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Review

GlycoRNA in Cancer: Biogenesis, Mechanistic Links to Tumor Biology, and Clinical Translation

Xuhui Chen[1,2,#], Haoran Yuan[1,2,#], Jia Shi[1,2,#], Guangdong Zeng[1,2,3,#], Xinjuan Fan[1,2,4,*], Yunlong Wang[1,2,*]

[1]State Key Laboratory of Metabolic Dysregulation & Prevention and Treatment of Esophageal Cancer, Tianjian Laboratory of Advanced Biomedical Sciences, School of Convergence Medicine, Zhengzhou University, Zhengzhou, Henan 450052, P.R. China

[2]Henan Provincial Key Laboratory of Radiation Medicine, Department of Radiation Oncology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, P.R. China

[3]Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Diseases, the Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong 510655, P.R. China

[4]Department of Pathology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, P.R. China

[*]Corresponding author: Xinjuan Fan, fanxjuan@zzu.edu.cn; Yunlong Wang, wangylong@zzu.edu.cn

[#]These authors contributed equally.

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Abstract

GlycoRNAs are an emerging class of glycoconjugates in which selected small noncoding RNAs carry glycans and can be displayed at the cell surface. Their discovery has expanded the conceptual scope of glycobiology by introducing RNA as a previously unrecognized glycosylated scaffold and by placing it within the extracellular glycan-rich interface involved in cell communication, immune regulation, and disease-associated signaling. Increasing evidence suggests that glycoRNAs may participate in several biologically important processes, including extracellular signaling, immune modulation, and intercellular communication, and may have particular relevance to cancer biology. In this review, we summarize current knowledge of glycoRNA biogenesis, molecular features, detection strategies, and biological functions, with a focus on their potential roles in tumor progression and their translational promise as biomarkers and therapeutic targets.

1. Introduction

Aberrant glycosylation is a hallmark of cancer biology and influences diverse processes, including signaling, invasion, metastasis, immune escape, and therapeutic vulnerability [1-5]. Rather than serving merely as a downstream consequence of malignant transformation, glycan remodeling often actively shapes tumor behavior by reorganizing receptor function, extracellular-matrix interactions, and communication with stromal and immune compartments [1,3-5]. These insights have established cancer glycobiology as a major framework for understanding how tumors adapt to and exploit their microenvironment.

Against this background, the discovery of glycoRNAs expanded the conceptual scope of the field by identifying RNA as a third major glycosylated scaffold alongside proteins and lipids [6,7]. Initial studies showed that selected small noncoding RNAs can carry complex N-glycans enriched in sialic acid and fucose and can be detected on the outer cell surface [7,8]. This finding was unexpected both chemically and biologically, because it placed RNA within the extracellular glycan-rich interface that tumors commonly exploit for signaling, immune regulation, and intercellular communication.

This observation raises an important question for cancer biology: does glycoRNA actively contribute to tumor progression, or is it simply an unusual molecular feature of the cell surface? Evidence accumulated to date supports a genuine connection between glycoRNA and cancer. Direct studies have linked glycoRNA to tumor-cell growth in glioma models and to cancer-associated glycosylated small-RNA signatures in pancreatic cancer and small extracellular vesicles (sEVs), supporting its relevance to both tumor biology and biomarker development [9-11]. In parallel, glycoRNA has also been implicated in additional processes-including extracellular signaling through cell-surface ribonucleoprotein assemblies, lectin-mediated immune regulation, and glycan-dependent shielding of endogenous RNAs from innate immune sensors-that have been demonstrated mainly in non-cancer systems [12,13]. Although these mechanisms remain to be directly validated in tumor

models, they provide plausible frameworks through which glycoRNA may also influence cancer progression.

The key question is therefore no longer whether glycoRNAs exist, but how their biogenesis, molecular interactions, and compartment-specific distribution translate into selective advantages for tumors and into clinically informative readouts. This question has become increasingly tractable with the rapid development of methods for chemical interrogation, spatial imaging, subclass enrichment, and detection of glycoRNAs in extracellular vesicles and biofluids [10,11,14-17]. In this review, we examine glycoRNA through a cancer-centered lens, focusing on its molecular features, biogenesis, and detection, and then discuss its potential roles in tumor growth, immune regulation, angiogenesis-associated signaling, extracellular vesicle biology, and biomarker development. Figure 1 summarizes major milestones in the field, Table 1 compares representative detection approaches, Figure 2 outlines the principal mechanistic axes discussed here, and Table 2 summarizes key studies together with their evidentiary limitations.

