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Review

The clinical utility of dysregulated microRNA expression in paediatric solid tumours

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Abstract MicroRNAs (miRNAs) are short, non-protein-coding genes that regulate the expression of numerous protein-coding genes. Their expression is dysregulated in cancer, where they may function as oncogenes or tumour suppressor genes. As miRNAs are highly resistant to degradation, they are ideal biomarker candidates to improve the diagnosis and clinical management of cancer, including prognostication. Furthermore, miRNAs dysregulated in malignancy represent potential therapeutic targets. The use of miRNAs for these purposes is a particularly attractive option to explore for paediatric malignancies, where the mutational burden is typically low, in contrast to cancers affecting adult patients. As childhood cancers are rare, it has taken time to accumulate the necessary body of evidence showing the potential for miRNAs to improve clinical management across this group of tumours. Here, we review the current literature regarding the potential clinical utility of miRNAs in paediatric solid tumours, which is now both timely and justified. Exploring such avenues is warranted to improve the management and outcomes of children affected by cancer.

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1. Introduction

Only a very small proportion, estimated to be <5%, of the genome encodes protein-coding messenger RNA (mRNA). The dogma that the remaining ~95% merely constitutes 'junk' DNA has long been dispelled, with the demonstration that the genome is pervasively transcribed, producing both protein-coding mRNAs and non-coding RNAs (ncRNAs) [1]. MicroRNAs (miRNAs) are small ncRNAs, typically 18–23 nucleotides (18–23 nt) in length, that regulate the expression of numerous protein-coding genes [2]. The first miRNA, *lin-4*, discovered in 1993 in the nematode *Caenorhabditis elegans*, regulates development via the protein lin-14 [3]. Since then, miRNAs have been shown to have crucial roles in diverse biological processes, such as proliferation, differentiation and development, and accordingly, their temporal and spatial expression is normally tightly regulated [4]. Consequently, miRNA dysregulation has been implicated in many disease processes, including tumorigenesis. Much recent research effort has, therefore, focused on the potential use of miRNAs as biomarkers in multiple diseases, including adult cancer, as reviewed elsewhere [5]. Studying dysregulated miRNA expression is of particular relevance to childhood cancers as they typically have a low mutational burden compared with their adult counterparts [6]. As a result, identifying DNA mutations and circulating tumour DNA (ctDNA) is likely to be of more limited use for this patient group.

As childhood cancers are rare, it has taken time to accumulate the necessary body of evidence showing the potential for miRNAs to improve clinical management across this group of tumours; a comprehensive review is now both possible and justified. This review, therefore, outlines our current understanding of miRNA biogenesis and describes their function. We emphasise the potential for elucidating the roles of specific miRNAs in paediatric solid tumours that are likely to impact on diagnosis, risk stratification and clinical management. Finally, we discuss opportunities to select dysregulated miRNAs for future therapeutic interventions.

2. MiRNA biogenesis

An overview of miRNA biogenesis is shown in Fig. 1. MiRNAs are transcribed from both intragenic and intergenic regions of the genome [7]. RNA polymerase II, or occasionally RNA polymerase III, transcribes DNA to produce pri-miRNAs, which are approximately one kilobase (1 Kb) in length. MiRNAs can be transcribed polycistronically to contain many miRNAs, termed a 'cluster', e.g., miR-17–92 and miR-371–373. The vast majority (~99%) of pri-miRNAs are processed via the canonical pathway, where pri-miRNAs are processed into pre-miRNAs by Drosha (a ribonuclease III enzyme) and DGCR8 (an RNA-binding protein [RBP]) [8]. The pre-miRNAs are exported from the nucleus into the cytoplasm by Exportin-5 [7] and further processed by Dicer, a ribonuclease (RNase) III enzyme,

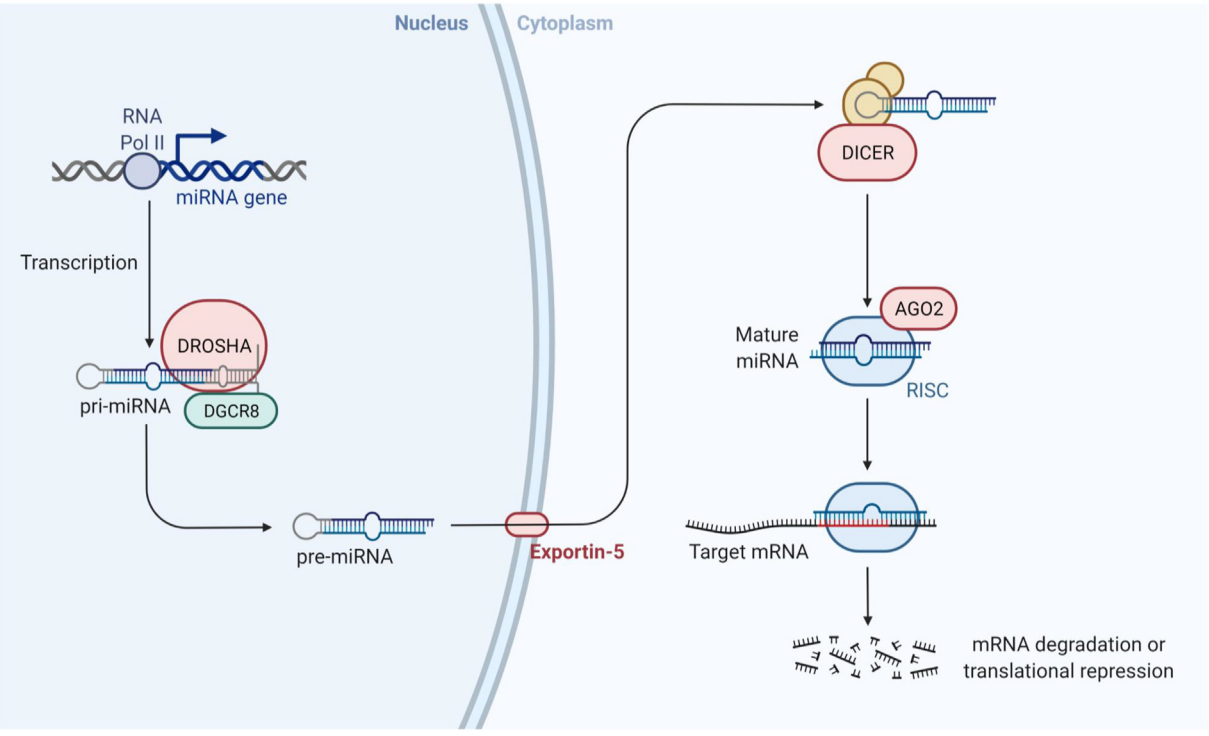

Fig. 1. Overview of microRNA biogenesis. Adapted from 'microRNA in cancer', by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.

to form a 22 nt mature miRNA duplex [8]. Either miRNA strand can be incorporated into the Argonaute protein-based RNA-induced-silencing-complex (RISC) to become the guide strand [9], then termed miRISC [7], and the non-incorporated strand is degraded by AGO2 [10]. In addition, multiple other 'non-canonical' pathways for miRNA biogenesis are being discovered and are reviewed elsewhere [9].

3. MiRNA function

MiRNAs control the activity of ~50% of all protein-coding genes [11], predominantly through miRNA-mediated gene silencing via miRISC. This interaction occurs via target sequences in the 3′ untranslated region (3′UTR) of mRNA strands, known as miRNA response elements (MREs). Functional interaction occurs via the 5' 'seed' region of the miRNA, comprising consecutive nucleotides at positions 2–8. Nucleotides 2–7 (2-7 nt) are most critical for determining binding specificity to the 3′UTR on target mRNA transcripts [12]. Once bound, the regulation of gene expression occurs via translational repression and/or mRNA degradation, with intra-cytoplasmic structures called P-bodies playing an important role [13]. Physiologically, miRNA-dependent gene regulation acts on the proteome to fine-tune protein output [14]. In humans, most miRNA gene regulation is now believed to be mediated through mRNA degradation [15,16], as detected from changes in mRNA expression [17]. It has also been proposed that miRNAs could potentially regulate genes by other mechanisms, which has again been reviewed elsewhere [7].

4. MiRNAs in cancer

MiRNAs play a key role in cancer development, both as oncogenes ('oncomiRs') and as tumour suppressor genes (TSGs) [18–23]. The earliest discovery of miRNA involvement in human cancer was in B-cell chronic lymphocytic leukaemia, where the frequently deleted chromosome 13q14 region contains miR-15a and miR-16-1 [24], which normally act as TSGs by repressing Bcl-2, inducing apoptosis [25]. Genomic amplification, for example, results in the overexpression of the miR-17–92 cluster in B-cell lymphoma [26]. Furthermore, genome-wide studies have shown that miRNA genes are located in 'cancer-associated genomic regions', with 50% of miRNAs in common fragile sites [27], suggesting that abnormal miRNA expression due to defects at the genome level promotes tumorigenesis. Such defects include translocations or specific DNA copy number gains/losses [28,29].

Epigenetic changes (heritable changes in gene expression that do not involve a change in DNA sequence) [30], where miRNA transcription is altered due to promoter DNA methylation [31] or chromatin remodelling through histone modifications [32], may lead to cancer. For example, the TSG miR-127 downregulates the proto-oncogene BCL6, but in malignant cells, it is within a CpG (methylation) island, and methylation results in reduced miR-127 expression. Inhibition of DNA methylation using a histone deacetylase inhibitor replenished levels of miR-127, with concomitant BCL6 downregulation [33].

