cancer outcome or predictive if they give information about which particular treatment the patient is most likely to respond to. In the last case, they may be used as a target for therapy[3].

The identification of robust biomarkers in NETs has proved challenging. Among circulating biomarkers, Chromogranin A (CgA) has been considered the most useful and widely used diagnostic and prognostic marker in the past decades[4]. However, the CgA assay has limitations including low reproducibility, poor sensitivity and modest specificity, and its overexpression in other diseases have led to diminished enthusiasm in its clinical utility[5]. Other monoanalyte biomarkers have also shown poor sensitivity, specificity and predictive ability, as summarized by Oberg et al[2]. Recently, on the basis of studies performed in a range of other cancers, there has been increasing interest in circulating tumour cells (CTCs), circulating tumour DNA (ctDNA) and microRNAs (miRNAs). Compared with traditional tissue biopsies, "liquid biopsies" are faster, less invasive, have the potential to reflect all metastatic sites, and can indicate therapeutic response or progression through serial sampling. Moreover, if we consider the potential of the genomic analysis, they offer an

alternative means of detecting genomic alterations and their evolution over time in a manner that is not feasible with invasive biopsy.

The aim of this chapter is to review the current knowledge about three new putative prognostic and predictive biomarkers for NETs, such as CTCs, ctDNA and miRNAs.

Circulating tumour cells

CTCs are released into the bloodstream from both primary tumour and secondary sites of disease, and are considered metastatic precursors[6]. CTCs were first detected in NET patients in 2011[7]. Khan et al. demonstrated EpCAM expression in NET by immunohistochemistry suggesting that NET CTCs might be detectable using the CellSearch platform for which EpCAM expression is a requirement. In 79 patients with metastatic NETs, CTCs were detected in midgut (43%), pancreatic (21%) and bronchopulmonary NETs (31%). Importantly, it was noted that the presence of CTCs was associated with disease progression while absence was strongly associated with stable disease. In a subsequent study, the same group defined the prognostic relevance of CTCs in a larger population of 175 NET patients[8]. Presence of CTCs was associated with increased burden, increased tumour grade and elevated CgA. There was a highly significant association between presence of CTCs and worse progression-free survival (PFS) and overall survival (OS). According to multivariate analysis, CTCs were demonstrated to be an independent prognostic factor for survival (HR 3.7 P=0.003) in contrast to CgA (HR 1.5 P=0.4). Subsequently the predictive role of CTCs was explored in 138 patients with metastatic NETs, in which CTCs were enumerated at baseline, 3-5 weeks and 10-15 weeks after commencing treatment[9]. The most commonly used therapies were somatostatin analogs (SSAs),

chemotherapy, peptide receptor radionuclide therapy and transarterial embolization. Early post-treatment dynamic changes in CTC count were significantly associated with radiological response and OS. Remarkably, patients who maintained undetectable CTCs post-therapy or had a ≥ 50% fall had a reduced chance of progression and superior survival compared with those that had a < 50% fall or a rise in CTC count. The authors also compared early and late post-treatment points and did not find a clear advantage for the later time point, consistent with similar studies in prostate and colorectal cancer. CTCs have also been evaluated for expression of therapeutic targets. For example, somatostatin receptor (SSTR) expression has been measured on CTCs isolated from metastatic gastroenteropancreatic NET patients[10]. In clinical practice, expression of SSTR2/5 is measured by nuclear medicine imaging but the resolution of these modalities is insufficient to define intra-tumoural heterogeneity of SSTR expression, nor is imaging the optimal method to track changes in expression that may arise during therapy. The authors showed that SSTR detection on CTCs is feasible and may provide insights into tumour heterogeneity as well as a means of tracking expression over time and during therapy, as compared with tissue expression. Finally, preliminary results from the same group show that presence of CTCs is associated with skeletal involvement and that the CXCR4/SDF-1 axis may be a potential mechanism of osteotropism for CTCs in NET patients (Rizzo et al unpublished data).