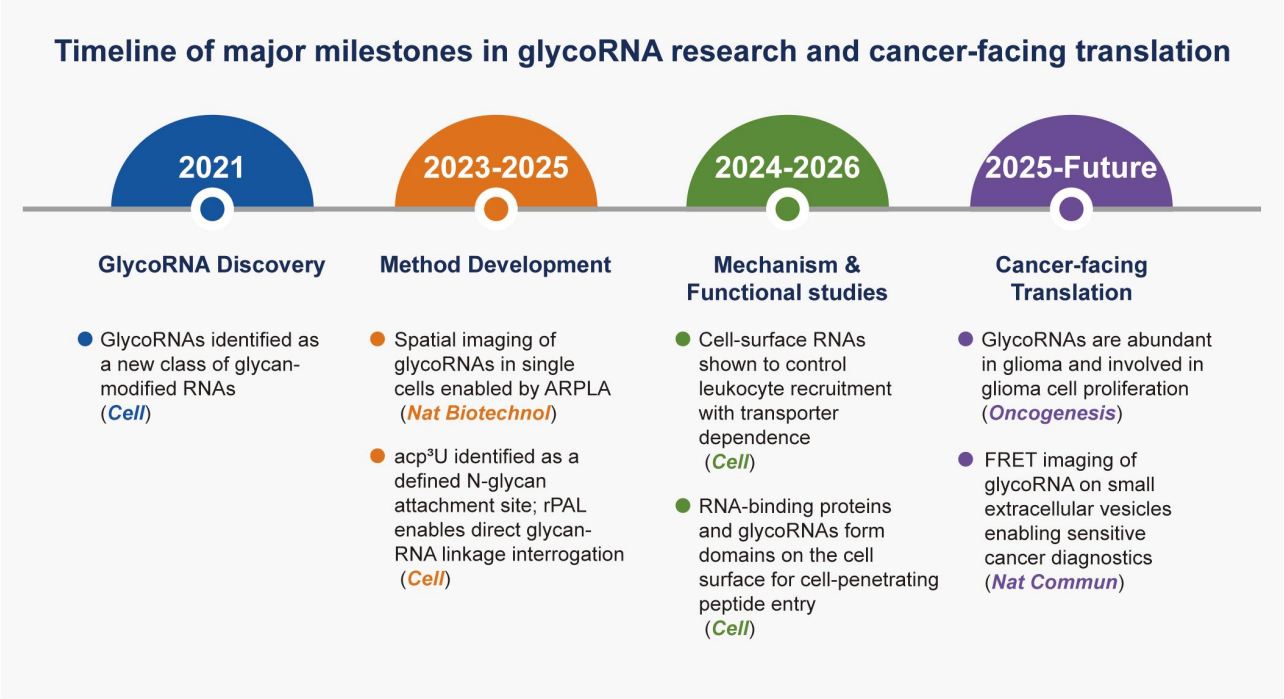

Figure 1. Timeline of major milestones in glycoRNA research and cancer-facing translation.

Timeline of major milestones in glycoRNA research and cancer-facing translation. The figure highlights the discovery of glycoRNAs in 2021, the development of detection methods from 2023 to 2025, ongoing mechanistic and functional studies from 2024 to 2026, and the emerging translation of glycoRNA research toward cancer biomarkers, liquid biopsy, therapeutic targeting, and clinical applications.

2. Definition and Biogenesis of GlycoRNA

2.1 Definition and Molecular Features

GlycoRNAs are small noncoding RNAs covalently modified with secretory-pathway N-glycans that are often highly sialylated and fucosylated and are frequently detected on the outer cell surface [7,8]. The foundational studies reported glycoRNA across multiple mammalian cell types and species, with production dependent on canonical N-glycan biosynthetic machinery and with evidence for interaction with Siglec-family receptors [7,8].

The currently described repertoire is enriched for canonical small RNA classes including snRNAs, snoRNAs, rRNA fragments, and tRNAs, although the exact composition depends on enrichment strategy and assay context. In glioma models, U2 and U4 snRNAs were highlighted as abundant glycoRNAs with complex sialylated and fucosylated glycan features [9].

2.2 Chemical Linkage and the Role of acp^{3}U

In glycoprotein biology, 'N-linked glycosylation' refers to a class of glycan modification defined by attachment through nitrogen and by its dependence on the canonical ER/Golgi N-glycosylation pathway [3,4]. In proteins, the glycan is classically attached to the amide nitrogen of asparagine residues, usually within the Asn-X-Ser/Thr consensus sequence. By extension, describing glycoRNAs as carrying N-linked glycans primarily refers to the glycan type and biosynthetic logic rather than, by itself, specifying the exact nucleotide-level attachment site on RNA.

Within glycoRNA, the currently best-validated attachment site is the modified RNA base acp^{3}U (3-(3-amino-3-carboxypropyl)uridine), a modified uridine bearing a carboxypropyl amino group. Using RNA-optimized periodate oxidation and aldehyde ligation (rPAL) together with mass spectrometry, Xie et al. identified acp^{3}U as a covalent attachment site for N-glycans on glycoRNA [16]. This finding did not redefine glycoRNA as a separate non-N-linked class; rather, it

provided molecular resolution for where an N-glycan can be installed on the RNA scaffold.