MiRNAs are also controlled by transcription factors, so the dysregulation of these alters miRNA expression. For example, the transcription factor c-Myc, which is upregulated in many cancers, has been shown to activate transcription of oncomiRs, such as the miR-17–92 cluster, and downregulate the expression of TSG miRNAs, such as miR-15a, miR-26, miR-29, miR-30, and *let-7* [34]. Alterations in the levels of miRNA biogenesis processing machinery may also result in cancer [35–37] or extensive post-transcriptional regulation of miRNAs [37,38]. For example, the upregulation of Drosha in cervical cancer, through gain of chromosome 5p, results in altered miRNA profiles [39], with the miRNAs most significantly associated with Drosha overexpression implicated in carcinogenesis [40]. Additionally, RBPs play an important role in miRNA biogenesis, through the binding to the stem-loop of miRNA precursors. For example, *KSRP* binds pre-miRNAs, promoting biogenesis [41], whilst *LIN28* specifically binds *let-7* pri- and pre-miRNAs, and blocks processing by Drosha and Dicer, respectively [42]. As *let-7* is a known miRNA tumour suppressor [43], the overexpression of *LIN28*, which occurs in ~15% of all human malignancies, results in reduced levels of mature *let-7* and tumorigenesis [44,45].

Alterations in the quantitative or qualitative nature of mRNA target 3′UTRs may also alter miRNA regulation. For example, proliferating cells express mRNAs with shortened 3′ UTRs and thus fewer miRNA-binding sites [46], and this phenomenon also occurs in cancer cells [47]. Additionally, polymorphisms or variants within 3′UTRs have been shown to increase cancer risk [48] and predict therapy response [49]. Finally, RBPs, such as *DND1,* bind to the 3′UTR of mRNAs and prevent access of, and subsequent regulation by, certain miRNAs [50], suggesting the dysregulated expression of such proteins has the potential to result in cancer formation.

The following section of the review specifically uses a biology-based thematic analysis to demonstrate the clinical utility of dysregulated miRNA expression in paediatric malignancies pertaining to potential biomarkers that may be used for diagnosis, disease monitoring, prognostication, and as candidate novel therapeutic targets.

5. Review methodology

It was not feasible to undertake a formal systematic review as the subject was too broad. Accordingly, after

appropriate expert advice (Dr Bob Philips, Senior Clinical Academic and Honorary Consultant Paediatric Oncologist, Centre for Reviews and Dissemination, University of York, York, UK) and after journal approval, a hybrid review methodology was undertaken. This involved two phases. The first phase was an initial review approach, with defined inclusion/exclusion criteria, to identify potentially relevant papers. The second phase then involved selecting key papers, based on a 'best evidence' fashion involving impact and relevance, that exemplified fundamental points in the review regarding clinical utility. This selection of representative papers from phase two was then used in the construction of a thematically driven synthesis, using a biology-based rather than disease-based approach.

The first review phase was undertaken in January 2022. For this, a detailed search strategy was developed using search terms that would capture relevant publications in the subject area of both paediatric malignancy and miRNAs. These search terms were specifically as follows: (((((((cancer*[tiab]) OR (neoplasm*[tiab])) OR (tumor*[tiab])) OR (tumour*[tiab])) OR (malignanc*[tiab])) OR ("Neoplasms" [Mesh]))) AND ((((("MicroRNAs" [Mesh]) OR (microRNA*[tiab])) OR (miRNA*[tiab]))))) AND (((((((("Pediatrics" [Mesh])) OR (child*[tiab])) OR (infant*[tiab])) OR (adolescen*[tiab])) OR (pediatric*[tiab])) OR (paediatric*[tiab]))). These terms were searched in MEDLINE, and the 'humans' filter was applied (n = 1205) (Fig. 2). Two independent reviewers (KC/EB) subsequently screened the manuscripts using the inclusion/exclusion criteria. We included all primary literature to allow us to detect discovery studies in small cohorts, given the rarity of some paediatric cancer subtypes. We excluded manuscripts that involved leukaemia or lymphoma (n = 244), as our focus was on paediatric solid tumours, those that were not relevant to the topic of the clinical utility of miRNAs (n = 155), those that were not primary literature (n = 139) or in English (n = 29), those that were not on cancer (n = 112) or paediatric cases (n = 27), and those that were subsequently retracted (n = 2). The first phase included 497 manuscripts, of which 101 were selected for

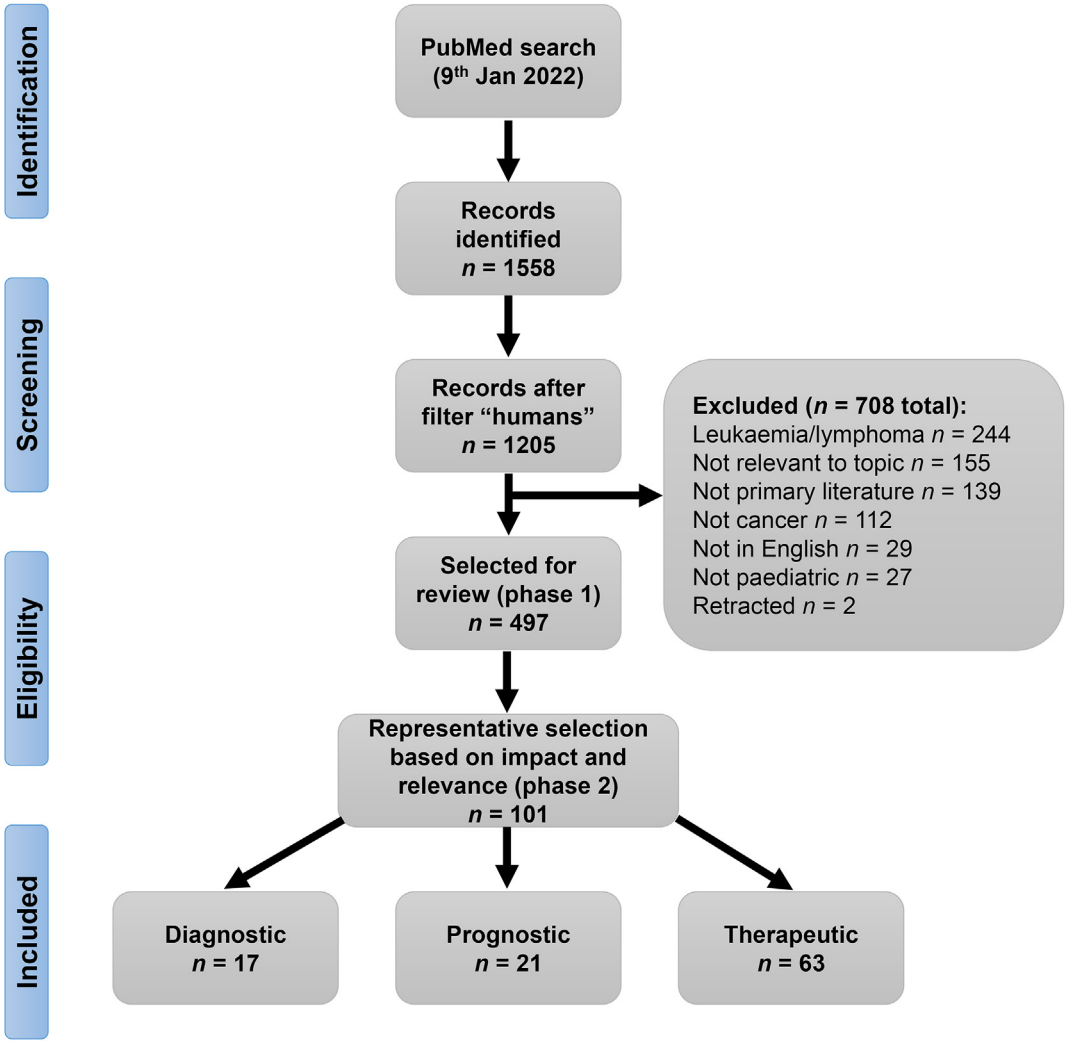

Fig. 2. PRISMA flow diagram of the literature search for the Review.

phase 2 based on impact and relevance, to highlight key principles and examples of the clinical utility of miRNAs in paediatric solid tumours.

6. MiRNAs in cancer diagnosis and treatment monitoring

It is likely that for each malignancy, a 'signature' comprising a small panel of key miRNAs will distinguish malignant tumours from benign tumours and normal tissue, as well as between different malignant tumours. Tumour tissue may be examined by *in situ* hybridisation (ISH) or polymerase chain reaction (qRT-PCR) to detect this differential expression, which may become routinely available for miRNAs in the future. However, the storage and preparation of fresh tissue is expensive and labour-intensive. Furthermore, a non-invasive method of detecting differential miRNA expression in malignancy, for example, from routine blood samples, would have advantages, such as potentially obviating the need for diagnostic surgical biopsy prior to definitive therapy (Fig. 3).

