Current Research in Pharmacology and Drug Discovery 3 (2022) 100079

Contents lists available at ScienceDirect

Current Research in Pharmacology and Drug Discovery

journal homepage: www.journals.elsevier.com/current-research-in-pharmacology-and-drug-discovery

Abnormal sialylation and fucosylation of saliva glycoproteins: Characteristics of lung cancer-specific biomarkers

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ARTICLE INFO

Keywords:
Glycoenzyme
Glycosylation
Saliva
Tumor biomarker
Mass spectrometry

ABSTRACT

Dysregulated surface glycoproteins play an important role in tumor cell proliferation and progression. Abnormal glycosylation of these glycoproteins may activate tumor signal transduction and lead to tumor development. The tumor microenvironment alters its molecular composition, some of which regulate protein glycosylation biosynthesis. The glycosylation of saliva proteins in lung cancer patients is different from healthy controls, in which the glycans of cancer patients are highly sialylated and hyperfucosylated. Most studies have shown that O-glycans from cancer are truncated O-glycans, while N-glycans contain fucoses and sialic acids. Because glycosylation analysis is challenging, there are few reports on how glycosylation of saliva proteins is related to the occurrence or progression of lung cancer. In this review, we discussed glycoenzymes involved in protein glycosylation, their changes in tumor microenvironment, potential tumor biomarkers present in body fluids, and abnormal glycosylation of saliva or lung glycoproteins. We further explored the effect of glycosylation changes on tumor signal transduction, and emphasized the role of receptor tyrosine kinases in tumorigenesis and metastasis.

Statement of significance

Fucosylation and sialylation of saliva glycoproteins distinguish lung cancer from cancer-free patients or healthy controls. Understanding the biosynthesis of fucosylation and sialylation of saliva glycoproteins paves the way for the diagnosis of lung cancer. Inhibition of oncogenic drivers that regulate fucosyltransferases and sialyltransferases may be the key to the treatment of lung cancer.

Rationale

Lung cancer is the leading cause of cancer death. This is mainly due to the lack of reliable biomarkers to diagnose lung cancer at an early stage. Previous studies have shown that body fluids such as saliva, serum, BALF, and urine are good clinical resources for the discovery of tumor markers. Our on-going work shows that the protein fucosylation of lung cancer saliva has unique characteristics compared with healthy controls. This may be used as a diagnosis marker in lung cancer. Therefore, it is of great importance to summarize the latest advances in the research of glycosylation in lung cancer saliva, and how these studies can promote the discovery of early lung cancer markers.

2. Glycosylation in disease and health

As one of the most common post-translationally modifications, protein glycosylation is related to the biological and physiological state of cells and organisms. Changes in glycosylation have been found in many diseases including cancers, influenza A virus (N3N2) (Wan et al., 2019), COVID-19 spike glycoprotein (Shajahan et al., 2020; Wang et al., 2021), cardiovascular (Gudelj and Lauc, 2018; Yang et al., 2018a) and neurodegenerative disease (Frenkel-Pinter et al., 2017; Xu et al., 2021). Abnormal glycosylation occurs in tumorigenesis and tumor progression (Hakomori, 1989, 2002), usually accompanied by branching and fucosylation changes of N-glycans, regulated mucin and its truncated O-glycans, and altered expression of sialic acids (Varki et al., 2015). Therefore, tumor-specific glycosylation (TSG) is often used as a diagnostic or prognostic biomarker. For example, α-fetoprotein (AFP) is a biomarker

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https://doi.org/10.1016/j.crphar.2021.100079
Received 25 October 2021; Received in revised form 31 October 2021; Accepted 15 December 2021