At present, acp^{3}U should be viewed as the leading experimentally supported glycoRNA attachment site rather than necessarily the only one. Given the biochemical diversity of small RNAs and the still limited depth of site-resolved mapping, additional attachment sites or context-dependent linkage chemistries cannot yet be excluded [16]. We therefore use cautious language throughout this review and treat attachment-site diversity in glycoRNA as an open question for future structural and mechanistic studies.

2.3 Trafficking Logic: Why GlycoRNA Biogenesis is Unusual

Canonical N-glycosylation occurs in the endoplasmic reticulum (ER) lumen and is elaborated in the Golgi, whereas RNA is synthesized in the nucleus and most small RNAs function in nuclear or cytosolic compartments. GlycoRNA biogenesis therefore requires that selected RNAs access secretory compartments or secretory-like enzymology [16,18]. A working model proposes formation of an acp^{3}U-linked handle, access of selected RNAs to the ER lumen, OST(oligosaccharyltransferase)-mediated transfer of N-glycans, and subsequent Golgi processing to yield mature surface glycoRNAs [16].

Consistent with this model, perturbation of N-glycan processing or OST function influences glycoRNA presentation. Work using Siglec11-Fc as a probe for surface sialylated glycoRNA showed dependence on catalytic OST isoforms and demonstrated that changing N-glycan biosynthesis or structure alters surface sialylated glycoRNA levels [8]. Even so, the trafficking route remains incompletely defined, and whether different tumor types use shared or distinct biogenetic routes is still unresolved.

2.4 Subcellular Locations Beyond the Plasma Membrane

Although surface localization is central to the current definition of glycoRNA, glycoRNAs are not restricted to the plasma membrane. They also appear in extracellular compartments, including extracellular vesicles [10,18,19]. Recent work suggests that glycoRNAs can be displayed on or associated with the exosomal membrane, extending the concept of RNA glycosylation from the cell surface to vesicle surfaces [10,19].

This distribution is particularly relevant in cancer because vesicle-surface molecules regulate vesicle recognition, uptake, and communication with recipient cells in the tumor microenvironment. Accordingly, vesicle-associated glycoRNAs may represent an additional layer of tumor intercellular signaling with implications for immune modulation, metastatic niche conditioning, and therapy response. Broader frameworks of cell-surface RNA biology increasingly situate glycoRNA within a wider cell-surface ribonucleoprotein landscape [18,20-22].

3. Detection and Enrichment Strategies for GlycoRNAs

Several experimentally validated approaches have now been developed for glycoRNA detection and enrichment. Because each method captures different aspects of glycoRNA biology, a comparative overview is provided in Table 1 before the methods are discussed individually.

Table 1. Comparative overview of representative glycoRNA detection and enrichment methods.

Method	Core principle	Typical input	Main strengths	Key limitations
Metabolic labeling	Unnatural sugar precursors enter glycan pathways and label glycoRNAs	Live cells	Live-cell compatibility; early discovery tool	Only for live-cell
rPAL	Periodate oxidation of sialic acids followed by aldehyde ligation	Purified RNA	High sensitivity, no live-cell limitation	Low specificity, lack for sequencing
SPCgRNA	Solid-phase capture enriches glycosylated RNAs	Purified RNA	No live-cell limitation, sequencing	Low specificity, difficult to quantitative
ARPLA	Aptamer–RNA probe proximity ligation	Fixed cells/tissues	Single-cell spatial resolution	Only for imaging and single sequencing
drFRET	Dual-recognition FRET detects glycoRNA on small extracellular vesicles	Serum or vesicle preparations	Simple operation for imaging	Only for imaging and single sequencing
Lectin/receptor assays	Lectins or glycan receptors capture/detect glycoRNA-associated glycans	Cells, tissues, biofluids	Simple operation	Usually semi-quantitative

3.1 Metabolic Labeling

Metabolic labeling was among the first strategies used to study glycoRNAs. In this approach, cells are fed unnatural sugar precursors such as Ac4ManNAz, which enter endogenous glycan biosynthetic pathways and become incorporated into glycans linked to RNA [7,23]. Using related approaches, Flynn et al. first identified sialylated small noncoding RNAs on the surface of living cells [7]. This strategy enables live-cell interrogation but requires careful control of labeling conditions and does not directly define RNA sequence identity or linkage chemistry.

3.2 Chemical Labeling and Mass Spectrometry

To define glycan-RNA linkage chemistry more directly, rPAL was developed to label sialylated glycoRNAs through mild oxidation of sialic acids followed by aldehyde derivatization and capture. When coupled to mass spectrometry, including SWATH-MS workflows, rPAL enabled identification of acp^3U as an attachment site for N-glycans on glycoRNA [16]. This method provides strong chemical evidence and high specificity but is technically demanding and less accessible for routine clinical deployment.