To be of use as non-invasive blood-based markers, miRNAs released from solid tumours into the bloodstream need to be protected from endogenous RNase activity that would result in their degradation and be robustly detectable in the cell-free fraction (either plasma [containing clotting factors] or serum [without]) [51]. MiRNAs released from tumour cells appear to be protected from RNase degradation by packaging within membrane-bound exosome particles, which are shed into the extracellular space from the cell membrane and then released into the bloodstream [52,53]. Furthermore, miRNA levels have been shown to be stable, even in serum samples subjected to multiple freeze–thaw cycles [54,55] or those left at room temperature for 24 h prior to processing [55]. These properties are critical when considering their potential use in routine clinical practice, where such variations in sample handling will occur. Additionally, serum miRNA levels in healthy individuals are stable and similar to that of circulating blood cells [54] and a good correlation exists between individual miRNA levels obtained from serum and plasma samples from the same patients [55].

It should be noted that miRNAs are not just released from tumour cells but also by cells of the tumour microenvironment and peripheral immune cells [56,57]. Furthermore, the use of miRNAs is not restricted to blood-based detection, with miRNAs reliably detected in a wide range of body fluids [58] (Fig. 3); for example, miRNAs are used for forensic examinations. Urinary levels of miR-126 and miR-152 have been used to identify cases of bladder cancer [59], and a recent study has highlighted the potential use of miR-204-5p in urinary exosomes, as an early diagnostic biomarker for Xp11.2 translocation-positive renal cell carcinoma [60]. Furthermore, miRNAs are detected within the cerebrospinal fluid (CSF) [61], offering the potential for facilitating the diagnosis and monitoring of central nervous system (CNS) malignancies [62].

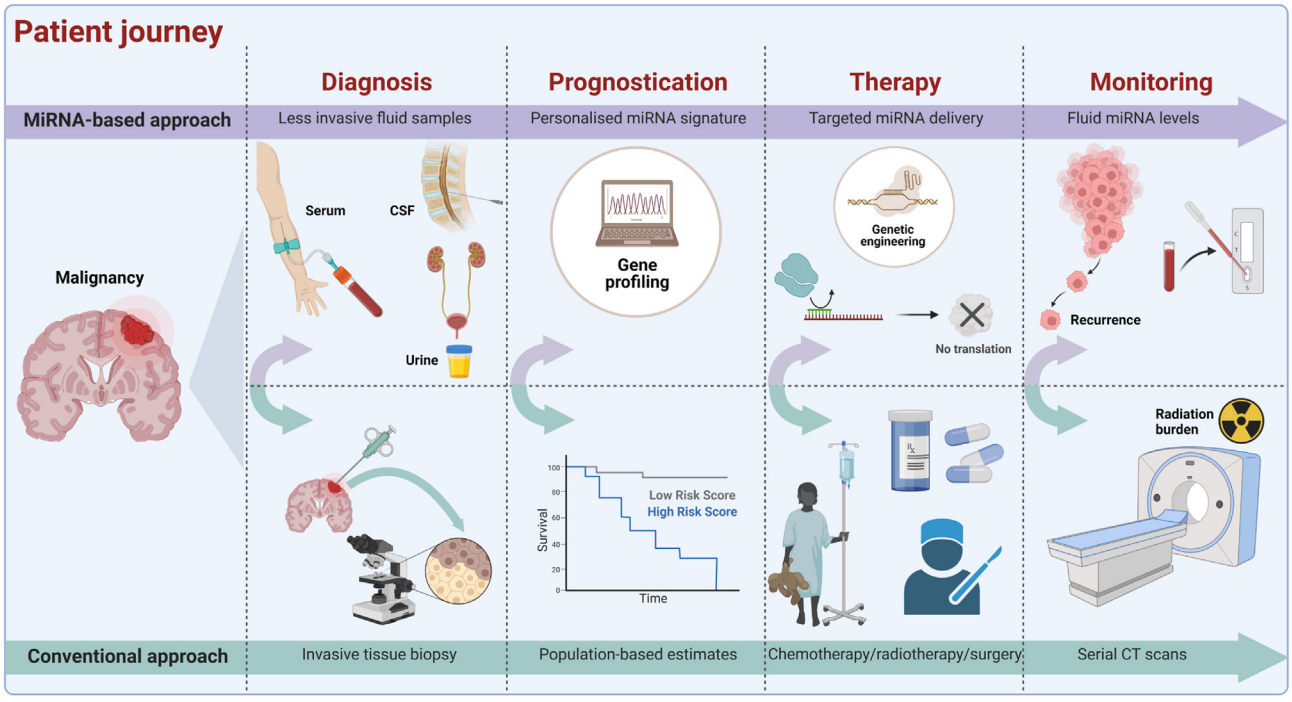

Fig. 3. A flow diagram illustrating the typical conventional patient journey (lower panel) through diagnosis, prognostication, therapy, and disease monitoring for a child with a solid tumour, versus a potential new approach (upper panel) with the use of miRNAs for these purposes. CSF, cerebrospinal fluid. Created with BioRender.com.

Certain technical issues still need to be resolved in blood-based miRNA detection before they can be used as a routine clinical tool, including clarification regarding the most appropriate quality control and/or normalisation methods. Whilst studies have investigated robust diagnostic serum miRNA profiling method [63], there is currently no universally accepted gold standard, and such issues are reviewed in more detail elsewhere [64]. However, they hold the substantial potential to revolutionise the diagnostic (D), prognostic (P), and therapeutic (T) approach for children with cancer, and many potential biomarkers have already been identified across various solid tumours (Table 1). Examples of where miRNAs may be used in patients' cancer pathways are shown in Fig. 3.

For medulloblastoma (MB), the most common paediatric brain malignancy, the miR-17–92 polycistron was highly upregulated, particularly in the MB subgroup associated with Sonic Hedgehog (SHH) signal pathway activation [65]. This increased miR-17–92 expression leads to SHH-mediated cerebellar granule neuron precursor (GNP) cell proliferation, suggesting that the amplification of this miRNA cluster imparts a selective growth advantage to MB tumour cells [65,66]. More generally, the upregulation of the miR-17–92 cluster and its paralogs were observed in three types of paediatric brain tumour (MB, ependymoma and pilocytic astrocytoma) and the level of expression correlated with histology and WHO grade [67]. Furthermore, miRNA expression analysis in MB cell lines and human MB tumours showed lower miR-34a expression compared with controls, suggesting that whilst miR-34a could be expendable for normal development, the loss of miR-34a promotes tumorigenesis [68]. Conversely, Braoudaki *et al.* found miR-34a to be upregulated in embryonal tumours and in their MB group alone, suggesting potential tissue-specific roles of miR-34a rather than global tumour suppressor functions [69], consistent with the observation that the functional role of miRNAs may be tumour- or context-specific [70].

Retinoblastoma (RB) is a malignancy arising from the retina in young children, with approximately 40% of cases being familial. Some patients with RB undergo enucleation due to late diagnosis and/or treatment resistance. Non-invasive markers would, therefore, be of benefit. A circulating miRNA study of RB and control cases revealed an average of 537 and 625 miRNAs detectable in plasma and extracellular vesicles, respectively [71]. A plasma signature of 19 miRNAs was identified that distinguished RB cases from controls [71]. Furthermore, Carvalho *et al.* identified a single nucleotide polymorphism (SNP) (rs4938723T>C) in the miR-34b/c gene as a potential biomarker for hereditary RB [72].

High-throughput screening initially demonstrated that the miR-371-373 cluster was overexpressed in adult gonadal malignant germ cell tumours (GCTs) [73]. Importantly, the miR-371-373 and miR-302/367 clusters were then shown to be universally overexpressed in all malignant GCTs, regardless of patient age (paediatric/adult), site (gonadal/extragonadal), or histological subtype [74]. The miR-302/367 cluster showed further overexpression in the GCT subtype yolk sac tumour (YST) compared with germinoma [75]. This is likely to be functionally significant as downregulated mRNAs in YSTs were enriched for complementary 3′UTR sequences to the common 2-7 nt seed region of miR-302ã miR-302d miRNAs, potentially contributing to their more aggressive nature [75].

Consequently, a panel of four miRNAs (miR-371a-3p/miR-372-3p/miR-373-3p/miR-367-3p) in serum, and CSF samples showed high sensitivity and specificity in the diagnosis of malignant GCTs and enabled early detection of relapse [62]. Of note, a multi-institutional pooled analysis has shown the single miRNA miR-371a-3p to be sufficient for non-invasive malignant testicular GCT diagnosis [76], as reviewed in detail elsewhere [77]. Miyachi *et al.* showed that circulating levels of the muscle-specific miRNA, miR-206, differentiated between rhabdomyosarcoma (RMS) tumours and non-RMS tumours with high sensitivity and specificity [78]. Another RMS study demonstrated that miR-486-5p was upregulated in both cells and exosomes and decreased to control value levels post-chemotherapy [79]. Several studies have also identified multiple miRNA level changes in osteosarcoma (OS) samples [80,81]. One particular study revealed levels of a four-plasma miRNA signature (miR-195-5p/miR-199a-3p/miR-320a/miR-374a-5p) were increased in OS patients at diagnosis and decreased significantly post-resection, suggesting a further use for monitoring disease response [82]. The potential for cancer recurrence surveillance using circulating miRNA is increasing and will offer major advantages by allowing non-invasive monitoring, reducing cumulative radiation exposure from serial scans and the associated potential second cancer risk [83]. Scans can be reserved for children with increasing markers in treatment and/or follow-up. Furthermore, for young children, a rational reduction in scans will also reduce the need for associated sedation and/or general anaesthetics required to obtain high-quality images without motion artefacts.

