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MicroRNA:Siglec crosstalk in cancer progression

D. Mustafov[1,2,a], M. S. Ahmad[1,a], A. Serrano[3], M. Braoudaki[1,b] and S. S. Siddiqui[1,b]

Abstract

Aberrant Siglec expression in the tumour microenvironment has been implicated in tumour malignancies and can impact tumour behaviour and patient survival. Further to this, engagement with sialoglycans induces masked antigen recognition and promotes immune evasion, highlighting deregulated immune function. This necessitates the elucidation of their expression profiles in tumour progression. MicroRNAs (miRNAs) mediated targeting represents a novel approach to further elucidate Siglec potential and clinical relevance. Although miRNA activity in Siglec expression remains limited, we highlight current literature detailing miRNA:Siglec interactions within the tumour landscape and provide insights for possible diagnostic and therapeutic strategies in targeting the Siglec/sialic acid axis.

Addresses

[1] School of Life and Medical Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK

[2] College of Health, Medicine and Life Sciences, Brunel University London, Uxbridge UB8 3PH, UK

[3] Francisco de Vitoria University, Ctra. M-515 Pozuelo-Majadahonda, Km. 1,800, Pozuelo de Alarcón, 28223, Madrid, Spain

Corresponding authors: Siddiqui, Shoib Sarwar (s.siddiqui3@herts.ac.uk); Braoudaki, Maria (m.braoudaki@herts.ac.uk)

(Serrano A.)

[a] Equal contribution.[b] Equal contribution and shared Correspondence.

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General overview

Human cells express a great variety of receptors and ligands on their membranes that enable optimal homeostasis and intact cellular processes. The aberrant expression of membrane glycolipids and glycoproteins in addition to the unique distribution of surface molecules, including sialic acid (Sia)-containing glycoconjugates, have attracted research attention due to their involvement in various pathologies including cancer [1]. The acquisition of further scientific findings and knowledge needs to be elucidated to fully understand their comprehensive functions and implications in health and pathogenesis.

Under normal homeostatic conditions, glycans exhibit multiple functions, such as enabling communication with both neighbouring cells and the immune system. In the tumour landscape, the role of sialic acid–binding immunoglobulin-type lectins (Siglecs) disrupts normal immune signalling, thus promoting tumour progression [2]. This review provides an opinion regarding recently studied miRNA-Siglec interactions and their effects on tumour progression.

Siglec structure, function, and ligand interaction

To date, a total of 15 human and 9 murine Siglec proteins have been identified and are classified into distinct groups based on sequence homology and domain conservation [3]. The groups that share high sequence and functional homology are outlined as Sialoadhesin (Siglec-1), cluster of differentiation 22 (CD22 or Siglec-2), myelin-associated glycoprotein (MAG or Siglec-4), and Siglec-15 with approximately 50%–80% similarity. In contrast, the remaining Siglec family members are characterised by lower sequence similarity (25–30%), due to structural heterogeneity related to less conserved Ig-domain-encoding due to rapid evolutionary events [4].

Siglecs are type-I transmembrane proteins consisting of several structural domains. Each Siglec possesses a variable number of C2-type domains and an N-terminal V–set binding domain located on the extracellular region, essential for sialoglycan binding [5]. Moreover, each Siglec has preferential complementarity towards respective sialoglycan ligands, such as those exhibiting α2,8-, α2,6- or α2,3 glycosidic linkages [6,7]. The intracellular constituent motifs displayed can be either immunoreceptor tyrosine-based inhibition or activation motifs (ITIM and ITAM, respectively) and define the overall functionality of the Siglec receptor in relation to immune function [8]. Upon sialoglycan engagement, activating Siglec signalling occurs via the recruitment and interaction with the adapter protein DNAX-activation protein 12 (DAP12). In comparison to their ITIM inhibitory Siglec counterparts, canonical downstream signalling is determined via the recruitment of

Src tyrosine phosphatases SHP1 and SHP2, reducing immune activation [9].

Siglec role in cancer

Aberrant expression of Siglecs and the overall Siglec/Sia axis are implicated in tumour onset and progression, displaying increased receptor–ligand interactions that pose detrimental effects on immune signalling [10]. Immune cells express Siglecs to interact with surface-exposed sialoglycans. This interaction triggers inhibitory signalling, hindering the detection and elimination of tumour cells via effective immune response generation [11]. Further to this, tumour cells highly express sialoglycans to induce Siglec engagement to evade immune surveillance, posing as a unique driver that emphasises enhanced binding and masked antigen recognition, preventing immune cell activation [11]. For instance, Siglec-15 expression is exhibited on the surface of tumours cells and TAMs. Upon binding with the STn antigen, Siglec-15 plays a role in immune modulation and the development of a heterogeneous TME [12]. Furthermore, Siglec-7/-9 activity modulates the activation of NK cell mediated cytotoxicity. Sialoglycan engagement with these inhibitory Siglecs stimulates downstream immunosuppressive signalling that down-regulates NK cell activation. Moreover, this interaction diminishes NK cells' ability to effectively release cytotoxic granules and IFN-γ, reducing the anti-tumour response [13]. Additionally, Siglec-10/CD24 activity facilitate monocytic differentiation to pro-tumorigenic polarised macrophage and enhances tumour growth and metastasis through the release of IL-10 and TGF-β [14].

Dysregulation of sialylation expression patterns and glycosylation post-translational modifications are typically associated with cancer hallmarks. These changes include erroneous branching of N-glycans, incomplete O-glycan sequences, truncation and hypersialylation resulting from the deregulation of sialyltransferase and neuraminidase activity [15]. As a result, modified sialoglycan production increases Siglec engagement on tumour cell membranes, leading to immune evasion, increased tumour growth and metastasis [16].

In addition to this, Siglecs exhibit distinct expression profiles across several myeloid cell populations, thus playing a key role in immune regulation. In particular, Siglec activity promotes tumour microenvironment (TME) development, including polarization of monocytic differentiation to pro-tumorigenic polarised macrophage phenotype or the altered cytotoxic functionality and activation of immune cells [12].