3.3 Solid-Phase Capture Enrichment

Solid-phase capture of glycoRNAs (SPCgRNA) was developed as an enrichment strategy prior to sequencing [11]. In this approach, total RNA is immobilized on a solid support and glycosylated RNA species are selectively released for downstream analysis. Li et al. used SPCgRNA to compare glycosylated small RNAs in nonmalignant and pancreatic cancer cells, revealing marked differences in glycosylated miRNA profiles [11]. The method improves sensitivity for discovery studies but may introduce enrichment bias and does not by itself prove functional causality for any specific glycoRNA species.

3.4 In Situ Imaging and Extracellular-Vesicle-Based Detection

More recently, glycoRNAs have been detected directly in intact cells and extracellular vesicles. ARPLA enables spatial visualization of glycoRNA at single-cell resolution and broadens the field beyond bulk biochemical enrichment [15]. Ren et al. further established a dual-recognition FRET (drFRET) strategy for sensitive detection of glycoRNAs on small extracellular vesicles from minute serum volumes [10]. These platforms are valuable for spatial biology and translational research, but they require specialized instrumentation and cross-laboratory standardization.

3.5 Lectin- and Receptor-Based Detection

Lectin- and receptor-based assays provide a simpler option for glycoRNA detection in cells and biofluids. Siglec11-Fc has been used to capture surface sialylated glycoRNAs, and lectin blotting combined with small-RNA blotting has been used to detect endogenous glycoRNAs in cells, tissues, and multiple human biofluids [8,17]. These approaches are practical and accessible, but they are generally semi-quantitative and typically do not directly identify the underlying RNA sequence.

3.6 Integrative Use of Orthogonal Methods

No single platform is sufficient for all glycoRNA questions. Metabolic labeling and rPAL are especially useful for mechanistic chemistry, SPCgRNA supports discovery-scale profiling, ARPLA adds spatial context, and drFRET and lectin-based assays broaden access to clinical materials. Future studies will benefit from combining orthogonal platforms, shared reference materials, and standardized reporting criteria for input type, glycan selectivity, throughput, and analytical sensitivity.

4. Potential Mechanisms of GlycoRNA in Cancer Biology

Current evidence linking glycoRNA to cancer-associated phenotypes is still uneven in both depth and model context. Direct functional support from cancer models is presently strongest for tumor cell growth and survival, particularly in glioma cells, and is further supported by cell-based studies showing altered glycosylated small-RNA profiles in pancreatic cancer models [9,11]. By contrast, several other proposed functions of glycoRNA-including roles in growth factor signaling, angiogenesis, adhesion, trafficking, and immune regulation-are supported mainly by mechanistic studies in non-cancer systems, such as endothelial models, inflammatory settings, and innate immune assays [12,13,21,24]. These observations are highly relevant to tumor biology and offer plausible frameworks for oncologic interpretation, but they should be distinguished from functions that have been directly demonstrated in cancer models. Accordingly, the following sections summarize glycoRNA-associated processes in cancer while indicating, where appropriate, whether the supporting evidence comes from tumor systems or from extrapolation of non-cancer studies.

4.1 GlycoRNA in Tumor Cell Growth and Survival

The clearest direct evidence for a functional role of glycoRNA in cancer currently comes from studies of tumor cell growth and survival. In glioma cell lines, including U87 and LN229, glycoRNAs were detected by metabolic labeling and Northern blotting, shown to carry complex sialylated and fucosylated glycans, and found to be enriched in small RNA species such as U2 and U4 [9]. In the same study, depletion of cell-surface glycoRNAs reduced glioma cell viability and proliferation at the tested time point [9]. These findings place tumor cell growth and survival among the few settings in which glycoRNA perturbation has been directly linked to a cancer-associated phenotype rather than inferred from non-cancer systems.

Additional support comes from pancreatic cancer cell models. SPCgRNA-based profiling identified differential glycosylated small-RNA signatures between MIA PaCa-2 cells and nonmalignant hTERT-HPNE cells, and pharmacologic inhibition of N-glycosylation was associated with reduced proliferation and migration together with increased apoptosis in MIA PaCa-2 cells [11]. However, these results do not yet demonstrate that a specific glycosylated RNA species is necessary and sufficient to drive the observed phenotype. Some of the reported effects may instead reflect broader disruption of glycosylation pathways rather than glycoRNA-selective mechanisms. Taken together, these findings support a cancer-relevant role for glycoRNA in tumor cell growth and survival, while also underscoring the need for more precise mechanistic dissection.

4.2 GlycoRNA in Growth Factor Signaling, Angiogenesis, and Microenvironmental Regulation

GlycoRNA may also participate in the regulation of extracellular signaling and tumor-associated microenvironmental responses. This possibility is especially relevant to angiogenesis, which is a fundamental enabling process in tumor progression [25,26]. A recent mechanistic study showed that cell-surface ribonucleoprotein (csRNP) clusters, including glycoRNA-containing assemblies, interface with heparan sulfate biology to regulate VEGF-A signaling [24]. In endothelial systems, these assemblies antagonized heparan-sulfate-mediated activation of ERK downstream of VEGF-A, whereas disruption of the VEGF-A165-RNA interaction enhanced ERK signaling and impaired vascular development [24].