Abbreviation

BAG	benzyl N-acetyl-α-D-galactosamide
CA	cancer antigen
CMP	cytidine monophosphate
FUT8	α1,6-fucosyltransferase
GalNAc	N-acetylgalactosamine
GALNTs	N-acetylgalactosaminyltransferases
GFRA1	GDNF receptor alpha-1
GlcNAc	N-acetylglucosamine
HILIC	hydrophilic interaction liquid chromatography
LADC	lung adenocarcinoma
MS	mass spectrometry
NSCLC	non-small cell lung cancer
PSA	prostate-specific antigen
RTK	receptor tyrosine kinase
SCLC	small cell lung cancer
TSG	tumor-specific glycosylation
TUN	tunicamycin

for the diagnosis of hepatocellular cancer, in which AFP-L3 carrying core fucosylated N-glycans greatly increases the AUC (Area under the receiver operating characteristic curve) from 86.7% to 95.4% (Yin et al., 2014). AFP is also used to help detect and diagnose testicular and ovarian cancers (de la Motte Rouge et al., 2016). Most tumor markers approved by Food and Drug Administration (FDA) are glycoproteins, such as cancer antigen 125 (CA 125), AFP, immunoglobulin, neuron-specific enolase (NSE), and prostate specific antigen (PSA) (Yang and Wang, 2017). This is based on the fact that glycoenzymes (glycosyltransferases and glycosidases) are regulated in the microenvironment as diseases occur and progress (Costa et al., 2020; Chugh et al., 2015). Mutations or alterations in glycoenzyme amino acids regulate the glycosylation of protein substrates, leading to changes in the function of the protein cascade in the cell. Thus, the analysis of TSG and their glycoenzymes may be the potential biomarkers for diagnosis and prognosis.

3. Glycoenzymes in the tumor microenvironment

Glycoenzyme refers to proteins involved in the biosynthesis of glycans and the transfer of oligosaccharide precursor to proteins or lipids. Common glycoenzymes include glycosyltransferases and glycosidases. The former synthesizes a variety of glycans, and the latter hydrolyzes monosaccharides from glycans or proteins. Studies have shown that changes in glycosyltransferase levels are found in the tumor tissue or serum of patients with gastric cancer (Bhat et al., 2018). In prostate cancer patients, proteomic analysis of tissues and serum found that α1,2-fucosyltransferase (FUT1) increased, leading to α1,2-fucosylation of non-core-GlcNAc in PSA (Dwek et al., 2010). α1,6-fucosyltransferase (FUT8) gene is also significantly elevated in prostate cancer (PCa) tissues, but the change in protein expression is negligible (Llop et al., 2016). Meanwhile, the sialyltransferases that synthesize α2,6-linked or α2,3-linked sialic acids are differentially regulated in the tumor tissues and serum of cancer patients (e.g., ST6Gal1 is upregulated in many cancers) (Garnham et al., 2019). Abnormal sialylation is usually associated with poor prognosis and metastasis (Vajaria et al., 2016a). The bisected GlcNAc on N-glycans often increases in tumor cells, as do branched N-glycans. The bisected GlcNAc is known to associate with cell growth control and tumor progression (Miwa et al., 2012). Indeed, the formation of bisected GlcNAc can effectively inhibit growth factor signaling and delay the progression of breast tumors (Song et al., 2010). Generally speaking, the higher the expression of glycoenzyme, the higher the degree of glycosylation.