The utility of CTC count has also been explored in patients with Merkel cell carcinoma (MCC). Despite some technical limitations of these studies, CTCs were found to reflect burden of disease and their presence showed a significant association with survival in 34 patients[11]. Gaiser et al. detected $EpCAM^{+}$ CTCs in

97% of 30 MCC patients by the Maintrac system and found that CTC count was elevated in patients with active disease[12].

Several but small trials explored the prognostic and predictive value of CTCs in small cell lung cancer (SCLC). After the first evidence of CTCs in the blood of a SCLC patients in 2009[13], several studies have shown that pretreatment CTC number and change in CTC number after chemotherapy or at relapse are independent prognostic factors for SCLC patients[14-20]. Along this line, a recent report highlighted that copy number alterations (CNAs) in CTCs of SCLC patients could correctly identify patients as chemorefractory or chemosensitive, indicating that CTC CNA may represent a further predictive and prognostic marker in these settings[21]. The most relevant findings on CTCs in NETs are summarized in Table 1.

Circulating tumour DNA

ctDNA is composed of short nucleic fragments (~166bp) released in the blood from apoptotic or necrotic cells[22]. Since the first report in 1977[23], several studies have investigated its prognostic significance in cancer[24-27]. ctDNA analyses can reveal important information about genomic aberrations relevant to the efficacy of targeted drugs, including EGFR mutations in non-small cell lung cancer[28], KRAS mutations in colorectal cancer[29], TP53 and PIK3CA mutations in breast cancer[30,31] and AR mutations in prostate cancer[32]. Consequently, ctDNA has clear applications for monitoring response to therapy in these patients.

A potential challenge with the application of ctDNA to the NET field is the relative lack of recurrent mutations in comparison with other tumours. Molecular profiling of small bowel (SB) NETs revealed the most common recurrent mutations was in cyclin dependent kinase inhibitor CDKN1B occurring in only 8% of cases[33]. Pancreatic

neuroendocrine tumours(pNETs) are also characterized by recurrent mutations in a relatively limited number of genes, which include tumour the suppressor gene MEN1, as well as ATRX and DAXX, genes implicated in chromatin remodeling[34]. An abstract presented by Pipinikas et al. at ENETS Conference in 2016 demonstrated that ctDNA can be detected in blood of pNET patients with a variable concordance between tissue and ctDNA somatic variants using whole exome sequencing (WES)[35]. A subsequent abstract presented by Beltran et al. at ASCO Conference in 2017 focused on WES of ctDNA in patients with neuroendocrine prostate cancer (NEPC) in order to develop a non-invasive tool to assess progression from adenocarcinomas to a NEPC phenotype[36]. WES of ctDNA and matched metastatic biopsies showed approximately 80% of shared mutations with a higher similarity of CNAs in NEPC compared to adenocarcinomas, suggesting less heterogeneity in NEPC. NEPC alterations were detectable in the circulation prior to the development of NEPC clinical features and, when different metastatic sites were compared with ctDNA, the contribution of tumour alterations in ctDNA was highest for the liver metastasis versus other sites of disease, with obvious implications for the interpretation of single site biopsies. The authors concluded that WES of ctDNA can be used to better understand intratumoural heterogeneity and to identify patients with predisposition towards NEPC transformation before clinical progression.

Very few case reports have been published so far on the possible use of ctDNA for personalized medicine in NETs. Recently, Wang et al. first reported an ALK traslocation revealed by ctDNA analysis in a patient with a metastatic atypical carcinoid tumour[37]. The ALK rearrangement occurred at the canonical intron 19 breakpoint and contained the intact kinase domain of ALK. The patient was started on the second-generation ALK inhibitor alectinib with rapid and lasting shrinkage of

his disease supporting the hypothesis that the ALK translocation was the driver mutation. In another case report, a woman with a high-grade, large-cell neuroendocrine cervical carcinoma was successfully started with Nivolumab combined with stereotactic body radiation therapy, based on blood ctDNA results showing alterations suspicious of high tumour mutational burden[38]. Tissue genomic results, available after initiating treatment, confirmed ctDNA results.