Because VEGF-A/ERK signaling and heparan-sulfate-dependent growth factor regulation are central to tumor angiogenesis, these findings provide a plausible framework through which glycoRNA-associated csRNP organization might influence vascular remodeling in cancer. At present, however, this interpretation remains largely extrapolated from non-cancer systems. Direct validation in tumor angiogenesis models-for example, by testing whether glycoRNA-selective perturbation alters vascular density, perfusion, or hypoxia *in vivo*-has not yet been reported.

Related work further showed that heparan-sulfate-dependent cell-surface RNA complexes can recruit immune receptors and form ternary assemblies composed of RNA, RNA-binding proteins, and heparan sulfate [21]. This broader framework supports the idea that tumor-associated remodeling of the glycocalyx and the surface RNP landscape may influence microenvironmental signaling. Nevertheless, glycoRNA-specific causality in cancer remains to be established.

4.3 GlycoRNA in Adhesion, Trafficking, and Metastasis-Associated Processes

Another plausible area of relevance is adhesion, trafficking, and metastasis-associated biology. Adhesion, rolling, and extravasation are central steps in metastatic dissemination and are strongly shaped by glycan-lectin interactions [27,28]. In non-cancer inflammatory models, cell-surface RNAs were shown to be required for neutrophil recruitment, and neutrophil glycoRNAs were found to interact with P-selectin [13]. Although these findings were not obtained in tumor systems, they offer a useful mechanistic parallel because selectin-dependent adhesion is also widely implicated in metastatic spread.

At present, however, there is no direct evidence that tumor-cell glycoRNA drives metastatic adhesion or colonization. The most cancer-relevant support remains indirect. In the drFRET study, glycoRNAs on small extracellular vesicles (sEVs) were reported to interact with Siglec proteins and P-selectin, and these interactions contributed to vesicle internalization [10]. These observations provide a concrete link between glycoRNA and trafficking or uptake biology, but whether glycoRNA directly regulates tumor-cell rolling, organotropism, or metastatic colonization remains an open question.

4.4 GlycoRNA in Immune Regulation and Tumor Immune Evasion

Immune regulation represents another major functional dimension of glycoRNA biology. Current evidence suggests that glycoRNA may influence tumor-immune interactions through at least two nonexclusive mechanisms: Glycan-dependent shielding of endogenous RNA from innate immune sensing, and engagement of sialic-acid-binding receptors that modulate immune-cell behavior [12,29-31]. These mechanisms are highly relevant to cancer, where suppression of inflammatory signaling and remodeling of glyco-immune checkpoints are both well-recognized features of immune escape.

One of the clearest mechanistic advances in this area comes from the demonstration that RNA N-glycosylation can limit innate immune sensing [12]. Graziano et al. showed that de-N-glycosylation of cell-culture-derived and circulating glycoRNA triggered inflammatory responses, including type I interferons, in a TLR3- and TLR7-dependent manner [12]. Mechanistically, N-glycans masked the immunostimulatory acp^3U base, and genetic deletion of DTWD2 abrogated innate activation by de-glycosylated small RNAs and apoptotic cells [12]. These findings provide strong causal support for glycan-dependent immune shielding, although the experiments were not performed in tumor models. In oncology, this mechanism is of particular interest because tumors frequently suppress nucleic-acid sensing and exploit immune-silent clearance of dying cells [32,33]. GlycoRNA may therefore represent an additional layer through which tumors restrain inflammatory responses, especially in settings associated with abundant cell death, such as chemotherapy or radiotherapy. However, this possibility remains a working hypothesis until tumor-specific studies directly determine whether glycoRNA unmasking can enhance antitumor immunity without causing unacceptable systemic inflammation.

GlycoRNA may also participate in immune regulation through the broader Siglec-sialic-acid immune axis, in which sialylated glycans engage Siglec receptors on immune cells to influence immune suppression, activation, and intercellular communication [29-31]. This axis is increasingly recognized as an important mechanism of tumor immune evasion driven by hypersialylation. GlycoRNAs are relevant in this context because they also carry sialylated glycans, and several studies have shown that glycoRNAs can bind sialic-acid receptors such as Siglec-5, Siglec-11, and Siglec-14 [7,8,10]. The dominant receptor interactions may vary according to the cellular or extracellular source of glycoRNA, suggesting context-dependent engagement. In addition, both alpha2-3- and alpha2-6-linked sialic acids have been detected on glycoRNAs [8], and these linkage types are known to differ in receptor preference and functional consequences [30,31]. However, whether distinct glycoRNA sialylation patterns generate different immunological effects remains unknown, and direct evidence in cancer models is still lacking.