The main glycoenzymes shown in Table 1 are derived from at least one of these organs, such as salivary glands, oral mucosa, bronchi, lungs, and stomach. Analysis of cancer cells or organs shows that aberrant glycosylation is associated with tumorigenesis, progression and metastasis (such as sialylation, fucosylation, and bisected GlcNAc N-glycans). Tumor tissues, serum and other human body fluids usually have high expression of sialylation, with specific linkages, namely α2,3-linked and/or α2,6-linked sialic acids (Dorsett et al., 2021). Different sialyltransferases are responsible for the synthesis of linkages of these sialic acids. ST6Gal1 or ST6Gal2 can catalyze the transfer of sialic acid monosaccharides from CMP-sialic acid to galactose-containing substrates, thereby forming α2,6-linked sialic acid (Vajaria et al., 2016b). According to the *Human Protein Atlas*, the protein expression of ST6Gal1 is present in the bronchi, lung and stomach, while ST6Gal2 expression is less in the lung and stomach (Table 1). The α2,3-linked sialic acid of N-glycans and O-glycans is also widely expressed in cancer. The synthesis of α2,3 linkage can be achieved by six different ST3Gal glycotransferases, all of which are highly expressed in a few human organs. ST3Gal1 is abundant in salivary glands, oral mucosa, bronchus, lung, and stomach (Kono et al., 1997a), and the protein encoded by ST3Gal1 is significantly enhanced in ovarian cancer (Wu et al., 2018). ST3Gal1 is an enzyme for synthesizing mucin-type core 1 O-glycan, especially sialyl Tn antigen (sTn) (Burchell et al., 1999; Yeo et al., 2019). It is upregulated in ovarian cancer tissue and cell lines; overexpression of ST3Gal1 can promote the growth, migration, and invasion of ovarian cancer cells (Wu et al., 2018). Vasorin (VASN) protein is the substrate of ST3Gal1, which regulates TGF-β1-mediated tumor angiogenesis and progression through α2,3 sialic acid on core 1 O-glycan (Yeo et al., 2019). Other known ST3Gal1 substrates, such as AXL (Pietrobono et al., 2020) and GFRA1 (Fan et al., 2018), were studied because of their preference for sialylation.

Other ST3Gals ($s = 2,3,4,5,6$) proteins are also present in the bronchi, lung, stomach, and salivary gland, but they form different cancer-specific glycosylation (Table 1). ST3Gal2 is mainly involved in the terminal sialylation of gangliosides (GD1a and GD1b), glycolipids, N-glycans and mucin-type O-glycans. The expression of ST3Gal2 can be used as a tumor predictor and prognostic marker (Aloia et al., 2015). The proteins encoded by ST3Gal3 or ST3Gal6 are found in salivary glands, oral mucosa and lung. Deleting ST3Gal3 or ST3Gal6 genes will reduce cell proliferation and colony formation, while knocking out ST3Gal4 has the opposite effect (Qi et al., 2020). These enzymes have different preferences for protein substrates, for example, ST3Gal4 sialylation for β1 integrin, ST3Gal6 for EGFR, ST3Gal3 for GD1a. Similar to ST3Gal3, ST3Gal5 is a ganglioside biosynthetic enzyme whose mutation can lead to neurocutaneous disease with altered glycolipid glycosylation (Boccuto et al., 2014). The loss of ST3Gal5 activity will reduce the production and diversity of brain gangliosides, and indirectly impact nerve cell function (Schnaar, 1991). Several miRNAs may directly target the expression of ST3Gal5, leading to tumor cell proliferation and metastasis in the progression of hepatocellular carcinoma (Cai et al., 2017).

Fucosyltransferases play an important role in regulating tumor cell morphology, proliferation, adhesion, migration and tumorigenicity. There are 13 types of fucosyltransferases (FUTs) in the human genome. The FUTs for N-glycan fucosylation are located in the Golgi apparatus, while the O-FUTs are usually located in the endoplasmic reticulum (ER) (Shan et al., 2019). The proteins of FUT2, FUT4, FUT6, FUT8, FUT10, FUT11 and POFUT1 are detected in salivary glands, oral mucosa, bronchus, lung and stomach (Table 1). These FUTs can form various fucose linkages. For example, FUT2 synthesizes α1,2-linked fucose at Globo H (Lai et al., 2019), while FUT4, FUT6 and FUT11 form α1,3-linked fucosylated *Lewis* X (CD15) (Jassam et al., 2019), and sialyl-*Lewis* X (Liu et al., 2011; Mollicone and Oriol, 2014). FUT8 is highly abundant in salivary glands, oral mucosa, bronchi, lung, and stomach. Its overexpression is related to tumor cell proliferation and progression (Chen et al., 2013). The enzyme catalyzes the addition of α1,6-fucose to a core GlcNAc residue. Since FUT8 can globally modify cell surface antigens, receptors and adhesion molecules, abnormal core fucosylation may lead to the malignancy of cancer cells and their ability to invade and metastasize. For