Blood ctDNA can be excreted into urine where it can be detectable[39]. Patient-matched tissue, plasma, and urine studies indicate concordance of DNA mutation status and comparable sensitivities across the three biospecimens[40]. Klempner et al. described a patient case with treatment-refractory metastatic high grade rectal NET harboring a $BRAF^{V600E}$ substitution who achieved a rapid and dramatic response to combination BRAF/MEK directed therapy and a concurrent decrease in urinary $BRAF^{V600E}$ ctDNA[41]. The correlation between clinical improvement and $BRAF^{V600E}$ urinary ctDNA detection provides evidence for the clinical utility of urine ctDNA in monitoring of tumour dynamics.

MicroRNAs

miRNAs are a family of 21-25-nucleotide small RNAs that regulate gene expression at the post-transcriptional level by binding to target RNAs, resulting in RNA degradation and inhibition of translation[42]. More than 1900 human miRNAs have been discovered since 1993, when the first miRNA was identified[43], and are annotated in the miRNA registry (http://mirbase.org). miRNAs are relatively stable in human tumour samples and can also be released in blood specimens by passive[44] or active secretion[45,46]. Several studies have recently profiled the expression of miRNAs in pulmonary carcinoids, reporting differences between normal lung tissue

and tumour, low- and high-grade bronchial NETs as well as localized and metastatic disease[47-49]. Ranade et al examined the prognostic role of 880 mature miRNAs and 473 pre-miRNAs in 31 SCLC samples and found that miR-92a2* levels inversely correlated with survival[50]. The authors also showed that expression levels of miR-92a-2*, miR-147 and miR-574-5p were significantly associated with chemoresistance. Downregulated miR-886-3p, which potentially repress cell proliferation, migration and invasion, correlated with shorter survival in 42 SCLC patients by Cao et al[51]. This result was subsequently confirmed in a study on 924 miRNAs from 42 SCLC patients where the authors found that miR-150/miR-886-3p signature significantly correlated with OS and PFS[52]. A prognostic role has also been described for miR-7, which targets the gene MRP1/ABCC1 involved in chemoresistance. Low expression level of miR-7 was significantly associated with drug responsiveness and OS in 44 patients with SCLC[53]. More recently, cytological samples from 50 patients with SCLC were analysed for the expression of a 3-miRNA panel (miR-192, miR-200c, and miR-205) and a better OS was described for patients with a low expression level of the 3-miRNA panel[54]. Lee et al. evaluated the expression pattern of three miRNAs (miR-21, miR-155 and miR let-7a) in a series of 63 lung NETs. The expression level of miR-21 in carcinoid tumours with lymph node metastases was significantly higher than in carcinoid tumours without lymph node metastases[49]. Mairinger et al. screened 763 miRNAs known to be involved in pulmonary carcinogenesis in 12 lung NETs and found that eight miRNAs showed a negative (miR-22, miR-29a, miR-29b, miR-29c, miR-367*; miR-504, miR-513C, miR-1200) and four miRNAs a positive (miR-18a, miR-15b*, miR-335*, miR-1201) correlation to tumour grade. Moreover, miRNAs let-7d, miR-19, miR-576-5p, miR-340*, miR-1286 were significantly associated with survival[55]. On the contrary, no

association between miRNAs expression levels and survival was described in other studies[47,56]. Rapa et al. evaluated 56 cases of lung NETs for the expression of 11 miRNAs (miR-15a, miR-22, miR-141, miR-497, miR-503, miR-129-5p, miR-185, miR-409-3p, miR-409-5p, miR-431-5p and miR-129*), selected on the basis of the results obtained in a previous pilot series. They found four miRNAs (miR-129-5p, miR-129*, miR-22, miR-141) down modulated in carcinoid cases with high pT3-4 stages and four (miR-129-5p, miR-409-3p, miR-409-5p, miR-431-5p) down modulated in carcinoid cases with vascular invasion as compared to cases without. Finally, the association with nodal status was statistically confirmed in the whole series for 3 microRNAs (miR-409-3p, miR-409-5p, miR-431-5p)[48].