The translational relevance of this pathway is underscored by the ongoing development of strategies targeting the Siglec-sialic-acid checkpoint, including antibodies against Siglec receptors and approaches that disrupt sialic-acid-dependent inhibitory signaling [30,31,34]. Within this framework, glycoRNA may represent an additional class of sialylated ligands contributing to immune suppression in the tumor microenvironment. Although its relative contribution remains to be defined, glycoRNA-directed analyses may help refine the mechanistic basis and biomarker potential of Siglec-targeted therapies. This pathway is also conceptually distinct from PD-1/PD-L1 blockade. Rather than duplicating existing immune checkpoint inhibitors, targeting glycoRNA-associated sialic-acid signaling would more likely provide a complementary strategy by modulating glycan-dependent immune regulation. Whether glycoRNA-rich tumors are particularly suitable for such combination approaches remains an open question.

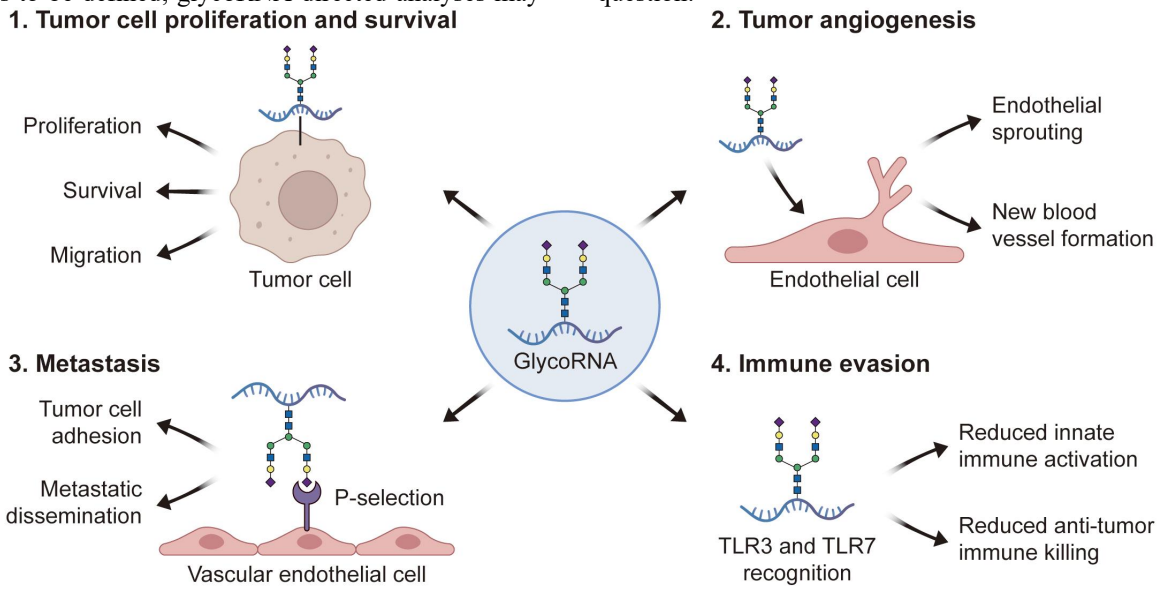

Figure 2. Proposed functions of glycoRNA in tumors.

Direct cancer evidence is presently strongest for tumor-cell proliferation in glioma models and for sEV-associated glycoRNA detection/uptake, whereas roles in angiogenesis, metastatic adhesion, and immune escape remain biologically plausible but are supported mainly by non-cancer or proof-of-principle systems.

5. Clinical Translation: Diagnostics, Prognostics, and Therapeutic Targeting

5.1 sEV GlycoRNA Liquid Biopsy

A dual-recognition FRET strategy enabled sensitive detection of N-acetylneuraminic-acid-modified RNAs on small extracellular vesicles from minimal biofluid input [10]. In a 100-sample cohort comprising six cancer types and non-cancer controls, sEV glycoRNA profiles distinguished cancer from non-cancer and showed encouraging performance in cancer-type classification [10]. These data support the translational promise of sEV glycoRNA, but the study is still best viewed as a pilot rather than a clinically mature assay.

Several practical barriers need to be addressed before clinical adoption. These include pre-analytical variation in vesicle isolation and storage, sample stability, batch effects, normalization strategies, inter-laboratory reproducibility, instrument accessibility, and the cost and technical expertise required for drFRET implementation. More broadly, multi-center cohorts with stage-stratified analysis, independent validation sets, and comparison against existing liquid-biopsy standards will be essential.

5.2 Native GlycoRNA Detection in Tissues and Biofluids

Lectin-based profiling has expanded the plausible specimen types for glycoRNA biomarker development by reporting endogenous glycoRNAs in tissues, cultured cells, and multiple human biofluids including plasma and urine [17]. These observations suggest that both vesicle-associated and free glycoRNA pools may eventually become analytically relevant in oncology, although disease specificity and background variation across inflammatory or benign conditions remain unresolved.