Table 1
Glycoenzymes present in human saliva. The glycoenzymes are responsible for protein sialylation (ST3Gal1/2/3/4/5/6, STGalNAc1/6), core fucosylation (FUT8), Gal or GlcNAc fucosylation (FUT2/4/6/10/11), bisecting GlcNAc (MGAT3), GlcNAc to high mannose (MGAT5), and Ser/Thr fucosylation (POFUT1). Glycoenzyme expression is based on data from *the Human Protein Atlas*.

Glycoenzyme	Gene	Substrate	Linkage	Protein expression	Cancer-specific glycosylation change	Cancer types	Phenotype	Reference
β-Galactoside α-2,3-Sialyltransferase 1	ST3Gal1	Gal	α2,3	Salivary gland, oral mucosa, bronchus, lung, stomach	Core 1 O-glycan, Sialyl Tn antigen, mucin	Breast, NSCLC, oral cancer	Reduced mRNA levels in lung cancer	Kono M. et al., 1997 (Kono et al., 1997b)
β-Galactoside α-2,3-Sialyltransferase 2	ST3Gal2	Gal	α2,3	Bronchus, lung, stomach	N-glycan and mucin-type O-glycan; GM, glycolipid; synthesis of GD1a and GT1b	Breast, lung, gastric cancer	Predictive and prognostic marker	Aloia A. et al., 2015 (Aloia et al., 2015)
β-Galactoside α-2,3-Sialyltransferase 3	ST3Gal3	Gal	α2,3	Salivary gland, oral mucosa, bronchus, lung, stomach	sLe^x expression; E-cadherin, claudin-1, β1 Integrin (enhanced)	NSCLC, Breast cancer, OSCC	ST3Gal3 knockout decreasing cell proliferation and colony formation	Qi F. et al., 2020 (Qi et al., 2020)
β-Galactoside α-2,3-Sialyltransferase 4	ST3Gal4	Gal	α2,3	Bronchus, salivary gland, stomach	sLe^x expression; E-cadherin, claudin-1, β1 Integrin (suppressed)	Gastric, lung cancer	ST3Gal4 knockout increasing cell proliferation and colony formation	Qi F. et al., 2020 (Qi et al., 2020)
β-Galactoside α-2,3-Sialyltransferase 5	ST3Gal5	Gal	α2,3	Lung, oral mucosa, stomach	miR-26a, miR-548I and miR-34a through ST3Gal5; ganglioside biosynthesis	Hepatocellular carcinoma	Cancer progression	Cai H. et al., 2017 (Cai et al., 2017)
β-Galactoside α-2,3-Sialyltransferase 6	ST3Gal6	Gal	α2,3	Salivary gland, oral mucosa, bronchus, lung, stomach	E-cadherin, claudin-1, β1 Integrin (enhanced); EGFR (suppressed)	Colorectal, bladder, lung cancer	ST3Gal5 knockou6 decreasing cell proliferation and colony formation; homing and survival in multiple myeloma	Qi F. et al., 2020[32]; Glavey S.V. et al., 2014 (Glavey et al., 2014)
β-Galactoside α-2,6-Sialyltransferase 1	ST6Gal1	Gal	α2,6	Bronchus, lung, stomach	Sialylation, CD75s and ST2H formation	NSCLC, breast, colorectal cancer	Poor prognosis, invasiveness and tumorigenicity, metastasis	Dorsett K.A. et al., 2021[23]; Vajaria B.N. et al., 2016 (Vajaria et al., 2016b)
α-N-acetylgalactosaminide α-2,6-sialyltransferase 1	ST6GalNAc1	GalNAc	α2,6	Salivary gland, bronchus, lung, stomach	O-glycan, sTn, sT in MUC1	NSCLC, prostate, breast, gastric, colon cancer	Tumor progression, cell proliferation, migration	Takamochi K. et al., 2016 (Takamochi et al., 2016)
α-N-acetylgalactosaminide α-2,6-sialyltransferase 6	ST6GalNAc6	GalNAc	α2,6	Salivary gland, bronchus, lung, stomach	Branched type disialyl structure to GalNAc or GlcNAc with a terminal 2,3-linked sialic acid on Gal (disialyl *Lewis*^{A}); GD1a and GT1b	Colon, pancreatic cancer	Tumor growth and proliferation	Furukawa K. et al., 2014 (Furukawa et al., 2014)
α−1,6-Fucosyltransferase 8	FUT8	Core GlcNAc	α1,6	Salivary gland, oral mucosa, bronchus, lung, stomach	N-linked fucosylation	NSCLC, Breast, Prostate cancer	Increased tumor metastasis, higher reoccurrence, and poorer survival	Agrawal P. et al., 2017 (Agrawal et al., 2017)
α-1,2-L-fucosyltransferase 2	FUT2	Fucα1-2Galβ1-3(sialylα2-6)GalNAc	α1,2	Salivary gland, oral mucosa, bronchus, lung, stomach	Globo H	Breast cancer, NSCLC	Cell proliferation	Lai T.Y. *et al.*, 2019 (Lai et al., 2019)
α-1,3-fucosyltransferase 11	FUT11	Branch GlcNAc	α1,3	Salivary gland, oral mucosa, bronchus, lung, stomach	Innermost GlcNAc of N-glycan	Pancreatic cancer, renal cell carcinoma	Colony, progression	Zodro E. et al., 2014 (Jassam et al., 2019)
α-1,3-fucosyltransferase 4	FUT4	Branch GlcNAc	α1,3	Stomach	*Lewis* X (CD15)	NSCLC, Hodgkin's lymphoma, breast cancer	Promoting tumor invasion and migration	Jassam S.A. et al., 2019 (Jassam et al., 2019)
α-1,3-fucosyltransferase 6	FUT6	Branch GlcNAc	α1,3		Sialyl-*Lewis* X			