Studies on miRNAs in pNETs are scarse. miR-21 levels were strongly associated with Ki67 index and liver metastases in a series of 40 pNENs[57]. Thorns et al. investigated the expression levels of 754 miRNAs in tissue samples of 37 pNET patients. They found that miR-642 and miR-210 correlated with Ki67 index and with metastatic spread, respectively, but could not provide information concerning survival[58]. Lee et al. evaluated the expression levels of eight miRNAs (miRNA-27b, 122, 142-5p, 196a, 223, 590-5p, 630 and 944) in 37 pNENs[59]. Only miR-196a level was significantly associated with stage and mitotic count. When pNETs were stratified into high and low miRNA-196a expression groups, miRNA-196a-high pNETs were significantly associated with advanced pathologic stage, higher mitotic counts and Ki67 index. In addition, high miRNA-196a expression was significantly associated with decreased OS and disease-free survival.

Also SBNET progression is characterized by a differential pattern of miRNA expression. Up-regulation of miR-183 and down-regulation of miR-133a were reported during tumour progression in two separate studies[60,61] on 8 and 24 SBNET

respectively, making these miRNAs appealing targets for future investigations. Other evidence suggests that miR-129-5p may have an anti-proliferative and anti-metastatic effect in midgut carcinoid tumours, and that its down-regulation during tumour progression might affect factors involved in RNA binding and nucleotide metabolism such as EGR1 and G3BP1[62]. Mandal et al. examined miR-96 and miR-133a expression in 51 gastrointestinal NETs and found increased expression of miR-96 and decreased expression of miR-133a during progression from primary to metastatic NETs, suggesting that a combination of both may serve as useful diagnostic and prognostic markers[63]. Miller et al. performed miRNA profiling experiments in 90 patient samples and discovered 39 miRNAs significantly deregulated in SBNETs compared with adjacent normal bowel[64]. Moreover, miR-1 and miR-143, which directly regulate FOSB and NUAK2 oncogenes, were found significantly downregulated in metastases compared with primary tumours.

Despite the evidence that tissue miRNAs can be detected in serum samples and that serum levels may correlate with tumour stage and treatment status[65], few data are available on circulating miRNAs. Bowden M et al. undertook a multi-stage study in SBNET patients[66]. They first developed a panel of 31 candidate miRNAs detectable in patient plasma, based on evaluation of SBNET samples and matched plasma samples. They refined the panel in an independent cohort of 40 cases and 40 controls and, among the 31 candidate miRNAs, they identified 4 miRNAs (miR-22-3p, miR-21-5p, miR-29b-3p and miR-150-5p) that were differently expressed between the patient and control groups. In particular, levels of miR-21-5p and miR-22-3p were higher, and miR-29b- 3p and miR-150-5p were lower in the plasma of the 40 patients compared to the 40 healthy controls. Then, they validated this panel in a second, large cohort of 120 patients and 120 matched independent controls and,

as in the previous cohort, they observed up-regulation of miR-21-5p and miR-22-3p and down-regulation of miR-150-5p in patients with metastatic SBNETs. Moreover, low plasma expression of miR-21-5p and miR-22-3p and high expression of miR-150-5p were significantly associated with prolonged OS. Finally, they generated a high/low risk index to combine miR-21-5p, miR-22-3p and miR-150-5p which was associated with shorter survival. The most relevant findings on prognostic miRNA in NETs are summarized in Table 2.

Conclusions

Despite the identification of CTCs, ctDNA and miRNAs as circulating biomarkers capable of providing prognostic and predictive information in NET patients, they have not been incorporated into routine clinical practice. This is due in part to technological limitations hampering routine analysis, as well as a limited data regarding the implications of clinical decision making based on these biomarkers. Therefore, more prospective evaluations are required to better understand the role of these biomarkers in neuroendocrine tumourigenesis. Incorporation of CTCs, ctDNA and miRNA analysis into clinical trials is highly recommended to allow greater generalizability and more impactful results, as already happened for CTCs (NCT02075606) and ctDNA (NCT02973204). The potential to interrogate the tumour genome sequentially using liquid biopsies promises to provide unique insights into tumour heterogeneity, cancer evolution and the emergence of tumour resistance in the coming years.

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Table 1. Prognostic and predictive role of CTCs in NETs.