7. MiRNAs in cancer prognostication

Numerous studies in paediatric patient populations have demonstrated the potential use of miRNAs as prognostic biomarkers, allowing for risk stratification, prediction of clinical outcome and the development of more personalised, tailored therapy. It has been suggested that the use of miRNAs in prognostication has several advantages over mRNAs. For example, miRNAs have a smaller size (18–23 nt) and are protein protected by the RISC complex, and thus are less prone to degradation. Furthermore, miRNAs are more likely to remain intact

Table 1
Dysregulated expression and clinical role of miRNAs in paediatric solid tumours. Ordered by ascending miRNA number and then by primary clinical use (D = diagnosis, P = prognostication, T = therapy).

Cancer	MiRNA	Key finding(s)	Conclusion(s)	Primary clinical use	Other clinical use(s)	Ref
Embryonal CNS tumours	C19MC	CNS tumours with C19MC amplification and/or *LIN28* expression span various histologies but comprise a single molecular disease	Identifies *LIN28*/*let-7*/PI3K/mTOR axis and DNMT3B as promising therapeutics for this distinct entity	T	D	[182]
ETMR	*C19*MC	Genomic and epigenomic alterations of *C19*MC drive multiple feedforward loops to drive *C19*MC-*LIN28A*-*MYCN* oncogenic circuit, which can be abrogated by bromodomain inhibitors	Highlights *C19*MC as a critical oncogene in ETMRs and offers therapeutic insights using bromodomain inhibitors	T		[179]
ETMR	*C19*MC, miR-17–92	Possible that tumours with *C19*MC or miR-17–92 amplification oversaturate the miRNA processing machinery	Oversaturation of the miRNA machinery could explain why all ETMRs, regardless of amplification status, show many structural aberrations	T	D	[181]
ETMR	*Let-7*	OE of *LIN28A* augments SHH and Wnt signalling in precursor cells via downregulated *let*-7-miRNA	*LIN28A*/*let-7a* interaction with the SHH pathway was detected at the level of Gli mRNA	T	D	[180]
GCT	*Let-7*	LIN28, the negative-regulator of let-7 biogenesis, was abundant in GCTs, regardless of age, site or histology	LIN28 depletion in GCT cells restored let-7 levels and repressed several oncogenic let-7 mRNA targets	T	D	[178]
WT	*Let-7*	Withdrawal of LIN28 expression reverts tumorigenesis; tumour formation is suppressed by enforced expression of let-7	*LIN28*/*let-7* pathway implicated in tumorigenesis	T		[173]
RMS	MiR-7, miR-3245p	MiR-7 and miR-324-5p OE reduce tumour growth in RMS models and miR-7 impaired metastatic lung colonisation	MiR-7 and miR-324-5p show anti-oncogenic and anti-metastatic potential	T		[165]
RMS	MiR-9-5p	MiR-9-5p reduction inhibited RMS cell migration	MiR-9-5p levels correlated with poor outcome and were higher in metastatic vs non-metastatic disease	P	D	[108]
NB	MiR-14q32	UE of 14q32 miRNAs in tumours associated with poor prognostic factors was confirmed in 226 primary NBs	Identification of miRNAs involved in the process of surviving treatment and gaining resistance in NB	P	D	[99]
NB	MiR-15a, miR-15b, miR-16	Induced expression of miR-15a, miR-15b and miR-16 reduced the proliferation, migration, and invasion of NB cells and repressed tumour formation *in vivo*	MiR-15a, miR-15b and miR-16 exert a TSG function in NB by targeting MYCN	T		[151]
OS	MiR-16-1-3p, miR-16-2-3p	Ectopic expression of these miRNAs affected tumour growth, metastasis, chemoresistance, and invasiveness	MiR-16-1-3p and miR-16-2-3p 'passenger' strands and 'lead' miR-16-5p strand act as TSG	T	D	[132]
MB	MiR-17–92	MiR-17–92 is highly up-regulated in MB. SHH treatment of primary cerebellar GNPs increase miR-17–92 expression	MiR-17–92 is a positive effector of SHH-mediated proliferation; aberrant expression/amplification of this miRNA confers a growth advantage	D		[66], [65]
NB, RMS	MiR-17–92	MiR-17–92 cluster host gene, *MIRHG1*, is correlated with tumours with MYCN amplification, higher stage, and poor prognosis	MiR-17–92 correlated with aggressive form of NB	P	D	[90]

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Table 1 (*continued*)

Cancer	MiRNA	Key finding(s)	Conclusion(s)	Primary clinical use	Other clinical use(s)	Ref
MB	MiR-17–92	Inhibition of miR-17 and miR-19a seed families by anti-miR-17 and anti-miR-19 diminished cell proliferation *in vitro* and reduced tumour growth *in vivo*	Inhibition of the miR-17–92 cluster has therapeutic implications for SHH MBs	T		[158]
MB	MiR-22	Forced expression of miR-22 reduced cell proliferation and induced apoptosis; knockdown of miR-22 increased proliferation	Downregulated miR-22 expression is associated with cell proliferation in MBs, potentially via PAPST1	T		[160]
OS	MiR-22	MiR-22 inhibited cell proliferation; miR-22 corroborated the effect of cisplatin	MiR-22 inhibited cell proliferative activity and decreased cisplatin resistance	T		[138]
RMS	MiR-22	MiR-22 decreased cell proliferation, anchorage-independent growth and invasiveness, and promoted apoptosis	Restoring miR-22 expression blocked tumour growth and prevented dissemination *in vivo*	T	D	[161]
NB	MiR-26a-5p, miR-26b-5p	*MYCN* regulates *LIN28B* expression in NB via two distinct parallel mechanisms: *MYCN*-miR-26a-5p-*LIN28B* and a direct *MYCN-LIN28B* regulatory axis	*MYCN*-regulated miRNAs have a role in the *MYCN*-driven oncogenic process	T		[152]
RMS	MiR-27a	Re-expression of miR-27a led to PAX3:FOXO1 mRNA destabilisation and chemotherapy sensitisation in alveolar RMS cells	Implicates a HDAC3-SMARCA4-miR-27a-PAX3:FOXO1 circuit as a driver of chemotherapy-resistant alveolar RMS	T		[164]
OS	MiR-27a-3p	Transfection with miR-27a-3p inhibitor decreased proliferative ability	MiR-27a-3p inhibition suppresses proliferation and invasion of OS cells	T		[133]
OS	MiR-29b	CDK6 downregulated by miR-29b in OS cells; inverse correlation between miR-29b and CDK6 protein levels	MiR-29b acts as a TSG of OS by targeting CDK6 in proliferation and migration processes	T		[123]
OS	MiR-29b-1	MiR-29b-1 OE causes proliferation, self-renewal and chemosensitivity, associated with downregulation of stem cell, cell cycle-related, and anti-apoptotic markers	MiR-29b-1 suppresses stemness properties of OS cells and is a potential therapeutic target	T		[122]
Embryonal CNS tumours	MiR-34a	MiR-34a up-regulated in both MB and AT/RT	Indicated tissue-specific roles rather than global TSG properties of miR-34a	D	P	[69]
MB	MiR-34a	Tumour incidence increased and formation accelerated in mice transgenic for SmoA1 and lacking miR-34a	MiR-34a is dispensable for normal development, but its loss accelerates tumour development	D		[68]
ES	MiR-34a	When miR-34a expression was enforced, cells were less proliferative and sensitised to doxorubicin and vincristine	Restoration of miR-34a activity may decrease malignancy and increase tumour sensitivity to current drugs	T	P	[169]
NB	MiR-34a	MiR-34a and *let-7b* NB-targeted nanoparticles, individually and synergistically, reduce cell division, proliferation, neoangiogenesis and tumour growth	MiR-34a may act as a TSG; miR-34a and *let-7b* combined replacement shows therapeutic efficacy	T	D	[141], [142–144]
OS	MiR-34a	OS xenograft tumour growth was inhibited *in vivo* when miR-34a prodrug and doxorubicin co-administered	Combination of doxorubicin and miR-34a replacement causes antiproliferative effects	T		[139,140]

Table 1 (*continued*)