(*continued on next page*)

Table 1 (*continued*)

Glycoenzyme	Gene	Substrate	Linkage	Protein expression	Cancer-specific glycosylation change	Cancer types	Phenotype	Reference
				Oral mucosa, bronchus, lung, stomach		NSCLC, gastrointestinal carcinoma	Cancer metastasis, suppressing EGFR dimerization and activation	Liu Y.C. et al., 2011 (Liu et al., 2011)
α-1,3-fucosyltransferase 10	FUT10	Branch GlcNAc	α1,3	Salivary gland, oral mucosa, bronchus, lung, stomach	Usually in plant but also found in human N-glycan	Gastrointestinal cancer	Tumor cell survival, proliferation and migration	Mollicone R. et al., 2014 (Mollicone et al., 2014)
β-1,4-mannosyl-glycoprotein 4-β-N-acetylglucosaminyltransferase	MGAT3	Bisecting GlcNAc	β1,4	Salivary gland, oral mucosa, bronchus, lung, stomach	Bisecting GlcNAc N-glycan (usually core-fucose)	Hepatoblastoma, lung cancer	Cell growth control, tumor progression and metastasis	Miwa H.E. et al., 2012 (Miwa et al., 2012)
β-1,6-mannosyl-glycoprotein 6-β-N-acetylglucosaminyltransferase	MGAT5	Mannose	β1,6	Salivary gland, oral mucosa, bronchus, lung, stomach	Addition of GlcNAc to α-mannose	Hepatocellular carcinoma, colorectal cancer, NSCLC	Cancer growth and metastasis	Dosaka-Akita H. et al., 2004 (Dosaka-Akita et al., 2004)
GDP-fucose protein O-fucosyltransferase 1	POFUT1	Ser/Thr	α	Salivary gland, oral mucosa, bronchus, lung, stomach	Addition of Fucose to Ser or Thr	Colorectal, lung cancer	Cancer progression	Du Y. et al., 2018 (Du et al., 2018)