Patients and primary site	Findings	Reference
79 metastatic NETs: - 19 pancreatic - 42 midgut - 13 bronchopulmonary - 5 unknown primary	• Significant association between CTC levels and burden of liver metastases ($P < 0.001$) • Moderate correlation between CTC levels and urinary 5-hydroxyindole acetic acid ($P = 0.007$) • No correlation between CTCs levels and Ki67 ($P = 0.59$) and low correlation between CTCs levels and CgA ($P = 0.03$) • Absence of CTCs associated with stable disease ($P < 0.001$)	7
175 metastatic NETs: - 42 pancreatic - 101 midgut - 17 bronchopulmonary - 12 unknown primary - 3 hindgut	• Significant association between CTC presence and grade ($P = 0.036$), tumour burden >25% ($P < 0.001$) and CgA >120 pmol/L ($P < 0.001$) • Presence of ≥ one CTC associated with worse PFS and OS ($P < 0.001$) • Within grades, presence of CTCs able to define a poor prognostic subgroup	8
138 metastatic NETs: - 31 pancreatic - 81 midgut - 12 bronchopulmonary - 11 unknown primary - 3 hindgut	• Significant association between the first post-treatment (after 3–5 weeks) CTC count and progressive disease (PD) ($P < 0.001$): PD in 8% of patients with 'favourable CTC response' (0 CTCs at baseline and after treatment, or ≥50% reduction from baseline) vs 60% in unfavourable group (<50% reduction or increase) • Strong association between changes in CTCs and OS ($P < 0.001$), the best prognostic group being patients with 0 CTCs before and after therapy, followed by those with ≥50% reduction in CTCs, with those with a <50% reduction or increase in CTCs having the worst outcome • In multivariate analysis, changes in CTCs strongly associated with OS ($P < 0.001$)	9
31 metastatic GEP-NETs - 12 pancreatic - 19 non-pancreatic	• Detection of CTCs in 68% of patients, of which 33% had evidence of heterogeneous expression of either SSTR2 or SSTR5 • In patients with $SSTR^+$ CTCs, fraction of $SSTR2^+$ or $SSTR5^+$ CTCs variable from 10 to 100% and 50 to 100%, respectively, indicating intra-patient heterogeneity of SSTR expression • Variable concordance between the IHC and CTC staining for SSTR 2 and 5	10
34 MCC	• Correlation between CTC presence and extent of disease ($P = 0.004$) • Significant difference in median OS between CTCs positive and CTCs negative	11

	samples ($P = 0.0003$)	
30 MCC	• Significantly higher CTC count in patients with active disease ($P < 0.05$) • Increasing CTC count associated with development of new metastases	[12]
50 SCLC	• Longer median survival for patients with <2 CTCs compared with patients with >300 CTCs ($P < 0.005$) • Persistently elevated CTC number at day 22 was an adverse prognostic factors in univariate analysis ($P < 0.01$)	[14]
97 SCLC	• Significantly shorter PFS and OS for patients with ≥50 CTCs compared with patients with less than 50 CTCs/7.5mLof blood ($P < 0.001$) • A favourable CTC number (<50) after one chemotherapy cycle was associated with significantly longer PFS and OS compared with an unfavourable CTC number (≥50) ($P < 0.001$) • Patients with less than 50 CTCs at both baseline and post-treatment time points had significantly better survival compared with other patients	[15]
59 SCLC	• Association between lack of measurable CTCs and prolonged survival ($P \leq 0.001$) • CTCs count decrease after the first cycle of therapy correlated with longer OS and PFS ($P \leq 0.001$) • CTCs count decrease after four cycles of therapy correlated with longer OS ($P = 0.05$) and PFS ($P = 0.007$) • CTCs count <2 after the first cycle of therapy was an independent prognostic factor for OS in multivariate analysis ($P = 0.09$)	[16]
60 SCLC	• Association between a reduction of CTC count higher than 89% following chemotherapy and a lower risk of death (HR 0.24, 95% CI 0.09–0.61)	[17]
30 SCLC	• Significantly longer median survival time for patients with a CTC count of <2 cells/7.5 ml compared with patients with a CTC count of ≥2 cells/7.5 ml prior to treatment ($P = 0.007$). Baseline CTC count was an independent prognostic factor for survival time in multivariate analysis ($P = 0.026$) • Longer median PFS for patients with a CTC count of <2 cells/7.5 ml following two cycles of chemotherapy compared with patients who had a CTC count of ≥2 cell/7.5 ml ($P = 0.07$)	[18]
89 SCLC	• Shorter OS in patients with ≥10 CTCs per 7.5 ml compared with patients with <10	[19]