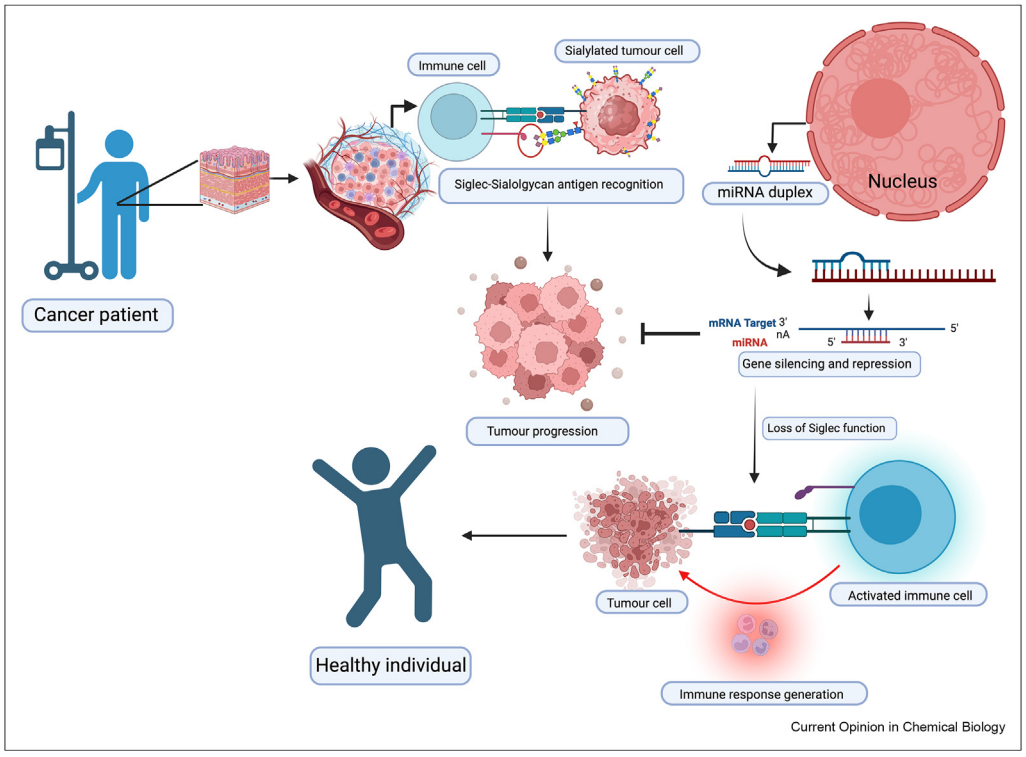
Mutually exclusive expression of Siglec-15 and PD-L1 on cancer populations underscores Siglec-15 targeting in PD-L1 negative patients. Encouraging patient response with a Siglec-15 monoclonal antibody (NC318) was shown in PD-L1 treatment resistant patients, leading to possible combination therapeutic strategies [17]. Furthermore, a humanised monoclonal antibody (Epratzumab) was shown to have a positive response in CD22 targeting and was well tolerated in non-Hodgkin's lymphoma patients in early phase I/II clinical trials [18]. Pre-clinical models also highlight potential implications of Siglec expression patterns in tumour progression. Blocking the Siglec-10/CD24 axis via a CD24-Fc vaccine emphasised reduced immune related adverse events in neuroblastoma murine models [19]. Similarly, CAR-T therapies targeting CD22 in B cell malignancies have shown promising patient response, having been investigated in pre-clinical and clinical trials, following patient relapses with CD19 CAR-T therapies [20].

Overall, the alteration of the homeostatic balance between Siglec/Sia engagement has been demonstrated to lead to an immunosuppressive TME, which corresponds to an increasingly described feature in cancer [21]. However, there is a need for further elucidation of their gene expression regulation in tumour progression. One such instance involves miRNA mediated Siglec regulation in cancer. Although the context of Siglec:miRNA interactions remains limited in the literature, there is a correlation linking deregulated Siglec expression patterns and miRNA activity on advanced tumour stage and tumour type. A previous study exhibited LINC00973 acting as a molecular sponge of miR-7109, preventing its binding to *SIGLEC15*. In addition, their interactions were associated with advanced tumour grades in clear cell renal cell carcinoma (ccRCC) and modulated *SIGLEC15* expression at the transcriptional level [22]. Similarly, overexpression of miR-494 in CD33 MDSCs constituted to greater tumour heterogeneity and significantly correlated with advanced tumour stage and histological grade [23]. Thus, this could outline the development of new miRNA-based therapeutic approaches targeting the Siglec/Sia axis in immunotherapy (Figure 1).

MicroRNA biogenesis and function

MicroRNAs (miRNAs) are small, non-coding single-stranded RNA molecules that regulate gene expression [24,25]. MiRNAs have been related to many biological processes, such as embryonal development, human cancer, neurodegenerative and cardiovascular disorders, diabetes, and metabolic diseases [26–29]. Given their multifunctional biological relevance, miRNAs have gained significant importance as potential candidates for diagnostic and prognostic biomarkers and possible therapeutic agents [26]. Thus, it is important to understand how miRNAs are synthesized and how they can modulate gene expression. There are two pathways that define the biogenesis of miRNAs, namely the canonical and non-canonical pathways (Figure 2).

Figure 1



MicroRNA mediated Siglec regulation. Within the TME, Siglec-sialoglycan interactions mask antigen recognition on tumour cells, promoting tumour progression and metastasis. The involvement of microRNA on Siglec gene regulation has the potential to impact Siglec loss of function and revert the activation of the immune response against tumour cells. This figure was created with BioRender.com.