example, FUT8 overexpression leads to the development of castration-resistant prostate cancer cells (Yang and Wang, 2017; Höti et al., 2018; Tu et al., 2017). It also contributes to tumor size, lymph node metastasis and advanced stage (Ito et al., 2003), while silencing FUT8 can reduce lung cancer metastasis and inhibit tumor growth (Agrawal et al., 2017). POFUT1 is involved in the transfer of O-fucose from GDP-β-L-fucose to *Ser* or *Thr* residues. This enzyme is essential for the Notch function, because once POFUT1 is knocked out, the Notch signal will be interrupted (Stahl et al., 2008).

Highly branched and bisected GlcNAc N-glycans are common features of cancer. Early studies have found that the increase in the expression of highly branched N-glycans present on cell surface is related to the malignancy of tumor cells (Asada et al., 1997). The synthesis of N-glycan branches is regulated by MGATs (mannosyl-glycoprotein β-N-acetylglucosaminyltransferase), such as MGAT1, MGAT2, MGAT4, MGAT5 (Lau et al., 2007). The MGAT5 protein is present in the salivary gland and lungs. Mgat5-deficient mice inhibit tumor growth and metastasis (Granovsky et al., 2000). The bisected GlcNAc on N-glycan is abnormally altered in cancer, which may be due to MGAT3 dysregulation in tumor tissues. Glycans with this structure has the functions of inhibiting growth factor signaling, slowing down tumor progression, and preventing tumor metastasis (Miwa et al., 2012; Song et al., 2010). N-glycan profiling of colorectal cancer cells also revealed that the unique bisected GlcNAc structure is associated with membrane glycoproteins in metastatic or invasive cell lines (Sethi et al., 2014).

4. Tumor biomarkers present in human body fluids

Proteins in body fluids are mostly glycosylated, because when they are secreted from cells, they can be post-transnationally modified by carbohydrates by cellular glycoenzymes. Therefore, human plasma, serum, urine and saliva can be used to discover TSG biomarkers. Tumor markers can be proteins or other substances, which are produced or shed by cancer cells in the body in response to immunity. Tumor markers, circulating or tissue-specific tumors, can be used for prognosis, diagnosis, staging, treatment evaluation, etc. It is worth noting that the three plasma tumor markers PSA, CA-125 and AFP have been used clinically for prostate, ovarian, and liver cancers, respectively (Meany et al., 2009).

According to its clinical application, disease biomarkers can be used for diagnosis, prognosis, treatment evaluation, and recurrence. Diagnostic biomarkers refer to those molecules that can predict the occurrence of diseases, and prognostic biomarker can monitor the effects of chemotherapy or immunotherapy. Despite advances in treatment, malignant tumors cause more than 18 million deaths from cancer worldwide each year. This may be due to the lack of reliable diagnostic biomarkers that can detect tumors at an early stage. For example, the 5-year survival rate of advanced lung cancer is about 15%, and the 5-year survival rate of early lung cancer has increased significantly by 45%. Free PSA in serum is a biomarker approved by the FDA for early diagnosis of prostate cancer. The 5-year survival rate for most men with localized prostate cancer is as high as 100% (Baade et al., 2009).

Other studies have revealed that the detection of circulating tumor DNA methylation in a longitudinal study in patients' plasma can provide early diagnosis of different cancers (Chen et al., 2020). Recent studies have found that serum proteins, CEA (Carcinoembryonic antigen), RBP (Retinol-binding protein), and α1 antitrypsin have 89.3% sensitivity and 84.7% specificity in the diagnosis of lung cancer patients (Patz et al., 2007). These results are based on analysis of serum proteins in 10 patients diagnosed with non-small-cell lung cancer (NSCLC). In order to obtain reliable biomarkers, more clinical cases are needed to prove whether these results are applicable to the statistical significance of different subtypes of NSCLC. Therefore, a set of biomarkers for early detection of NSCLC remain to be discovered.