The current literature also hints at cancer-type-specific glycoRNA profiles, but the evidence remains preliminary. In glioma, the strongest published signal emphasizes cell-surface enrichment of U2- and U4-associated glycoRNAs linked to growth phenotypes [9]. In contrast, pancreatic cancer studies have mainly highlighted differential glycosylated miRNA profiles identified by enrichment and sequencing approaches [11]. These differences are intriguing and may reflect tumor-type-specific repertoire usage, but cross-platform comparisons and larger tumor panels are needed before any stable cancer-type signature can be defined.

5.3 Therapeutic Targeting Strategies

Because direct glycoRNA therapeutics remain early, current opportunities are best framed as strategy classes rather than near-term drugs. One approach is to interfere with glycoRNA biogenesis or processing through

modulation of OST activity or N-glycan-processing pathways, both of which influence surface sialylated glycoRNA levels [8]. However, these pathways are also essential for normal protein N-glycosylation, so on-target toxicity in healthy tissues is a major concern and a central translational barrier.

A second approach is to block glycoRNA-lectin interactions within the Siglec-sialic-acid axis [29-31]. Here, glycoRNA should be viewed as one potentially important sialylated scaffold among several. Such interventions may complement existing immune checkpoint therapy, including PD-1/PD-L1 blockade, by reducing glycan-dependent inhibitory signaling that is not directly addressed by conventional checkpoint inhibitors.

A third concept is therapeutic unmasking of immunostimulatory RNA features. Because removal of N-glycans can expose acp^3U and trigger TLR3/TLR7-dependent type I interferon responses [12], a cancer-directed version of this strategy could in principle convert glycoRNA from an immune-shielded to an immune-stimulatory state. The challenge is selectivity: Tumor-targeted delivery systems such as nanoparticles, antibody-guided delivery platforms, intratumoral formulations, or other locally restricted approaches would likely be required to avoid systemic immune activation.

Finally, targeting exosomal glycoRNA biology, including sorting, transfer, and uptake, may influence tumor-stroma communication and drug-resistance pathways [10,19]. In parallel, glycoRNA-based liquid-biopsy signatures may eventually prove useful for treatment monitoring if they can be validated across stage, therapy type, and longitudinal sampling.

Table 2. Representative glycoRNA studies relevant to cancer biology and translation.

Year	Cancer context	Model	Main findings	Evidence level / key limitations	Reference
2021	Multiple cell types including cancer lines	Cell lines; *in vivo* detection contexts	Established glycoRNAs as sialylated N-glycan-modified small RNAs enriched at the cell surface; showed Siglec interactions	Foundational discovery; limited functional causality	Flynn et al. [7]
2023	Pancreatic cancer	Cell lines; enrichment + RNA-seq; functional assays	SPCgRNA identified differential glycosylated small-RNA profiles between MIA PaCa-2 and hTERT-HPNE cells; NGI-1/B4GALT1 linked to proliferation/migration-associated phenotypes	Cancer-relevant but mainly *in vitro*; specific glycoRNA causality unresolved	Li et al. [11]
2024	Not cancer-specific	Single-cell imaging platform	ARPLA enabled spatial imaging of glycoRNA in single cells	Methodological advance; limited molecular identity/glycoform mapping	Ma et al. [15]
2024	Multiple contexts	Cell lines; mass spectrometry	Identified acp^3U as an N-glycan attachment site and introduced rPAL-based chemistry	Strong chemical evidence; trafficking model still partly inferential	Xie et al. [16]
2024	Not cancer-specific	Mouse neutrophils; *in vivo* inflammation models	Cell-surface RNAs including glycoRNAs contributed to neutrophil recruitment and P-selectin recognition	Useful analogy for metastasis biology; direct tumor relevance untested	Zhang et al. [13]
2025	Glioma	Glioma cell lines; sequencing; phenotyping	Glioma cells were enriched in glycoRNAs; depletion of cell-surface glycoRNAs reduced viability/proliferation	Strongest direct cancer functional evidence so far; no *in vivo* tumor validation	Xin et al. [9]
2025	Not cancer-specific	Human/mouse cells; innate immune assays; *in vivo* injection	N-glycans shielded acp^3U and prevented TLR3/TLR7 sensing; de-glycosylation triggered type I IFN responses	Strong mechanistic evidence for immune shielding; tumor translation untested	Graziano et al. [12]
2025	Breast, pancreatic, liver, intestinal/colorectal, lung, cervical	Clinical serum cohort + cancer cell lines	drFRET detected sEV glycoRNAs from 10 uL serum; pilot cohort showed promising discrimination and uptake-related Siglec/P-selectin interactions	Pilot translational evidence; standardization and multi-center validation needed	Ren et al. [10]
2025	Cancer cell line	Cell systems; exosomes	Exosomal glycoRNAs were transferable and linked to exosome biology	Important EV biology; direct cancer-specific implications remain inferential	Sharma et al. [19]
2026	Not cancer-specific	Tissue/cell profiling; biofluids	Lectin-based detection of native glycoRNAs in tissues and multiple biofluids	Broad analytical expansion; oncology specificity remains undefined	Li et al. [17]
2026	Not cancer-specific	Endothelial / signaling systems	GlycoRNA-containing csRNP assemblies regulated VEGF-A signaling through heparan sulfate	Mechanistically important; direct tumor angiogenesis validation lacking	Chai et al. [24]