Canonical miRNA biogenesis

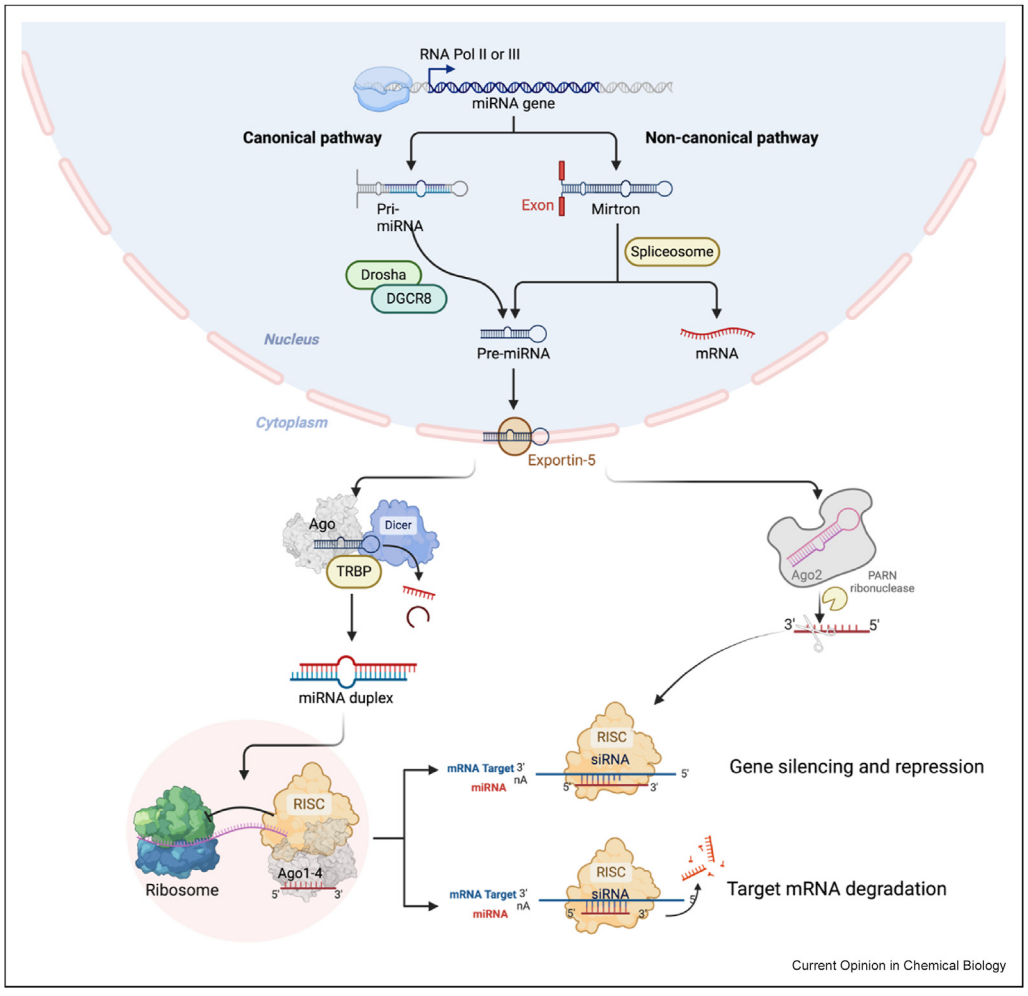
The canonical miRNA biogenesis is a multistep process that transforms primary miRNA transcripts (pri-miRNAs) into functional, mature single-stranded miRNAs that associate with Argonaute (Ago) proteins to form the Ago complex, which usually is referred to as the RNA-induced silencing complex (RISC) [30].

MiRNA synthesis begins within the nucleus, where RNA polymerase II transcribes a DNA sequence encoding a miRNA gene. This results in the formation of a long, 5′ capped, and 3′ polyadenylated pri-miRNA transcript characterised with a hairpin structure [31]. Within the nucleus, the pri-miRNA encounters the microprocessor complex, a trimer formed by one Drosha and two DiGeorge syndrome critical region gene 8 (DGCR8) molecules [32]. Drosha, an RNase III enzyme, recognizes and cleaves the pri-miRNA, releasing a shorter, hairpin-shaped RNA molecule called a precursor miRNA (pre-miRNA) [30]. Following Drosha-DGCR8 processing, the pre-miRNA is exported from the nucleus to the cytoplasm. This step is facilitated by the Exportin-5 (XPO5)/RanGTP complex. XPO5 interacts with pre-miRNA in a sequence-independent manner and in the presence of a GTP-binding nuclear protein Ran (RanGTP) [33].

Once in the cytoplasm, the pre-miRNA encounters the Dicer complex. Dicer, another RNase III enzyme, recognises the pre-miRNA and cleaves it at a defined position. This cleavage event generates a short, double-stranded miRNA duplex containing the mature miRNA and its passenger strand [34]. Once the miRNA duplex is formed, it is loaded into an Ago protein with the help of the Hsc70/Hsp90 chaperone machinery in an ATP dependent manner [35]. This miRNA duplex can be loaded into any of the four proteins of the Ago proteins, with preferential loading on the most abundant Ago2 paralog [36]. Upon Ago complex association, one miRNA duplex strand (mature guide miRNA) is retained, while the other (passenger strand) is discarded, forming a single-stranded RNA-induced silencing complex (RISC). Notably, both 5' (5p) and 3' (3p) derived miRNAs could exert functionality on target mRNAs [37].

The mature miRNA loaded onto the RISC complex is guided towards its complementary mRNA targets within the cytosol. Target recognition primarily relies on base-pairing interactions between the miRNA seed region (nucleotides 2–7/8 of the mature miRNA) and the complementary sequence within the mRNA 3′ untranslated region (UTR) [38]. This interaction leads to

Figure 2



An overview of the canonical and non-canonical pathways of miRNA biogenesis. The canonical miRNA biosynthesis commences with the transcription of miRNA genes by RNA polymerase II or III, producing primary miRNA transcripts (pri-miRNA), which can be several hundred to thousands of nucleotides (nts) long and might contain more than one hairpin structure. In the nucleus, the pri-miRNA transcripts are recognised and cleaved by the Drosha enzyme (RNase III enzyme) and its co-factor, the Di-George Syndrome Critical Region 8 (DGCR8) enzyme. Once bound to each other, they form a microprocessor complex which recognises the RNA stem loop and cleaves pri-miRNA to form 65–70 nts long precursor-miRNA (pre-miRNA). The produced pre-miRNA is characterised by a two-nucleotide-long 3′ overhang, which is subsequently recognised by a transmembrane protein, exportin 5, which facilitates the export of the pre-miRNA to the cytoplasm, where its processing continues with the aid of Dicer. Dicer associates with the TAR-RNA binding protein (TRBP) cofactor forming the Dicer-TRBP, which is incorporated in the removal of the terminal loop of the pre-miRNA and thus the production of the mature double-stranded miRNA (dsRNA). The formed mature dsRNA is approximately 18–22 nts long and constitutes of a miRNA-guide strand and a miRNA-passenger strand (miRNA/miRNA* or miR-3p/miR-5p, respectively). The strand with a lower 5′-end stability, usually the guide strand, subsequently associates with an RNA-induced silencing complex (RISC), which contains Argonaute (AGO 1–4) proteins. The miRNA-RISC complex is then guided to a complementary mRNA sequence where it acts as a post-transcriptional regulator via inducing degradation or translational inhibition of the target mRNA. This translational silencing occurs at the 3′ untranslated region (UTR) region of the target mRNA. The non-canonical miRNA biosynthesis includes pathways such as mirtron (a non-coding RNA molecule derived from introns) processing, omitting the Drosha/DGCR8 microprocessor complex interaction and thus expanding the diversity of miRNA origins. This involves introns cleavage by spliceosomes or other debranching enzymes present in the nucleus, resulting in the exclusion of the pre-miRNA hairpin, thus making the pre-miRNA ready for nucleus export via exportin 5 and further processing by Dicer. Another non-canonical miRNA biogenesis mechanism aids the generation of miR-3p strands via omitting the Dicer cleavage. Instead, Ago2 and PARN ribonucleases remove the hairpin structure of the pre-miRNA and produce a miR-3p strand which can then act upon its mRNA targets. This figure was created with BioRender.com.

various modes of translational repression based on the complementarity binding between the miRNA:mRNA complex. During full complementarity binding, the RISC complex can directly cleave the target mRNA, leading to its full degradation. In contrast, partial complementarity induces a wider range of regulatory effects such as partial gene silencing and mRNA destabilisation (Figure 2) [39].