Table 2 shows cancer biomarkers that are highly abundant in saliva, lung tissue, or serum. Most biomarkers are used in targeted therapy for a variety of cancers and are listed by the National Cancer Institute

Table 2
List of saliva biomarkers used for cancer monitoring, diagnosis, treatment determination, recurrence, or prognosis. The biomarkers are detected by their proteins in saliva or other body fluids. CA15-3, CA27-29, and CA19-9 have been tested for their O-glycans. NSCLC = non-small cell lung cancer, OSCC = oral squamous cell carcinomas, PDAC = pancreatic ductal adenocarcinomas, SCLC = small cell lung cancer. MUC1* is one of the CA19-9 substrates.

Proteins	Gene	Type of detection	Disease	Clinical applications	References
Myeloid cell surface antigen CD33	CD33	Protein	Acute myeloid leukemia	Determining treatment	Ehninger et al. (2014)
Estrogen receptor	ESR1	Protein	Breast cancer	Determining treatment	Bretschneider et al. (2008)
CA15-3	MUC1	Sialyl O-glycan on MUC1	Breast cancer	Monitoring, detection recurrence	Streckfus et al. (2000)
CA27-29	MUC1	O-glycan on MUC1	Breast cancer	Detection metastasis, recurrence	Asiago et al. (2010)
HER2	ERBB2	Protein	Breast, ovarian, pancreatic, gastric cancer	Therapy selection	Loibl and Gianni (2017)
β-2 microglobulin	B2M	Protein	Colorectal cancer	Diagnosis	Prizment et al. (2016)
Proliferating cell nuclear antigen	PCNA	Protein	Colorectal cancer	Prognosis	Mayer et al. (1993)
Carcinoembryonic antigen (CEA)	CEACAM5	Protein	Colorectal cancer, OSCC	Diagnosis, monitoring, detection recurrence	Saeland et al. (2012)
Trisephosphate isomerase	TPI1	Protein	Gastric cancer	Diagnosis	Xiao et al. (2016)
Gastrin	GAST	Protein	Gastrinoma	Diagnosis, monitoring, detection recurrence	Dockray et al. (2001)
CD117	KIT	Protein	Gastrointestinal stromal tumor	Diagnosis	Sarlomo-Rikala et al. (1998)
α-fetoprotein	AFP	Protein and core fucosylation	Hepatocellular carcinoma	Diagnosis, staging, detecting recurrence, monitoring	Sato et al., 1993)
Calcitonin	CALCA	Protein	Medullary thyroid cancer	Diagnosis, recurrence	Elisei et al., 2004)
Epidermal growth factor receptor	EGFR	Protein	NSCLC	Diagnosis, monitoring, prognosis	Pirker et al. (2010)
Programmed death ligand 1 (PD-L1)	CD274	Protein	NSCLC	Diagnosis, monitoring	Kerr et al., 2015)
Annexin A1	ANXA1	Protein	NSCLC	Diagnosis	Xiao et al. (2016)
Tissue-type plasminogen activator	PLAT	Protein	NSCLC	Recurrence	Foa et al. (1999)
Catenin β1	CTNNB1	Protein	NSCLC	Prognosis	Woenckhaus et al. (2008)
Cyclin D1	CCND1	Protein	NSCLC	Prognosis	Betticher et al. (1996)
WAP four-disulfide core domain protein 2	WFDC2	Protein	Ovarian cancer	Prognosis, detection recurrence	Hellström et al., 2003)
CA125	MUC16	Protein	Ovarian cancer	Diagnosis, detection recurrence, monitoring	Van Gorp et al. (2011)
CA19-9 or sialyl *Lewis* epitopes on mucins	MUC1*	sLewA on mucin	PDAC	Monitoring	O'Brien et al., 2015)
Salivary leptin	LEP	Protein	Salivary gland tumor	Diagnosis'	Schapher et al. (2009)
Neuron-specific enolase	ENO2	Protein	SCLC	Diagnosis, monitoring	Ando et al. (2004)
Chromogranin A	CHGA	Protein	SCLC	Diagnosis, assessment of treatment response, recurrence	Lamy et al. (2000)