Cancer	MiRNA	Key finding(s)	Conclusion(s)	Primary clinical use	Other clinical use(s)	Ref
NB	MiR-34b	DLL1 ligand identified as the Notch pathway component OE in MYCN-amplified NB cells	MiR-34b downregulated DLL1 mRNA expression levels to arrest cell proliferation and induce neuronal differentiation in NB cells	T		[145]
RB	MiR-34b/c	Mir-34b/c rs4938723T > C was sequenced; age at diagnosis lower in CC carriers than TT genotype in hereditary RB	Mir-34b/c rs4938723T > C may represent a candidate biomarker for hereditary RB	D		[72]
OS	MiR-34c-5p	MiR-34c-5p was UE in OS tissues and cells and inhibited proliferation, migration, and invasion	MiR-34c-5p inhibited proliferation, migration and invasion, potentially via targeting FLOT2	T		[127]
OS	MiR-107	MiR-107 OE increases cell viability, migration, and invasion and inhibits apoptosis	MiR-107 inhibitor transfection abolished these effects	T		[134]
MB	MiR-124	CDK6 is present in approximately one-third of MBs and is an independent poor prognostic marker	MiR-124 OE inhibits the proliferation of MB through inhibiting CDK6, *in vitro* and *in vivo*	T		[157]
pLGG	MiR-125b	MiR-125b OE resulted in decreased growth and invasion, as well as apoptosis	MiR-125 is frequently UE in pLGG; OE decreases cell growth and induces apoptosis	T	D	[185]
ES	MiR-130b	Small molecule inhibition of PAK1 blocked miR-130b activation of JNK and downstream AP-1 target genes	MiR-130b induces proliferation, invasion and migration *in vitro* and increased metastatic potential *in vivo*	T		[171]
NB	MiR-137	Low miR-137 in NB is associated with poor prognosis; re-expressing miR-137 *in vitro* increased apoptosis; miR-137 downregulated KDM1A mRNA	MiR-137 acts as a TSG through targeting KDM1A mRNA; re-expression of miR-137 could be a therapeutic strategy	T	P	[94]
pLGG	MiR-139-5p	OE of miR-139-5p inhibited cell proliferation	MiR-139-5p downregulation drives cell proliferation by derepressing PI3K/AKT signalling	T	D	[184]
WT	MiR-140-5p	MiR-140-5p was UE in WT	High levels of miR-140-5p inhibited cellular proliferation and metastasis	T		[172]
ES	MiR-143, miR-145	TARBP2 restoration and systemic delivery of miR-143 or miR-145 inhibited EWS CSC clonogenicity and tumour growth *in vivo*	CSC self-renewal and tumour maintenance may depend on the deregulation of TARBP2-dependent miRNA expression	T		[167]
OS	MiR-143-3p	MiR-143-3p expression was lower in OS tissues compared with normal and associated with poor prognosis	MiR-143-3p inhibited OS cell proliferation and metastasis whilst promoting apoptosis	T	D, P	[125]
MB	MiR-183∼96∼182	MiR-183∼96∼182 expression is associated with lower rates of EFS and overall survival	Identifies a previously unrecognised molecular subgroup with poor clinical outcome	P		[111]
NB	MiR-184	MiR-184 levels increase with ATRA treatment and *MYCN* knockdown *in vitro;* pro-apoptotic when OE *in vitro*	MiRNA profiles distinguish subtypes of NB; miR-184 UE important in NB pathogenesis	T	D, P	[153]
NB	MiR-184	Pro-apoptotic effects of miR-184 ectopic OE in cells reproduced by	MYCN may contribute to tumorigenesis miR-184	T		[154]

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Table 1 (*continued*)

Cancer	MiRNA	Key finding(s)	Conclusion(s)	Primary clinical use	Other clinical use(s)	Ref
		the siRNA inhibition of AKT2	repression, increasing AKT2; miR-184 could have therapeutic implications for MYCN-amplified NB			
WT	MiR-185	MiR-185 UE in WT, allowing the de-repression of the oncogene SIX1	MiR-185 reduces growth and cell migration *in vitro* and tumour growth *in vivo*	T	D	[175]
MB	MiR-193a	MiR-193a expression in MYC amplified group 3 MB cells inhibited growth and tumorigenicity, as well as increased radiation sensitivity	MiR-193a has therapeutic potential in the treatment of group 3 MBs and other MYC overexpressing cancers	T		[159]
HB	MiR-193a-5p	MiR-193a-5p/DPEP1 axis participated in HB progression via regulating the PI3K/Akt/mTOR	MiR-193a-5p was UE in HB tissues and associated with a poor clinical prognosis	P	T	[115]
WT	MiR-195	Forced expression of miR-195 impaired tumour survival and metastasis; this could be restored by LINC00473	Loss-of-function of LINC00473 *in vivo* caused regression of WT via miR-195/IKKα-mediated growth inhibition	T		[174]
OS	MiR-199a-3p	MiR-199a-3p is decreased in OS cells; transfection of miR-199a-3p increased drug sensitivity via the downregulation of CD44	CD44-miR-199a-3p axis has a role in metastasis, recurrence, and drug resistance of OS	T	D, P	[137]
OS	MiR-199a-5p	MiR-199a-5p OE in OS; miR-199a-5p inhibition decreased cell proliferation and growth	MiR-199a-5p reduction by stable antisense oligonucleotides of miR-199a-5p inhibited the OS tumour growth in nude mice	T	D	[135]
OS	MiR-200c	MiR-200c OE in lung metastases, implicating an inhibitory feedback loop to PI3K-AKT	Identified a new role for miR-200c as a mediator of lung metastasis in OS	P	T	[103]
NB	MiR-204	MiR-204 expression was predictive of patient EFS and overall survival; ectopic miR-204 expression increased sensitivity to cisplatin and etoposide *in vitro*	MiR-204 is a prognostic marker in NB, functioning, partly by increasing sensitivity to cisplatin through anti-apoptotic BCL2 downregulation	P	T	[93]
NB	MiR-204	MiR-204 directly bound MYCN mRNA and repressed MYCN expression; miR-204 OE inhibited NB cell proliferation *in vitro* and tumorigenesis *in vivo*	Identifies miR-204 as a TSG and negative regulator of MYCN	T		[149]
RCC	MiR-204-5p	MiR-204-5p was OE in cell lines from Tg mouse tumours and from tissue from 2 Xp11 tRCC patients	MiR-204-5p in urinary exosomes is a potential biomarker for early diagnosis of Xp11 tRCC	D		[60]
RMS	MiR-206	Muscle-specific miR-1, miR-133a/b and miR-206 are lower in RMS compared to normal skeletal muscle; miR-206 OE inhibited cell growth and migration and induced apoptosis *in vitro*	Low miR-206 expression is associated with poor prognosis, high tumour stage and presence of metastases at diagnosis	P	D, T	[107]
RMS	MiR-206	Both *in vitro* and *in vivo* miR-206 acts as a TSG in fusion negative RMS, potentially through the downregulation of PAX7	MiR-206 relieves the differentiation arrest in fusion negative RMS and miR-206 replacement could be a potential therapeutic strategy	T		[162]
GCT	MiR-214-3p	Showed 27 miRNA candidates with differential expression	MiR-214-3p expression contributed to cisplatin	T	D	[183]

Table 1 (*continued*)

Cancer	MiRNA	Key finding(s)	Conclusion(s)	Primary clinical use	Other clinical use(s)	Ref
		between germinomas and nongerminomatous malignant GCTs	resistance by targeting the pro-apoptotic protein BCL2L11.			
OS	MiR-216a	MiR-216a expression predicted improved outcomes; miR-216a OE suppressed proliferation, migration, and invasion *in vivo* and *in vitro* by CDK14 inhibition	Suggested possibility that miR-216a activation and CDK14 inhibition may be therapeutic strategies in OS	T	D, P	[130]
OS	MiR-216a-5p	MiR-216a-5p OE exerted inhibition effects via downregulating SOX5 expression; DANCR-regulated SOX5 expression by sponging to miR-216a-5p	LncRNA DANCR silence inhibits SOX5-medicated progression and autophagy in OS via miR-216a-5p	T		[136]
OS	MiR-221/222	Identified and validated 29 deregulated miRNAs in OS	MiR-221/miR-222 were associated with time to metastasis	P	D	[101]
GCT	MiR-302/367	Genes downregulated by miR-302 family involved in key biological processes, e.g., apoptosis regulators	MiRNA profiles distinguish the most common pure malignant GCT subtypes, YST and germinoma	D		[75]
GCT	MiR-302/367, miR-371-373	Genes downregulated by these miRNA clusters are involved in key biological processes, e.g., signalling pathways	MiR-371-373 and miR-302/367 clusters segregate malignant GCTs from benign GCTs and controls, regardless of patient age, anatomical site or histological subtype	D		[74]
NB	MiR-323a-5p, miR-342-5p	MiR-323a-5p and miR-342-5p reduced cell proliferation *in vitro* and *in vivo*	New vulnerabilities of high-risk NB through the combined inhibition of targets such as CCND1, CHAF1A, INCENP and BCL-XL	T		[150]
NB	MiR-340	Identified 67 epigenetically regulated miRNA; 42% of these were associated with poor survival when UE; miR-340 induced either differentiation or apoptosis in a cell context-dependent manner and represses *SOX2*	Extensive epigenetic silencing of miRNAs that target a large repertoire of genes that are OE in unfavourable NB	P	T	[98]
OS	MiR-340	Low miR-340 and high ROCK-1 were associated with metastasis, poor response to pre-operative chemotherapy and the shortest overall and progression-free survival	MiR-340 downregulation and ROCK1 upregulation may be associated with poor prognosis in OS	P		[104]
GCT	MiR-371-373	MiR-371-373 cluster highly expressed in seminomas, embryonal carcinoma, and yolk sac tumour	Previous miRNA 371–373 cluster finding was confirmed.	D		[73]
OS	MiR-377	MiR-377 OE or HAT1 silencing inhibited tumour growth and reduced size *in vivo*	MiR-377 may promote OS cell apoptosis through the inactivation of HAT1-mediated Wnt signalling	T		[131]
NB	MiR-410, miR-487b	Fifteen miRNAs of the 14q32.31 cluster discriminated high-risk from low-risk NB; miR-487b and miR-410 expression was associated with disease-free survival of the non-MYCN-amplified favourable NB	MiR-487b and miR-410 are potential biomarkers of relapse in favourable NB	P		[95]

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Table 1 (*continued*)