Non-canonical miRNA biogenesis

In brief, non-canonical biogenesis utilises the Drosha/DGCR8-independent and Dicer-independent pathways in pre-miRNA transcript production. Mirtrons and viral tRNA-like structures bypass the Drosha/DGCR8 microprocessor complex to generate the pre-miRNA transcript. Additionally, m7G-capped pre-miRNAs utilise a distinct export pathway and favour 3p-miRNA incorporation into the silencing complex (Figure 2) [40]. Meanwhile, the Dicer-independent pathway requires pre-miRNA cleavage supervised by Ago2, resulting in the formation of a mature miRNA strand. Further processing via 3′ trimming is mediated by the PARN ribonuclease enzyme, which can only generate functional miRNA-3p [41].

MiRNA–Siglec interaction network

MiRNA–Siglec interactions represent an exciting avenue for research in elucidating their therapeutic potential and clinical relevance across the tumour landscape (Table 1). This type of association has been recently reported in bladder cancer. The upregulation of *BACH1-IT2* and its consequential impact on Siglec-15 expression, orchestrated by miR-4786-5p, offers a compelling glimpse into the molecular intricacies underlying cancer development [42]. *BACH1-IT2* was seen to promote tumour progression by suppressing miR-4786, consequently increasing *SIGLEC15* expression on tumour cells facilitating greater immunosuppression and heterogenous TME.

Similarly, the involvement of miRNAs such as miR-582-5p and miR-7109 in the negative regulation of Siglec-15 expression in hepatocellular carcinoma (HCC) and ccRCC respectively, has been reported [22,43]. In HCC, the effect of sponging upon miR-582-5p by *TUG1* resulted in elevated *SIGLEC15* expression patterns, indicating enhanced immunosuppression within HCC cells and a greater number of inactivated T-cells [43]. Similarly, in ccRCC, the sponging of miR-7109 by LINC00973 prevented effective miRNA binding and regulation of the *SIGLEC15* gene. This prompted greater *SIGLEC15* expression and immunosuppression in the tumour cells [22]. These findings not only shed light on the molecular interactions between miRNAs and Siglecs in cancer progression but also hold promise for the identification of potential diagnostic and therapeutic biomarkers.

Ahmad et al. explored the interplay between Siglec-15 and miRNAs in colorectal cancer (CRC) progression. Utilisation of a small molecule inhibitor (SHG-8) was predicted to bind to the Siglec-15 protein via the ARG143 residue. Exposure to SHG-8 reduced tumour progression and stimulated cytokine production from differentiated macrophages. Therefore, this suggested a potential activation of immune cells for an effective immune response. Simultaneously, small RNA sequencing analysis demonstrated significant upregulation of miR-6715b-3p, potentially targeting *PTTG1IP*, a possible oncogene in CRC. This suggested a potential regulatory axis involving Siglec-15 inhibition and subsequent miRNA-driven oncogene suppression. Further investigation into this regulatory axis is necessitated to elucidate its clinical relevance to CRC progression. However, its therapeutic potential holds promise for novel CRC treatment strategies [44].

MiRNA–Siglec interactions can also act as a potential therapeutic avenue in acute myeloid leukaemia (AML). The interplay between miRNA-125b with the cell surface marker *CD33/SIGLEC3* is of growing interest. A study investigated the potential of red blood cell-derived extracellular vesicles (RBCEVs) for directed delivery of anti-FLT3-ITD and anti-miR-125b oligonucleotides in AML. CD33 conjugated antibody-coated RBCEVs allowing for $CD33^{+}$ specific targeting of AML cells were loaded with antisense oligonucleotides (ASOs) to inhibit FLT3-ITD or miR-125b. The observed results demonstrated that the engineered EVs reduced cellular viability and suppressed leukaemia progression *in vitro* and *in vivo* due to the significant increase in the uptake of RBCEVs. These results highlight the importance of miR-125b and its regulation of the *CD33* gene [45].

Another report suggested that miRNA-Siglec interactions have emerged as a valuable tool for cholangiocarcinoma (CCA) diagnosis and treatment [46]. MiR-3913-5p was identified as an independent prognostic factor for CCA, with lower expression correlating with poor survival outcomes. Further *in vitro* experiments validated the differential expression of miR-3913-5p in CCA, whereas computational target gene prediction analysis identified *RNF24* and *SIGLEC10* as potential downstream effectors of miR-3913-5p, warranting further investigation [46]. MiR-3913-5p was associated with the negative regulation of cell metabolism, thus promoting tumour growth. Conversely, *SIGLEC10* potentially influenced metastasis in CCA by altering cellular adhesion, whilst *RNF24* affected tumour cell proliferation. Furthermore, a computational approach highlighted miRNA involvement in *SIGLEC12* mediated tumorigenesis in kidney renal papillary cell carcinomas (KRCC). Deregulated expression of miR-133b/miR-654 and miR-574 was predicted to modulate *SIGLEC12* expression patterns. Moreover, data may suggest *Siglec-12*$^{+}$ KRCC tumours upon miRNA exertion exhibit greater sensitisation to immunotherapeutic checkpoint blockade [47]. Further investigations highlighting the synergistic therapeutic potentials of miR-319-5p and *SIGLEC10*, as well as the miR-133b/miR-654 and miR-574/Siglec-12 axis, are necessitated to validate their clinical relevance in CCA and KRCC onset, respectively. Emerging technologies such as small RNA-sequencing (sRNA-seq) and spatial

Table 1

miRNA involvement in Siglec regulation in cancer.