6. Limitations

Despite rapid progress, several limitations constrain both interpretation and translation. First, glycoRNA biogenesis remains incompletely mapped. Although acp^{3}U is now a validated attachment site, the full diversity of attachment chemistries, the rules governing RNA substrate selection, and the route by which selected RNAs access secretory compartments remain unresolved [16,18].

Second, the field still lacks enough cancer-specific causal evidence. At present, the strongest direct cancer functional data are concentrated in glioma cell lines, with pancreatic cancer studies adding supportive but still indirect evidence [9,11]. Many additional mechanisms discussed in the literature-including angiogenesis-related signaling, selectin-linked trafficking, and immune-silent efferocytosis-are mechanistically compelling but derive largely from non-cancer systems [12,13,24]. This evidence gap should be made explicit to avoid overstating what is already established in oncology.

Third, analytical translation faces substantial barriers. For sEV glycoRNA assays, pre-analytical variables such as vesicle isolation methods, storage conditions, hemolysis, sample stability, batch effects, normalization, and instrument-dependent sensitivity may all influence results [10]. Cross-platform harmonization among rPAL, enrichment-based sequencing, lectin-based detection, and FRET-based assays will also require shared reference materials and reporting standards.

Fourth, the structural and biological heterogeneity of glycoRNA remains underdefined. We still do not know which glycoRNA species are dominant across tumor types, whether the most functionally important species reside on cells or vesicles, or how linkage composition, glycan branching, and local scaffold context affect receptor binding and downstream signaling [10,16,30].

Finally, therapeutic translation must contend with specificity and safety. Strategies that inhibit OST or broader N-glycan processing risk substantial on-target toxicity because these pathways are essential in normal tissues [8]. Conversely, strategies that unmask glycoRNA may trigger potent innate immune activation [12]. Progress will therefore depend on tumor-selective targeting, carefully staged preclinical modeling, and biomarkers that distinguish desirable local immune activation from systemic inflammatory risk.

7. Conclusions and Discussion

GlycoRNAs have moved from a surprising biochemical observation to a coherent interface linking RNA biology, glycobiology, and cancer-relevant extracellular signaling. The field now has a stronger chemical foundation through identification of acp^{3}U-linked glycosylation, multiple orthogonal detection platforms, and early but still limited evidence that glycoRNAs may shape tumor-cell growth, vesicle biology, and immune regulation [9,10,12,15-17].

The most important next step is not simply to accumulate more descriptive datasets, but to build a firmer evidence hierarchy. Future work should prioritize glycoRNA-selective perturbation strategies, *in vivo* tumor models that distinguish cancer-validated functions from extrapolated ones, cross-platform analytical standards, and prospective cohorts that test biomarker performance under clinically realistic conditions. In parallel, integration with glyco-immune checkpoint biology may clarify whether glycoRNA-aware biomarkers can help stratify response or resistance to emerging Siglec-directed approaches and to existing immunotherapies [29-31,34,35].

Overall, glycoRNA is a promising but still early field. Its value for cancer research will depend on maintaining conceptual precision, calibrating claims to the strength of available evidence, and pairing technological innovation with rigorous tumor-focused validation.

Abbreviations

Abbreviation	Definition
acp^{3}U	3-(3-amino-3-carboxypropyl)uridine
ARPLA	Spatial imaging platform for glycoRNA
csRNP	Cell-surface ribonucleoprotein
drFRET	Dual-recognition fluorescence resonance energy transfer
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
EV	Extracellular vesicle
IFN	Interferon
OST	Oligosaccharyltransferase
rPAL	RNA-optimized periodate oxidation and aldehyde labeling
sEV	Small extracellular vesicle
Siglec	Sialic-acid-binding immunoglobulin-like lectin
SPCgRNA	Solid-phase capture of glycoRNAs
TLR	Toll-like receptor
VEGF	Vascular endothelial growth factor

Author Contributions

YL W, XH C, HR Y, J S, and GD Z performed the literature review, organized the evidence, and drafted the manuscript. XJ F contributed to manuscript revision, conceptual refinement, and figure planning. YL W conceived and supervised the study. All authors reviewed the manuscript, approved the final version, and take responsibility for the integrity of the work.

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Competing Interests

No potential conflict of interest was reported by the authors.

Generative AI Statement

The authors used generative AI assistance only for language polishing and take full responsibility for the manuscript.

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