Cancer	MiRNA	Key finding(s)	Conclusion(s)	Primary clinical use	Other clinical use(s)	Ref
HB	MiR-483	MiR-483-5p and miR-483-3p inclusion into the four-miR signature predicted poor outcome associated with large tumours and vessel invasive growth	Expansion of the four-miR signature by miR-483 serves as a prognostic biomarker in HB	P		[114]
WT	MiR-483-5p	IGF2 mRNA is transcriptionally up-regulated by miR-483-5p, embedded within the IGF2 gene; ectopic expression of miR-483-5p in IGF2-dependent sarcoma cells increases tumorigenesis *in vivo*	Functional positive feedback loop of an intronic miR-483-5p on transcription of IGF2	T		[177]
OS	MiR-486	Analysis of 40 OS tissues showed miR-486 UE; low miR-486 was associated with shorter survival	MiR-486 inhibited the proliferation and migration of OS cells	T	D, P	[124]
RMS	MiR-486-5p	MiR-486-5p is OE in both RMS cells and exosomes; RMS serum samples showed miR-486-5p is enriched in exosomes and follow-up after chemotherapy showed a reduction to control values	Identified miR-486-5p in exosome-mediated oncogenic paracrine effects of RMS and its use as a potential biomarker	D		[79]
RMS	MiR-486-5p	MiR-486-5p is activated by PAX3-FOXO1 and promotes proliferation, invasion and growth	Inhibition of miR-486-5p in xenografts decreased tumour growth	T		[163]
NB	MiR-490-5p	Decreased miR-490-5p levels correlated with stage, metastasis and poor survival in NB patients; miR-490-5p OE suppressed cell proliferation, migration, invasion, and induced cell apoptosis	MiR-490-5p functions as a TSG in NB by targeting MYEOV; low levels are associated with a poor prognosis	P	T	[97]
MB	MiR-495	MiR-495 expression is repressed in MB samples and an independent predictor of overall survival	MiR-495 may be a prognostic marker in MB	P		[109]
NB	MiR-497	Low miR-497 expression is associated with worse EFS and overall survival; miR-497 OE reduced cell viability	MiR-497 acts as a TSG through direct targeting of WEE1	T	P	[96]
OS	MiR-509-3p	MiR-509-3p OE inhibited migration of OS cells and sensitised cells to cisplatin; AXL, which plays a role in cisplatin resistance, was downregulated upon miR-509-3p treatment	MiR-509-3p/AXL and miR-509-3p/ARHGAP1 axes have the potential therapeutic implications for resistant metastatic OS	T		[128]
NB	MiR-542-5p	Thirty-seven miRNAs correlated with TrkA expression, and 6 miRNAs further analysed *in vitro* were regulated upon TrkA transfection, suggesting a functional relationship	MiR-542-5p discriminated local vs metastatic disease and was inversely correlated with MYCN amplification and EFS	P		[86]
NB	MiR-542-5p	MiR-542-5p ectopic OE decreased the invasive potential of NB cell lines *in vitro*; miR-542-3p-loaded nanoparticles decreased proliferation and induced apoptosis *in vivo*	Functional evidence for miR-542-5p as a TSG potentially through targeting Survivin	T		[147,148]
NB	MiR-558	Knockdown of miR-558 decreased growth, invasion, metastasis and angiogenesis of NB cells *in vitro* and *in vivo*	MiR-558 induces the transcriptional activation of heparanase facilitating the tumorigenesis and aggressiveness of NB	T		[155]
OS	MiR-590-3p	MiR-590-3p was decreased OS tissues and cell lines	MiR-590-3p inhibits proliferation and metastasis in OS cells via SOX9	T	D	[126]

Table 1 (*continued*)

Cancer	MiRNA	Key finding(s)	Conclusion(s)	Primary clinical use	Other clinical use(s)	Ref
OS	MiR-598	MiR-598 was UE in OS tissues, serum and cell lines; OE suppressed proliferation, migration, and invasion of cells	Inhibitory role of miR-598 in OS progression *in vivo*, by targeting PDGFB and MET	T	D	[129]
ES	MiR-708	EWS/FLI1 represses miR-708, resulting in EYA3 OE, causing chemoresistance by decreased DNA repair	EYA3 inhibitors and/or re-introduction of miR-708 could sensitise EWS to chemotherapeutics	T	P	[170]
GBM	MiR-1300	Observed cytokinesis failure followed by apoptosis in miR-1300 transfected cells; ectopic expression of miR-1300 decreased tumour growth	Identified miR-1300 as a regulator of endomitosis with therapeutic potential	T	D	[186]
MB	MiR-4521	MiR-4521 transfection reduced proliferation and invasion of cell lines and induced cell death through caspase 3/7 activation	MiR-4521 restoration may suppress the effects of aberrant FOXM1 expression	T		[156]
CNS tumours	Various	MiR-17-5p and miR-20a obtained a high level of expression in MBs and EPs but not in PA samples	MiRNA expression depended on tumour grade and histology	D		[67]
GCT	Various	MiR-371a-3p/miR-372-3p/miR-373-3p/miR-367-3p in serum and CSF samples showed high sensitivity and specificity in the diagnosis of malignant GCTs and enabled the early detection of relapse	A robust pipeline for diagnosis and monitoring of extracranial and intracranial paediatric malignant GCTs	D		[62]
NB	Various	MiR-124-3p/miR-9-3p/miR-218-5p/miR-490-5p/miR-1538 were highly OE in MYCN-amplified high-risk NB	A pipeline for diagnostic serum miRNA profiling in childhood solid tumours	D		[63]
OS	Various	MiR-195-5p/miR-199a-3p/miR-320a/miR-374a-5p were increased in OS patients and decreased in plasma post-surgery; miR-195-5p and miR-199a-3p correlated with metastasis status	Four plasma miRNA signature as a non-invasive biomarker for OS	D	P	[82]
OS	Various	MiR-199a-5p targeted the highest number of genes	Identified 36 UE miRNAs and 182 OE miRNAs in OS samples compared to controls	D		[80]
OS	Various	MiR-205-5p was decreased and miR-574-3p/miR-214/miR-335-5p were increased in OS samples; in metastatic patients at diagnosis, low levels of miR-214 were associated with better overall survival	Validated a signature profile of plasma miRNAs that distinguish OS from healthy animals in retrospective and prospective studies; translated these findings to 40 human plasma samples	D	P	[81]
RB	Various	537 detectable miRNAs in plasma and 625 in extracellular vesicles; identified plasma signature of 19 miRNAs present in all Rb cases	Identified plasma signature of 19 miRNAs that discriminate RB cases from controls	D		[71]
EP	Various	Multivariate analysis adjusted for age, sex, grade and localisation showed miR-17-5p as prognostic marker	High expression of miR-17-5p was associated with reduced EFS and overall survival	P		[112]
NB	Various	25-miRNA signature discriminates test patients with respect to progression-free and overall survival	Established a miRNA classifier to identify high-risk NB patients at greater risk for adverse outcome	P		[91]
NB	Various	Drosha or Dicer knockdown promotes cell growth *in vitro*;	Combination of 15 biomarkers delineates risk	P	T	[92]

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Cancer	MiRNA	Key finding(s)	Conclusion(s)	Primary clinical use	Other clinical use(s)	Ref
		reduced miRNA biogenesis results in the global UE of miRNAs observed in advanced NB and is associated with poor prognosis	groups of NB and predicts clinical outcome			
OS	Various	Higher expression of miR-27a and miR-181c pre-treatment correlated with metastatic disease; higher expression of miR-451 and miR-15b pre-treatment correlated with positive chemotherapy response	MiRNA signature-associated OS and pre-treatment biomarkers of metastasis and therapeutic response	P	D, T	[105]
OS	Various	MiR-155-5p/miR-135b-5p/miR-146a-5p were OE, and miR-199b-5p/miR-100-3p were UE in highly aggressive cell lines	MiR-135b-5p and miR-146a-5p were predictive of metastatic capacity	P		[106]

Abbreviations: ATRA, all-*trans*-retinoic acid; AT/RT, atypical teratoid/rhabdoid tumour; CNS, central nervous system; CSC, cancer stem cell; DANCR, differentiation antagonising non-protein-coding RNA; EFS, event-free survival; EP, ependymoma; ETMR, embryonal tumour with multilayered rosettes; ES, Ewing sarcoma; GBM, glioblastoma multiforme; GCT, germ cell tumour; GNP, granule neuron precursor; HB, hepatoblastoma; MB, medulloblastoma; NB, neuroblastoma; OE, over-expressed; OS, osteosarcoma; pLGG, paediatric low-grade glioma; RB, retinoblastoma; RCC, renal cell carcinoma; RMS, rhabdomyosarcoma; SHH, Sonic Hedgehog; TSG, tumour suppressor gene; UE, under-expressed; WT, Wilms tumour; YST, yolk sac tumour.

in formalin-fixed, paraffin-embedded clinical samples [84,85].

Neuroblastoma (NB) accounts for ~15% of childhood cancer deaths and is clinically heterogeneous, with those with favourable prognosis potentially undergoing spontaneous regression, whereas unfavourable NB can lead to a high mortality, despite intensive multimodality treatment [86]. Unfavourable NB is particularly associated with MYCN amplification and particular chromosomal aberrations [87]. Studies have demonstrated that MYCN upregulates the miR-17–92 cluster [88], through direct binding to the promoter [89]. Wei *et al.* showed that the high expression of miR-17–92 cluster host gene, MIRHG1, correlated with poor prognosis and higher disease stages, as well as the expected MYCN amplification [90]. The miR-17–92 cluster is, therefore, implicated in unfavourable NB, potentially through the promotion of cell growth [89]. A previous study identified a 25-miRNA signature that identified high-risk NB patients with a poorer prognosis in fresh frozen and archived tissue samples [91]. Interestingly, all but one of the miR-17–92 cluster miRNAs were part of this prognostic signature, and 16 of the 25 miRNAs were MYC/MYCN driven [91]. Another study showed that a combination of 12 miRNAs, age at diagnosis, and mRNA expression levels of the miRNA biogenesis enzymes *Dicer* and *Drosha* could serve as a predictor of clinical outcome and separate NB patients into prognostic groups [92]. Low expression levels of *Dicer* and *Drosha* resulted in global downregulation of miRNAs that correlated with poor prognosis [92].