Siglec protein	Observed myeloid expression in situ	Cytoplasmic tail	Preferential Sialoglycan binding	miRNA involvement	Reference.
Siglec-1	Monocytes, Macrophages, Dendritic cells	ITIM	α2,3 linked sialoglycans	N/A	
Siglec-2	B-cells, Mast cells, Dendritic cells	ITIM	α2,6 linked sialoglycans	miR-17~92	[49]
Siglec-3	Monocytes, Macrophages, NK cells, Myeloid progenitor cells, Microglia	ITIM	α2,3 and α2,6 linked sialoglycans GD1a and GT1b gangliosides	miR-125b, miRNA-494	[23,45,50]
Siglec-4	Mast cells, Oligodendrocytes, Schwann cells	ITIM	α2,3 linked sialoglycans	N/A	
Siglec-5	Neutrophils, Mast cells, B-cells, Monocytes, Activated T-lymphocytes	ITIM	α2,3 linked sialoglycans	N/A	
Siglec-6	Mast cells, B-cells, Basophils	ITIM	α2,6 linked sialoglycans Leptin Glycodelin-A STn antigen	N/A	[51]
Siglec-7	NK cells, Monocytes, Mast cells	ITIM	α2,8 linked sialoglycans CD43 Gangliosides (GD3, GD2, GD1b, GT1b, DSGb5 Disialyl-T antigen	N/A	[52–54]
Siglec-8	Eosinophils, Mast cells, Basophils	ITIM	α2,3 linked sialoglycans	N/A	
Siglec-9	Monocytes, Neutrophils, NK cells	ITIM	α2,3, α2,6 linked sialoglycans	N/A	
Siglec-10	NK cells, B-cells, activated $CD4^+$ T-cells, Monocytes, Eosinophils	ITIM	α2,3, α2,6 linked sialoglycans	miR-561-5p	[55,56]
Siglec-11	Macrophages, Brain microglial cells	ITIM	α2,8 linked sialoglycans	N/A	
Siglec-14	Granulocytes, Monocytes	Interact with DAP-containing ITAM	α2,3 linked sialoglycans	N/A	
Siglec-15	Osteoclasts, TAMs	Interact with DAP-containing ITAM	Sialyl-Tn antigen CD11b α2,3, α2,6 linked sialoglycans	miR-6715b-3p, miR-4786, miR-582-5p, miR-7109	[6,22,42–44,57,58]
Siglec-16	Macrophages, Microglia	Interact with DAP-containing ITAM	α2,8 linked sialoglycans	N/A	

transcriptomics approaches have provided insights to our understanding of miRNA:Siglec interactions. Sequencing analysis in combination with a multiomics approach offered comprehensive insights into the complex regulatory networking involving miRNAs and Siglecs. These approaches identified regulatory pathways and characterisation of oncogenic targets that impact the Siglec/Sia axis and its interactions on tumour progression. The findings of Ahmad et al. suggested the interplay of miR-6715b-3p with *SIGLEC15* via *PTTG1IP* through the use of sRNA-seq analysis, which suggested a regulatory pathway associated with CRC progression [44]. Furthermore, Rodriguez et al. bulk tissue transcriptomics analysis revealed that the expression of Siglec-7/-9 both correlated with myeloid cell markers (TYROBP, FCER1G, C1QR and CD14) and co-expressed on $CD14^+$ myeloid cells in pancreatic ductal adenocarcinoma (PDAC), suggesting these sialylated tumour cells can play a part in controlling immune suppression and provide new targets for cancer immunotherapy [48].

Deregulated Siglec/miRNA networks may play a crucial role in reducing sensitisation to immunotherapeutic treatment. MiRNAs can modulate the expression of Siglec genes to inhibit T-cell activation and promote immunosuppression. It has been demonstrated that miRNAs could impact immune checkpoint inhibitor (ICI) efficacy. MiR-21, let-7a/b and miR-155 were shown to regulate the polarisation of tumorigenic macrophages [59–61]. In contrast, miR-28, miR-138 and miR-149 exhibited greater association for enhanced T-cell exhaustion [62–64]. This may suggest possible implications of miRNA activity in enhancing treatment resistance in cancer. Nevertheless, preclinical studies suggest a combination of miRNA-based therapies with ICIs could overcome resistance mechanisms and potentiate the treatment response to immune checkpoint blockade in cancer patients. The miRNA/Siglec network in initiating resistance to immunotherapy remains largely unexplored. However, further investigations on the Siglec/miRNA mediated interactions could provide potential avenues for the development of targeted immunotherapies that could effectively overcome resistance.

However, miRNA-mediated Siglec regulation is not solely limited to cancer. One study indicated upregulated sialoadhesin expression in chronic obstructive pulmonary disease (COPD) and presented miR-195-5p targeting sialoadhesin expression, possibly emphasising as a potential target in COPD treatment [65]. Similarly, an *in vivo* study demonstrated that miR-1260 blocking impeded sialoadhesin activity and inhibited the occurrence of inflammation in COPD murine models [66]. This highlights the interplay of miRNA-Siglec involvement having significant implications in patient health. The pathogenesis of Hirschsprung's disease (HSCR), a rare congenital disorder characterised by the absence of enteric neurons, has been linked to the interplay between miR-215 and its host gene, isoleucyl-tRNA synthetase 2 (*IARS2*), along with their target *SIGLEC8*. Expression analyses revealed that miR-215 levels were significantly lower in the colon tissues of HSCR patients, positively correlating with *IARS2* levels and negatively with *SIGLEC8* expression. These findings suggest that the *IARS2*/miR-215/*SIGLEC8* axis is a critical factor in the development of HSCR [67]. A recent study has demonstrated that in chronic hepatitis C virus (HCV), miR-124 expression was associated with STAT3 signalling promoting $CD33^+$ MDSC differentiation. They also suggested that the interaction between miR-124 and RUNX1 stimulated the release of immunosuppressive markers, such as iNOS and STAT3. Silencing these interactions prevented MDSC differentiation and enhanced $CD4^+$ T-cell activity, suggesting a possible therapeutic application in conjunction with antiviral treatment [68].

Elucidating miRNA:Siglec interactions present several challenges within the clinical setting. Firstly, the interactions involved between miRNA and Siglec activity are highly complicated, leading to the difficult identification of downstream therapeutic targets. Furthermore, tissue specific expression patterns of miRNAs and Siglecs pose challenges related to non-specific interactions and off-target effects. Moreover, delivery systems for miRNA-based therapeutics require further optimisation for efficient and efficacious delivery to the tumour site. In addition, variability in patient response and individual differences related to genetic polymorphisms and gene heterogeneity may influence the miRNA:Siglec interactive networks across patients. Addressing these possible challenges is crucial for elucidating the clinical relevance of miRNA:Siglec interactions to enable their transition to clinical settings.

Conclusion

The intricate interplay among Siglecs, miRNAs, and cancer progression unveils a compelling narrative within the domains of immunology and oncology research. Through meticulous investigations into various malignancies, scientists have illuminated the crucial role of miRNAs in modulating Siglec expression. This modulation significantly impacts tumour behaviour and patient prognosis. While these examples suggest miRNA-Siglec interactions can influence gene regulation, the field is still developing. In conclusion, continued exploration of these intricate molecular networks promises to unravel possible diagnostic and therapeutic avenues to enhance our understanding of cancer biology and immune regulation.

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Declaration of competing interest

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.