	CTCs per 7.5 ml ($P < 0.0001$) • After the second cycle of chemotherapy, worse OS and PFS in the group with ≥10 CTCs per 7.5 ml ($P < 0.0001$ and $P = 0.0002$, respectively) • On disease progression, shorter median OS in patients with ≥10 CTCs per 7.5 ml ($P = 0.0053$) • Both PFS and OS of patients with a CTC count <10 per 7.5 ml at baseline or a drop in CTCs to <10 per 7.5 ml after the second cycle of chemotherapy longer than in patients with a CTC count ≥10 per 7.5 ml after the second cycle of chemotherapy or patients in whom the CTC count increased to 10 per 7.5 ml after treatment	
50 SCLC	• Longer PFS in patients with <5 CTCs at baseline ($P = 0.0259$) • Significant correlation with both OS ($P = 0.0116$) and PFS ($P = 0.0002$) if a higher cutoff (CTC <50 or CTC ≥50) was used .• Longer PFS and OS in patients with <5 CTC on day 1 of the second cycle of chemotherapy ($P = 0.0001$)	[20]
31 SCLC	• Identification of chemosensitive and chemorefractory patients by CTCs copy-number aberrations profile and observation of significant difference ($P = 0.0166$) in PFS between the two groups • Difference in CTCs copy-number aberrations profile between initial and acquired chemoresistance	[21]

Abbreviations: NET, neuroendocrine tumours; CTCs, circulating tumour cells; CgA, chromogranin A; OS, overall survival; PFS, progression-free survival, GEP-NETs, gastroenteropancreatic NETs; SSTR, somatostatin receptor; IHC, immunohistochemistry; MCC, Merkel cell carcinoma; SCLC, small cell lung cancer.

Table 2. Prognostic miRNAs in NETs.

miRNA	NET histology	Source	De-regulation	References
miR-92a2*	SCLC	Tissue	Up-regulated	[50]
miR-886-3p	SCLC	Tissue	Down-regulated	[51]
miR-150 and miR-886-3p	SCLC	Tissue	Down-regulated	[52]
miR-7	SCLC	Tissue	Up-regulated	[53]
miR-192, miR-200c, miR-205	SCLC	Tissue	Up-regulated	[54]
miR-21	Lung NETs	Tissue	Up-regulated	[49]
let-7d, miR-19, miR576-5p, miR-340*, miR-1286	Lung NETs	Tissue	Up-regulated	[55]
miR-409-3p, miR-409-5p, miR-431-5p, miR-129-5p, miR-129*, miR-22, miR-141, miR-431-5p	Lung NETs	Tissue	Down-regulated	[48]
miR-21	pNETs	Tissue	Up-regulated	[57]
miR-642, miR-210	pNETs	Tissue	Up-regulated	[58]
miR-196a	pNETs	Tissue	Up-regulated	[59]
miR-183 miR-133a	SBNETs	Tissue	Up-regulated Down-regulated	[60,61]
miR-129-5p	SBNETs	Tissue	Down-regulated	[62]
miR-96 miR-133a	GI-NETs	Tissue	Up-regulated Down-regulated	[63]
miR-1, miR-143	SBNETs	Tissue	Down-regulated	[64]
miR-200a	SBNETs	Blood	Up-regulated	[65]
miR-21-5p, miR-22-3p miR-150-5p	SBNETs	Blood	Up-regulated Down-regulated	[66]

Abbreviations: NET, neuroendocrine tumours; SCLC, small cell lung cancer; pNETs, pancreatic NETs; SBNETs, small bowel NETs; GI-NETs, gastrointestinal NETs.