In contrast to MYCN, TrkA shows higher levels of expression in tumours with a more favourable prognosis. Schulte *et al.* demonstrated that miR-542-5p expression, the most significant TrkA-correlated miRNA, was positively correlated with event-free survival (EFS) and is repressed in MYCN-amplified tumours [86]. Furthermore, miR-204 expression has also been correlated with EFS and overall survival, potentially via increasing sensitivity to cisplatin and etoposide through targeting the 3′UTR of BCL2 and NTRK2 (TrkB) [93]. Conversely, the downregulation of several miRNAs has been associated with poor prognosis, including, but not limited to, miR-137 [94], miR-487b and miR-410 [95], miR-497 [96], miR-490-5p [97], miR-340 [98], and 14q32 miRNAs [99].

Whilst the use of miRNAs as prognostic markers in NB has been particularly well studied, there is growing research in other paediatric solid tumours. OS typically has a poor prognosis, and treatment failure is usually due to metastasis before or after diagnosis [100], and so prognostic biomarkers, particularly of metastatic potential, would have substantial clinical utility. One study demonstrated that miR-221/miR-222 was associated with time to metastasis [101]. Interestingly, these same miRNAs have been shown to be linked with metastasis in other adult tumours, including gastric, colorectal and breast cancer [101]. Furthermore, in OS, the miR-221/PTEN/PI3K/AKT signalling pathway has been implicated for drug resistance [102]. Berlanga *et al.* identified a new role for miR-200c as a mediator of lung metastasis in OS and suggested this may be via the upregulation of the reverse process of mesenchymal-to-epithelial transition (MET) [103]. Another study showed that miR-340 downregulation correlated with mRNA ROCK1 upregulation, and this combination occurred more often in

OS with metastases and poor chemotherapy response [104]. Furthermore, miR-27a, miR-181c, miR-135b-5p, and miR-146a-5p have all been identified as potential biomarkers of OS metastasis [105,106].

Similar to OS, several potential prognostic miRNA biomarkers have been found in, e.g., RMS, MB, hepatoblastoma (HB), and ependymoma (EP). Low tissue miR-206 levels have been shown to be an independent predictor of shorter survival in *PAX3/7-FOXO1* fusion gene negative RMS and are associated with the presence of metastases at diagnosis [107]. In contrast, high levels of miR-9-5-p were seen in metastatic compared with non-metastatic RMS cases and correlated with poor clinical outcome [108]. In MB, low tissue levels of miR-495 expression were an independent predictor of overall survival [109]. The same study showed that miR-495 directly interacts with the *Gfi1* 3′UTR, and previous studies have identified a pro-oncogenic role of *Gfi1* in MB groups 3/4 with poor outcome [109,110]. Increased miR-183–96–182 cluster miRNA levels, which is specific for MB cases expressing photoreceptor transcriptional genes, a MB molecular subgroup, were linked with lower EFS and overall survival [111]. For EP, the third most common paediatric brain tumour, high expression of miR-17-5p was associated with reduced EFS and overall survival [112]. The role of miR-17-5p is context-specific and has been shown to act as either a TSG or oncogene depending on the cellular environment [112,113]. Of note, miR-17-5p derives from the miR-17–92 cluster, a prognostic biomarker for NB. For HB, the most common primary liver neoplasm of childhood, the addition of miR-483 to a previously identified four-miRNA HB biomarker signature predicted patients with poorer outcomes [114]. Dipeptidase 1 (DPEP1) plays an important oncogenic role in multiple tumours, and the miR-193a-5p/DPEP1 axis has been shown to be a prognostic predictor, as well as a potential therapeutic target, in HB patients [115].

8. MiRNAs as cancer therapeutic targets

Whilst the potential clinical utility of diagnostic/prognostic biomarkers can be highlighted without necessarily elucidating any underlying functional relevance, an understanding of the mechanistic relationship between particular miRNA changes and tumorigenesis does offer new therapeutic opportunities. One of the major limitations to the success of conventional chemotherapy, and even targeted agents such as tyrosine kinase inhibitors, is the development of drug resistance. Although investigators have so far identified only a small number of protein-coding targets of miRNAs, one of the potential advantages of using miRNA-mediated therapy is that miRNAs themselves target numerous mRNAs [12,16], which are often members of multiple, key cellular pathways. As a result, mutations or variations in the 3′UTR sequence of a single mRNA, which may increase the risk of developing cancer *per se* [48], are unlikely to be responsible for tumours becoming resistant to miRNA therapy.

The therapeutic potential of miRNAs has been recognised for a number of years [116,117]. Mechanisms through which this may be realised include the delivery of a single, antisense RNA strand (‘antagomir’) which binds overexpressed miRNAs or a miRNA ‘mimic’ to replace under-expressed miRNAs. The first demonstration of the potential of this technique was published in 2008, when an antagomir to miR-122 (a liver-expressed miRNA important in cholesterol and lipid metabolism) was delivered intravenously to non-human primates, resulting in a long-lasting and reversible reduction in plasma cholesterol without any evidence of toxicity [118]. Generally, however, there are potential concerns that trying to normalise levels of highly overexpressed miRNAs in cancer cells using antagomirs may lead to deleterious side effects in normal cells, unless (a) those overexpressed miRNAs found in cancer cells are virtually non-expressed in normal tissues/organs, e.g., Ref. [74], and/or (b) effective target delivery is utilised. This may involve using molecules or antibodies that bind to receptors only expressed on the surface of cancer cells. Perhaps, a more tractable approach to miRNA-mediated therapy will be to identify miRNAs that are downregulated in cancer cells, but expressed at relatively high levels in normal cells, so that the latter are less susceptible to unwanted side effects caused by increases in miRNA concentration through miRNA mimic replenishment methodology. Indeed, this approach was demonstrated in a murine model of hepatocellular carcinoma, where miR-26a is downregulated [119]. Systemic administration of miR-26a, using an adeno-associated virus, led to the inhibition of cancer cell proliferation, apoptosis, and protection from disease progression without concomitant toxicity [119]. The recent years have seen the translation of miRNA therapeutics in human trials. For example, anti-miR-122 (miravirsen) administration caused dose-dependent reduction in hepatitis C virus (HCV) RNA levels with no evidence of viral resistance in patients with chronic HCV genotype 1 infection [120]. In mesothelioma, a miR-16-based mimic entered a human phase 1 clinical trial with an acceptable safety profile [121]. The translation of our understanding of miRNA expression in childhood cancers to *in vitro* studies, and subsequently to appropriate *in vivo* models (Table 1), will be essential if we are to fully realise the role that these small ncRNAs play in paediatric malignancy.

OS is highly malignant and overcoming drug resistance is important for advances to be made in clinical outcomes [102]. Recently, the therapeutic potential of many miRNAs identified in the pathogenesis of OS through *in vitro* and *in vivo* experiments has been highlighted. The overexpression of multiple miRNAs has

been shown to reduce OS cell proliferation and/or migration, via targeting of mRNA targets, including miR-29b-1 [122], miR-29b via CDK6 [123], miR-486 via PKC-δ signalling pathways [124], miR-143-3p via FOS-like antigen 2 (FOSL2) [125], miR-590-3p via SOX9 [126], miR-34c-5p via FLOT2 [127], miR-5093p via ARHGAP1 [128], miR-598 via PDGFB and MET [129], miR-216a via CDK14 [130], miR-377 via HAT1-mediated Wnt signalling [131] and miR-16-1-3p and miR-16-2-3p via FGFR2 [132]. Similarly, the inhibition of some miRNAs has been shown to reduce proliferation in OS cells, including miR-27a-3p via TET1 [133], miR-107 via TPM1 (tropomyosin 1) [134], and miR-199a-5p via PIAS3 and p27 [135]. Aberrant expression of differentiation antagonising non-protein-coding RNA (DANCR) has been reported in OS, with DANCR silencing suppressing OS cell progression through targeting of miR-216a-5p and downregulation of SOX [136]. Some studies on OS have shown that miRNAs can increase sensitisation to chemotherapy agents, thus offering the potential of combined therapy that may combat drug resistance and reduce the toxicity of conventional therapy. For example, Gao *et al.* demonstrated that miR-199a-3p transfection increased the sensitivity of OS cells to doxorubicin, potentially via the downregulation of CD44 [137]. MiR-22 has been shown to inhibit the proliferation of OS cells and increase the anti-proliferative action of cisplatin, both *in vitro* and *in vivo* [138]. Bioengineered miR-34a prodrug, with and without doxorubicin, has been shown to suppress OS xenograft tumour growth, representing a putative novel therapeutic agent [139,140].