Data availability

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Filipsky F, Läubli H: **Regulation of sialic acid metabolism in cancer**. *Carbohydr Res* 2024, **539**, 109123.
2. Jiang K-Y, Qi L-L, Kang F-B, Wang L: **The intriguing roles of Siglec family members in the tumor microenvironment**. *Biomark Res* 2022, **10**:22.
3. Stanczak MA, Läubli H: **Siglec receptors as new immune checkpoints in cancer**. *Mol Aspect Med* 2023, **90**, 101112.
4. Van De Wall S, Santegoets KCM, Van Houtum EJH, Büll C, Adema GJ: **Sialoglycans and Siglecs can shape the tumor immune microenvironment**. *Trends Immunol* 2020, **41**: 274–285.
5. Van Houtum EJH, Büll C, Cornelissen LAM, Adema GJ: **Siglec signaling in the tumor microenvironment**. *Front Immunol* 2021, **12**, 790317.
6. Lenza MP, Egia-Mendikute L, Antoñana-Vildosola A, Soares CO, Coelho H, Corzana F, Bosch A, Manisha P, Quintana JI, Oyenarte I, *et al.*: **Structural insights into Siglec-15 reveal glycosylation dependency for its interaction with T cells through integrin CD11b**. *Nat Commun* 2023, **14**:3496.
7. Zheng Y, Ma X, Su D, Zhang Y, Yu L, Jiang F, Zhou X, Feng Y, Ma F: **The roles of Siglec7 and Siglec9 on natural killer cells in virus infection and tumour progression**. *Journal of Immunology Research* 2020, **2020**:1–9.
8. Siddiqui SS: **Non-canonical roles of Siglecs: beyond sialic acid-binding and immune cell modulation**. *Mol Aspect Med* 2023, **90**, 101145.
9. Murugesan G, Weigle B, Crocker PR: **Siglec and anti-Siglec therapies**. *Curr Opin Chem Biol* 2021, **62**:34–42.
10. Läubli H, Nalle SC, Maslyar D: **Targeting the siglec–sialic acid immune Axis in cancer: current and future approaches**. *Cancer Immunol Res* 2022, **10**:1423–1432.
11. Zhou X, Chi K, Zhang C, Liu Q, Yang G: **Sialylation: a cloak for tumors to trick the immune system in the microenvironment**. *Biology* 2023, **12**:832.
12. Huang Z, Guo Y, Li B, Shen M, Yi Y, Li L, Zhao X, Yang L: **Siglec-15 on macrophages suppress the immune microenvironment in patients with PD-L1 negative non-metastasis lung adenocarcinoma**. *Cancer Gene Ther* 2023, https://doi.org/10.1038/s41417-023-00713-z.
13. Daly J, Sarkar S, Natoni A, Stark JC, Riley NM, Bertozzi CR, Carlsten M, O'Dwyer ME: **Targeting hypersialylation in multiple myeloma represents a novel approach to enhance NK cell–mediated tumor responses**. *Blood Advances* 2022, **6**: 3352–3366.
14. Barkal AA, Brewer RE, Markovic M, Kowarsky M, Barkal SA, Zaro BW, Krishnan V, Hatakeyama J, Dorigo O, Barkal LJ, *et al.*: **CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy**. *Nature* 2019, **572**:392–396.
15. Munkley J: **Aberrant sialylation in cancer: therapeutic opportunities**. *Cancers* 2022, **14**:4248.
16. Stanczak MA, Siddiqui SS, Trefny MP, Thommen DS, Boligan KF, Von Gunten S, Tzankov A, Tietze L, Lardinois D, Heinzelmann-Schwarz V, *et al.*: **Self-associated molecular patterns mediate cancer immune evasion by engaging Siglecs on T cells**. *J Clin Invest* 2018, **128**:4912–4923.
17. Wang J, Sun J, Liu LN, Flies DB, Nie X, Toki M, Zhang J, Song C, Zarr M, Zhou X, *et al.*: **Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy**. *Nat Med* 2019, **25**:656–666.
18. Lim J, Sari-Ak D, Bagga T: **Siglecs as therapeutic targets**. ***Cancer***. *Biology* 2021, **10**:1178.
19. Wu X, Srinivasan P, Basu M, Zimmerman T, Li S, Wang Y, Zheng P, Liu Y, Sandler AD: **CD24-Fc suppression of immune related adverse events in a therapeutic cancer vaccine model of murine neuroblastoma**. *Front Immunol* 2023, **14**, 1176370.
20. Adeel K, Fergusson NJ, Shorr R, Atkins H, Hay KA: **Efficacy and safety of CD22 chimeric antigen receptor (CAR) T cell therapy in patients with B cell malignancies: a protocol for a systematic review and meta-analysis**. *Syst Rev* 2021, **10**:35.
21. Läubli H, Kawanishi K, George Vazhappilly C, Matar R, Merheb M, Sarwar Siddiqui S: **Tools to study and target the Siglec–sialic acid axis in cancer**. *FEBS J* 2021, **288**: 6206–6225.
22. Liu Y, Li X, Zhang C, Zhang H, Huang Y: **LINC00973 is involved in cancer immune suppression through positive regulation of Siglec-15 in clear-cell renal cell carcinoma**. *Cancer Sci* 2020, **111**:3693–3704.
23. Moaaz M, Lotfy H, Elsherbini B, Motawea M, Fadali G: **TGF-β enhances the anti-inflammatory effect of tumor- infiltrating CD33+11b+HLA-DR myeloid-derived suppressor cells in gastric cancer: a possible relation to MicroRNA-494**. *Asian Pac J Cancer Prev APJCP* 2020, **21**:3393–3403.
24. Mustafov D, Siddiqui SS, Klena L, Karteris E, Braoudaki M: **SV2B/miR-34a/miR-128 axis as prognostic biomarker in glioblastoma multiforme**. *Sci Rep* 2024, **14**:6647.
25. Braoudaki M, Sarafidis M, Koutsouris DD, Koutsouri G, Lambrou GI: **Bioinformatics analysis reveals ki-67 specific microRNA functions in pediatric embryonal tumors**. In *2019 41st annual international conference of the IEEE engineering in medicine and biology society (EMBC)*. IEEE; 2019:1346–1349.
26. Ho PTB, Clark IM, Le LTT: **MicroRNA-based diagnosis and therapy**. *IJMS* 2022, **23**:7167.
27. Braoudaki M, Koutsouris D-D, Kouris I, Paidi A, Koutsouri G, Lambrou GI: **Bioinformatics and regression analyses manifest tumor-specific miRNA expression dynamics in pediatric embryonal malignancies**. In *2018 40th annual international conference of the IEEE engineering in medicine and biology society (EMBC)*. IEEE; 2018:5834–5837.
28. Lykoudi A, Kolialexi A, Lambrou GI, Braoudaki M, Siristatidis C, Papaioanou GK, Tzetis M, Mavrou A, Papantoniou N: **Dysregulated placental microRNAs in early and late onset preeclampsia**. *Placenta* 2018, **61**:24–32.
29. Ntoumou E, Tzetis M, Braoudaki M, Lambrou G, Poulou M, Malizos K, Stefanou N, Anastasopoulou L, Tsezou A: **Serum microRNA array analysis identifies miR-140-3p, miR-33b-3p and miR-671-3p as potential osteoarthritis biomarkers involved in metabolic processes**. *Clin Epigenet* 2017, **9**:127.
30. Shang R, Lee S, Senavirathne G, Lai EC: **microRNAs in action: biogenesis, function and regulation**. *Nat Rev Genet* 2023, **24**: 816–833.
31. Bofill-De Ros X, Vang Ørom UA: **Recent progress in miRNA biogenesis and decay**. *RNA Biol* 2024, **21**:1–8.

32. Nguyen HM, Nguyen TD, Nguyen TL, Nguyen TA: **Orientation of human microprocessor on primary MicroRNAs**. *Biochemistry* 2019, **58**:189–198.

33. Wu K, He J, Pu W, Peng Y: **The role of exportin-5 in MicroRNA biogenesis and cancer**. *Dev Reprod Biol* 2018, **16**:120–126.

34. Luo Q-J, Zhang J, Li P, Wang Q, Zhang Y, Roy-Chaudhuri B, Xu J, Kay MA, Zhang QC: **RNA structure probing reveals the structural basis of Dicer binding and cleavage**. *Nat Commun* 2021, **12**:3397.

35. Lee S, Jee D, Srivastava S, Yang A, Ramidi A, Shang R, Bortolamiol-Becet D, Pfeffer S, Gu S, Wen J, *et al.*: **Promiscuous splicing-derived hairpins are dominant substrates of tailing-mediated defense of miRNA biogenesis in mammals**. *Cell Rep* 2023, **42**, 112111.

36. Nakanishi K: **Anatomy of four human Argonaute proteins**. *Nucleic Acids Res* 2022, **50**:6618–6638.

37. Medley JC, Panzade G, Zinovyeva AY: **microRNA strand selection: unwinding the rules**. *WIREs RNA* 2021, **12**, e1627.

38. Gan HH, Gunsalus KC: **The role of tertiary structure in MicroRNA target recognition**. In *MicroRNA target identification*. Edited by Laganà A, New York: Springer; 2019:43–64.

39. Iwakawa H, Tomari Y: **Life of RISC: formation, action, and degradation of RNA-induced silencing complex**. *Mol Cell* 2022, **82**:30–43.

40. Mustafov D, Seriah S, Malik R, Braoudaki M: **MicroRNA biomarkers in primary brain malignancies**. In *Epigenetics - regulation and new perspectives*. Edited by Huang T, IntechOpen; 2023.

41. Lee Y-Y, Lee H, Kim H, Kim VN, Roh S-H: **Structure of the human DICER–pre-miRNA complex in a dicing state**. *Nature* 2023, **615**:331–338.

42. * Li X, Liang Z, Pan J, Zhang M, Liu J, Hu R, Liao C: **Identification of BACH1-IT2-miR-4786-Siglec-15 immune suppressive axis in bladder cancer**. *BMC Cancer* 2024, **24**:328.
This study identified a molecular pathway involving the *BACH1-IT2*/miR-4786/*SIGLEC15* axis that contributes to immune evasion and progression of bladder cancer. This pathway suggested potential targets for new therapeutic approaches and prediction for patient survival outcomes.

43. ** Ren Y, Lyu J, Guo Y, Yao Y, Hu L: **Long noncoding RNA TUG1 inhibits tumor progression through regulating siglec-15-related anti-immune activity in hepatocellular carcinoma**. *Journal of Immunology Research* 2022, **2022**:1–24.
Researchers demonstrated the involvement of miR-582-5p in directly targeting *SIGLEC15* and *TUG1*, implicated in liver cancer tumorigenesis. By acting as a "sponge" for miR-582-5p, *TUG1* allowed *SIGLEC15* to suppress the immune response in liver cancer. Silencing *TUG1* with miR-582-5p-RNA inhibitor therapy proposes alternative treatment strategies for normalising the immune response and reduction of tumour growth.

44. ** Ahmad MS, Braoudaki M, Patel H, Ahmad I, Shagufta Siddiqui SS: **Novel Siglec-15-Sia axis inhibitor leads to colorectal cancer cell death by targeting miR-6715b-3p and oncogenes**. *Front Immunol* 2023, **14**, 1254911.
This research incorporated the development and utilisation of a small molecule inhibitor (SHG-8) that targeted Siglec-15 *in vitro* in colon cancer. Small RNA sequencing analysis identified deregulated expression of miR-6715b-3p, possibly acting as a tumour suppressor following SHG-8 exposure. In addition, miR-6715b-3p targeted *PTTG1IP* emphasising possible interaction with the *SIGLEC15*/Sia axis. These findings suggested a combination strategy with PD-L1 antagonists for colon cancer patients.

45. * Chen H, Jayasinghe MK, Yeo EYM, Wu Z, Pirisinu M, Usman WM, Pham TT, Lim KW, Tran NV, Leung AYH, *et al.*: **CD33 -targeting extracellular vesicles deliver antisense oligonucleotides against FLT3-ITD and miR -125b for specific treatment of acute myeloid leukaemia**. *Cell Prolif* 2022, **55**, e13255.
Chen et al. exhibited engineered vesicles (RBCEVs) for drug delivery capable of gene and miRNA targeting in AML. Moreover, the designed carriers also targeted CD33 (Siglec-3), a highly expressed protein on AML cells. Therapeutic agent loaded RBCEVs directly targeting the *FLT3-ITD* gene or miR-125b exerted tumour suppression of AML *in vitro* and *in vivo*. This provided a promising avenue for effective AML treatment.

46. Yao Y, Jiao D, Liu Z, Chen J, Zhou X, Li Z, Li J, Han X: **Novel miRNA predicts survival and prognosis of cholangiocarcinoma based on RNA-seq data and in vitro experiments**. *BioMed Res Int* 2020, **2020**:1–14.