In NB, exogenous miR-34a administration decreased cell proliferation, supporting the role for miR-34a as a TSG [141–143]. Recently, delivery of both miR-34a and *let-7b* via NB-targeted nanoparticles, resulted in synergistic reduction in proliferation, neo-angiogenesis, and tumour growth/burden in orthotopic xenografts and increased survival [144]. Another study showed that among miR-34 family members, miR-34b downregulated DLL1 expression and arrested NB cell proliferation, thus suggesting that the Notch ligand DLL1 could be an attractive therapeutic target in NB through miRNA interaction [145]. However, it should be noted that a phase 1 clinical trial of a liposomal miR-34a mimic, MRX34, in adult patients with advanced solid tumours, was stopped early due to serious immune-related adverse events, so the clinical application of these *in vitro* and *in vivo* studies remains a key barrier to overcome [146]. Other miRNAs identified as potential TSGs in NB pathogenesis in functional experiments include miR-542-3p and miR-542-5p [147,148], miR-204 [149], miR-137 [94], miR-497 [96], miR-323a-5p and miR-342-5p [150], miR-15a-5p, miR-15b-5p and miR-16-5p [151], miR-26a-5p and miR-26b-5p [152], and miR-184 [153,154]. Conversely, miR-558 has been shown to have an oncogenic role as overexpression increased growth, invasion, metastasis and angiogenesis of NB cells via enhancing heparanase, an endogenous endoglycosidase that degrades heparan sulphate proteoglycans [155].

In MB studies, the transfection of miR-4521 reduced cell proliferation *in vitro* [156], and overexpression of miR-124 reduced proliferation both *in vitro* and *in vivo* [157]. Conversely, the inhibition of miR-17–92 cluster miRNAs reduced cell proliferation *in vitro* and tumour growth *in vivo* [158]. Many of the miRNA therapeutic studies described above have focused on increasing tumour sensitisation to chemotherapy regimens. Interestingly, Bharambe *et al.* observed that miR-193a expression increased the radiation sensitivity of MB cells [159]. MiR-22 inhibits the proliferation of MB [160], OS [138] and RMS [161] cells. RMS is classified molecularly as PAX3/7-FOXO1 fusion positive or negative [162]. Of note, differentiation therapy represents a possible therapeutic avenue for tumours, such as RMS, and miR-206 acts as a TSG promoting cell cycle exit and myogenic differentiation in fusion-negative RMS through various targets, including CCND2, NOTCH3, PAX3 and PAX7 [162]. In fusion-positive RMS, miR-486-5p is activated by PAX3-FOXO1 and promotes proliferation, invasion and growth. Thus, miR-486-5p inhibition *in vivo* reduced tumour growth [163]. In contrast, the re-expression of miR-27a promoted chemotherapy sensitisation both *in vitro* and for the more aggressive alveolar RMS subtype [164]. Interestingly, the overexpression of miR-7 has been shown to impair metastatic lung colonisation in RMS models, thus offering a novel potential therapeutic approach [165].

The *EWS-FLI-1* fusion gene, which occurs as a result of the t(11;22)(q24;q12) translocation and is present in approximately 85–90% of all Ewing sarcomas (ES), initiates reprogramming towards cancer stem cells *in vitro*, through the modulation of miR-145 and the pluripotency factor SOX2 [166]. Vito *et al.* showed that the systemic delivery of synthetic miR-143 or miR-145 -reduced ES cell clonogenicity and tumour growth *in vivo* [167]. Unfortunately, ES has a tendency for metastasis and, as a result, has a poor survival rate that has not improved significantly over the last 30 years [168]. Nevertheless, miRNAs have a potential clinical utility in reducing metastatic potential in ES and improving chemotherapy sensitisation. For example, enforced miR-34a expression in ES cell lines made the cells less proliferative and sensitised them to vincristine and doxorubicin [169]. The re-introduction of miR-708 and/or use of transcriptional cofactor, EYA3, inhibitors could re-sensitise ES cells to chemotherapy [170]. Finally, miR-130b has been shown to increase ES metastatic potential *in vivo,* and thus miR-130b and its mRNA targets represent novel approaches for preventing metastasis [171].

Wilms tumour (WT), also known as nephroblastoma, is the most common paediatric renal malignancy [172], in which the *lin28/let-7* pathway has been implicated in tumorigenesis [173]. Furthermore, the overexpression of miR-195 inhibited WT survival *in vitro*, the effect of which was restored by LINC00473, a long ncRNA (lncRNA), implicating LINC00473 as an oncogene [174]. Both miR-140-5p and miR-185 have been identified as having TSG functions in WT [172,175]. Conversely, miR-483-3p and miR-483-5p, intragenic miRNAs located within intron 2 of the *IGF2* gene, are overexpressed in WT and appear to have anti-apoptotic roles [176,177]. Interestingly, the *IGF2*/miR-483 locus is located at 11p15.5, a common region of genetic and epigenetic abnormality in WT [176]. At present, there have been few studies systematically examining miRNA expression in WT and linking such findings with clinicopathological data and outcome. However, such work will facilitate the identification of miRNA/mRNA networks that drive tumorigenesis.

Malignant GCTs are clinically complex and heterogeneous yet share functionally important molecular abnormalities. In malignant GCTs, regardless of histological subtype, site, or patient age, *LIN28* is abundantly expressed with *let-7* family downregulation [178]. Thus, the *LIN28/let-7* axis is a promising therapeutic target, offering strategies including protective small molecule targeting of pre-*let-7* stem-loop binding motifs, induction of stem-loop binding protein KSRP, promoting maturation of miRNA subsets including *let-7*, use of *let-7* mimics and inhibition of the terminal-uridyltransferase (TUTase) ZCCHC11, which ultimately leads to *pre-let-7* degradation [178]. *LIN28* and the C19MC oncogenic miRNA cluster have also been identified as potential therapeutic targets for certain CNS embryonal tumours, such as those with multilayered rosettes (ETMRs) [179–182]. CNS GCTs can be histologically subdivided into germinomas and non-germinomatous malignant GCTs (NGMGCT) [183]. MiR-214-3p overexpression has been shown *in vitro* to reduce the expression of the pro-apoptotic protein BCL2-like 11 and induce cisplatin resistance, offering new mechanistic insight into underlying treatment resistance [183]. Paediatric low-grade gliomas (pLGGs) have the tendency to recur, and supratentorial lesions are difficult to resect [184]. Catanzaro *et al.* showed that miR-139-5p was significantly downregulated in supratentorial pLGGs, and this drives cell proliferation by derepressing PI3K/AKT signalling pathways [184]. MiR-125 is also downregulated in pLGG, and its overexpression leads to reduced cell growth and apoptosis induction [185]. In glioblastoma (GBM), miR-1300 ectopic expression reduced GBM growth in an orthotopic model, and it was identified as a regulator of endomitosis [186].

Thus, many *in vitro* and *in vivo* studies across a broad range of paediatric solid tumours have elucidated critical roles for miRNAs in tumorigenesis and highlighted many as possible therapeutic targets via the use of inhibitors or mimics. Nevertheless, despite many preclinical experiments, few miRNA candidates have reached clinical development and trials, and further research is needed. One important exception is for malignant GCTs, where circulating miRNA biomarkers are embedded in clinical trials which include paediatric enrolment, e.g., AGCT1531 (ClinicalTrials.gov identifier: NCT03067181) and P3BEP (NCT02582697). There are many challenges that need addressing for the delivery of miRNA-based therapeutics, which include but are not limited to miRNA degradation by nucleases, poor target tissue delivery, immune reactions, unwanted off-target effects, and poor binding affinity for complementary sequences [70,187].

9. Conclusion

This review highlights key published observations regarding miRNA expression/profiles across a range of paediatric cancers observed in clinical practice. It will be important to extend and further the observations described here in order to translate these findings for clinical benefit. The physical properties of miRNAs, which make them resistant to degradation in body tissues and fluids, make them ideal candidates to explore as biomarkers of paediatric malignancies. MiRNA biomarkers are of particular relevance for paediatric malignancies, which typically have a low mutational burden compared with their adult counterparts; thus, the identification of DNA mutations and ctDNA as biomarkers are of more limited use. This review has demonstrated the opportunities that miRNAs hold for diagnosis, monitoring of treatment response, detection of early asymptomatic disease recurrence, prognostication, and as potential therapeutic targets of paediatric solid tumours. Further research is required to improve tissue-specific delivery of miRNA therapeutics and minimise off-target effects. It is not unreasonable to envisage the future use of miRNA mimics or antagonists, based on miRNA signatures, to deliver personalised, targeted therapy. A systematic investigation of the roles of dysregulated miRNAs across all paediatric malignancies, involving international collaboration where necessary, is warranted to afford substantial improvements in the management and outcomes of children affected by cancer.

CRediT roles

Nicholas Coleman, Matthew J. Murray - *Conceptualisation;* Karan R. Chadda, Ellen E. Blakey, Matthew J. Murray - *Data curation;* Karan R. Chadda, Ellen E. Blakey, Nicholas Coleman, Matthew J. Murray

- *Formal analysis;* Karan R. Chadda, Ellen E. Blakey, Nicholas Coleman, Matthew J. Murray - *Investigation and methodology;* Nicholas Coleman, Matthew J. Murray - *Supervision;* Karan R. Chadda, Matthew J. Murray - *Roles/Writing – original draft;* Karan R. Chadda, Ellen E. Blakey, Nicholas Coleman, Matthew J. Murray - *Writing – review & editing.*

Conflict of interest statement

The authors 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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