47. ** Ogbodo AK, Mustafov D, Arora M, Lambrou GI, Braoudaki M, Siddiqui SS: **Analysis of SIGLEC12 expression, immunomodulation and prognostic value in renal cancer using multiomic databases**. *Heliyon* 2024, **10**, e24286.
This research focused on the investigation of Siglec-12 expression profiles in kidney cancer (KRCC). While the mechanisms of Siglec-12 action within KRCC remain unclear, it was suggested that high protein levels might be associated with poor patient prognosis. Further compactional analyses predicted that miR-133b/miR-654/miR-574 were involved in the modulation of *SIGLEC12* expression. The researchers proposed that miRNA blockade of *SIGLEC12* might inhibit cancer-promoting pathways and induce sensitisation to immunotherapeutic treatment within KRCC patients.

48. Rodriguez E, Boelaars K, Brown K, Eveline Li RJ, Kruijssen L, Bruijns SCM, Van Ee T, Schetters STT, Crommentuijn MHW, Van Der Horst JC, *et al.*: **Sialic acids in pancreatic cancer cells drive tumour-associated macrophage differentiation via the Siglec receptors Siglec-7 and Siglec-9**. *Nat Commun* 2021, **12**:1270.

49. Psathas JN, Doonan PJ, Raman P, Freedman BD, Minn AJ, Thomas-Tikhonenko A: **The Myc-miR-17-92 axis amplifies B-cell receptor signaling via inhibition of ITIM proteins: a novel lymphomagenic feed-forward loop**. *Blood* 2013, **122**:4220–4229.

50. Gonzalez-Gil A, Schnaar RL: **Siglec ligands**. *Cells* 2021, **10**:1260.

51. Robida PA, Rische CH, Morgenstern NB-B, Janarthanam R, Cao Y, Krier-Burris RA, Korver W, Xu A, Luu T, Schanin J, *et al.*: **Functional and phenotypic characterization of siglec-6 on human mast cells**. *Cells* 2022, **11**:1138.

52. Büll C, Nason R, Sun L, Van Coillie J, Madriz Sørensen D, Moons SJ, Yang Z, Arbitman S, Fernandes SM, Furukawa S, *et al.*: **Probing the binding specificities of human Siglecs by cell-based glycan arrays**. *Proc Natl Acad Sci USA* 2021, **118**, e2026102118.

53. Yamakawa N, Yasuda Y, Yoshimura A, Goshima A, Crocker PR, Vergoten G, Nishiura Y, Takahashi T, Hanashima S, Matsumoto K, *et al.*: **Discovery of a new sialic acid binding region that regulates Siglec-7**. *Sci Rep* 2020, **10**:8647.

54. Chang L-Y, Liang S-Y, Lu S-C, Tseng HC, Tsai H-Y, Tang C-J, Sugata M, Chen Y-J, Chen Y-J, Wu S-J, *et al.*: **Molecular basis and role of siglec-7 ligand expression on chronic lymphocytic leukemia B cells**. *Front Immunol* 2022, **13**, 840388.

55. Liu J, Yan C, Xu S: **LncRNA IL21-AS1 facilitates tumour progression by enhancing CD24-induced phagocytosis inhibition and tumorigenesis in ovarian cancer**. *Cell Death Dis* 2024, **15**:313.

56. Bandala-Sanchez E, Bediaga NG, Naselli G, Neale AM, Harrison LC: **Siglec-10 expression is up-regulated in activated human CD4+ T cells**. *Hum Immunol* 2020, **81**:101–104.

57. Läubli H: **Unraveling the impact of a glyco-immune checkpoint in bone metastasis**. *Proc Natl Acad Sci USA* 2024, **121**, e2400499121.

58. Boelaars K, Van Kooyk Y: **Targeting myeloid cells for cancer immunotherapy: siglec-7/9/10/15 and their ligands**. *Trends in Cancer* 2024, **10**:230–241.

59. Xi J, Huang Q, Wang L, Ma X, Deng Q, Kumar M, Zhou Z, Li L, Zeng Z, Young KH, *et al.*: **miR-21 depletion in macrophages promotes tumoricidal polarization and enhances PD-1 immunotherapy**. *Oncogene* 2018, **37**:3151–3165.

60. Yu D, Liu X, Han G, Liu Y, Zhao X, Wang D, Bian X, Gu T, Wen L: **The let-7 family of microRNAs suppresses immune evasion in head and neck squamous cell carcinoma by promoting PD-L1 degradation**. *Cell Commun Signal* 2019, **17**:173.

61. Huffaker TB, Lee S-H, Tang WW, Wallace JA, Alexander M, Runtsch MC, Larsen DK, Thompson J, Ramstead AG, Voth WP,

et al.: **Antitumor immunity is defective in T cell–specific microRNA-155–deficient mice and is rescued by immune checkpoint blockade**. *J Biol Chem* 2017, **292**:18530–18541.

62. Li Q, Johnston N, Zheng X, Wang H, Zhang X, Gao D, Min W: **miR-28 modulates exhaustive differentiation of T cells through silencing programmed cell death-1 and regulating cytokine secretion**. *Oncotarget* 2016, **7**:53735–53750.

63. Wei J, Nduom EK, Kong L-Y, Hashimoto Y, Xu S, Gabrusiewicz K, Ling X, Huang N, Qiao W, Zhou S, *et al.*: **MiR-138 exerts anti-glioma efficacy by targeting immune checkpoints**. *Neuro Oncol* 2016, **18**:639–648.

64. Zhang M, Gao D, Shi Y, Wang Y, Joshi R, Yu Q, Liu D, Alotaibi F, Zhang Y, Wang H, *et al.*: **miR-149-3p reverses CD8 $^{+}$ T-cell exhaustion by reducing inhibitory receptors and promoting cytokine secretion in breast cancer cells**. *Open Biol* 2019, **9**, 190061.

65. Li S, Jiang L, Yang Y, Cao J, Zhang Q, Zhang J, Wang R, Deng X, Li Y: **MiR-195-5p inhibits the development of chronic obstructive pulmonary disease via targeting siglec1**. *Hum Exp Toxicol* 2020, **39**:1333–1344.

66. Li S, Jiang L, Yang Y, Cao J, Zhang Q, Zhang J, Wang R, Deng X, Li Y: **Siglec1 enhances inflammation through miR-1260-dependent degradation of IκBα in COPD**. *Exp Mol Pathol* 2020, **113**, 104398.

67. Lei H, Li H, Xie H, Du C, Xia Y, Tang W: **Role of MiR-215 in hirschsprung's disease pathogenesis by targeting SIGLEC-8**. *Cell Physiol Biochem* 2016, **40**:1646–1655.

68. Thakuri BKC, Zhang J, Zhao J, Nguyen LN, Nguyen LNT, Schank M, Khanal S, Dang X, Cao D, Lu Z, *et al.*: **HCV-associated exosomes upregulate RUNXOR and RUNX1 expressions to promote MDSC expansion and suppressive functions through STAT3–miR124 Axis**. *Cells* 2020, **9**:2715.