(carcer.gov/about-cancer/diagnosis-staging/diagnosis/tumor-markers-list). Biomarkers can be classified by detecting proteins or glycans attached to proteins. The quantification of protein levels in saliva or serum is carried out by enzyme-linked immunosorbent assay (ELISA) with chemiluminescence or fluorescent detection. β-2 microglobulin (B2M) levels are often elevated in the serum of patients with colorectal cancer or myeloma (Prizment et al., 2016), while in patients with NSCLC, salivary annexin A1 levels are increased (Xiao et al., 2016). These molecules, CALCA (Elisei et al., 2004), CCND1 (Betticher et al., 1996), CD274 (Kerr et al., 2015), CD33 (Ehninger et al., 2014), CEACAM5 (Saeland et al., 2012), CHGA (Lamy et al., 2000), CTNNB1M (Woenckhaus et al., 2008), EGFR (Pietrobono et al., 2020), ENO2 (Ando et al., 2004), ERBB2 (Loibl and Gianni, 2017), ESR1 (Pirker et al., 2010), GAST (Dockray et al., 2001), KIT (Sarlomo-Rikala et al., 1998), LEP (Schapher et al., 2009), MUC16 (Van Gorp et al., 2011), PCNA (Mayer et al., 1993), PLAT (Foa et al., 1999), TPI1 (Xiao et al., 2016), and WFDC2 (Hellström et al., 2003), are quantified based on their protein level in serum or saliva. On the other hand, several glycoproteins encoded by MUC1 may require measurement of protein and attached glycans, including CA15-3 (Streckfus et al., 2000), CA27-29 (Asiago et al., 2010), and CA19-9 (O'Brien et al., 2015). Glycan structure or abundance of these proteins often changes in cancer, such as truncated O-glycans on mucins in tumor microenvironment (Chia et al., 2016). Noticeably, tumor cells carry dense Tn or sTn antigens that are covalently bound to the *Ser* or *Thr* residues of glycoproteins. If only the protein is analyzed without characterizing their glycan structure or glycoform, the antibodies used in the ELISA based only on proteins may compromise the accuracy and sensitivity of diagnosis. Since abnormal glycosylation changes have been observed in cancer cells, CSG biomarkers are ideal for better diagnosis or prognosis.

5. Abnormal glycosylation of saliva and lung proteins in lung cancer

Although serum or plasma are commonly used to discover tumor biomarkers, saliva has become one of the essential body fluids. This is because using saliva as a non-invasive diagnostic sample can avoid the risk of pain, anxiety or infection, and it is easy to store and collect multiple subsequent samples. Saliva has been utilized to diagnose oral diseases and monitor disease progression, such as patients suspected of COVID-19 (Fakheran et al., 2020). Proteomic analysis of human saliva found that 48 of the 500 proteins were significantly differentially expressed between normal controls and gastric cancer patients. Among them, STAT2 (signal transducer and activator of transcription 2) was up-regulated, and tumor suppressor gene of DMBT1 (deleted in malignant brain tumors 1 protein) was down-regulated (Xiao et al., 2016). A meta-analysis of 29 articles from more than 10,000 subjects showed that the diagnostic accuracy of biomarkers in saliva for lung cancer is approximately 88% (Rapado-González et al., 2020). Thus, saliva represents a promising non-invasive source for the discovery of novel biomarkers for lung cancer.

Saliva is mainly composed of proteins, urea, ammonia, and

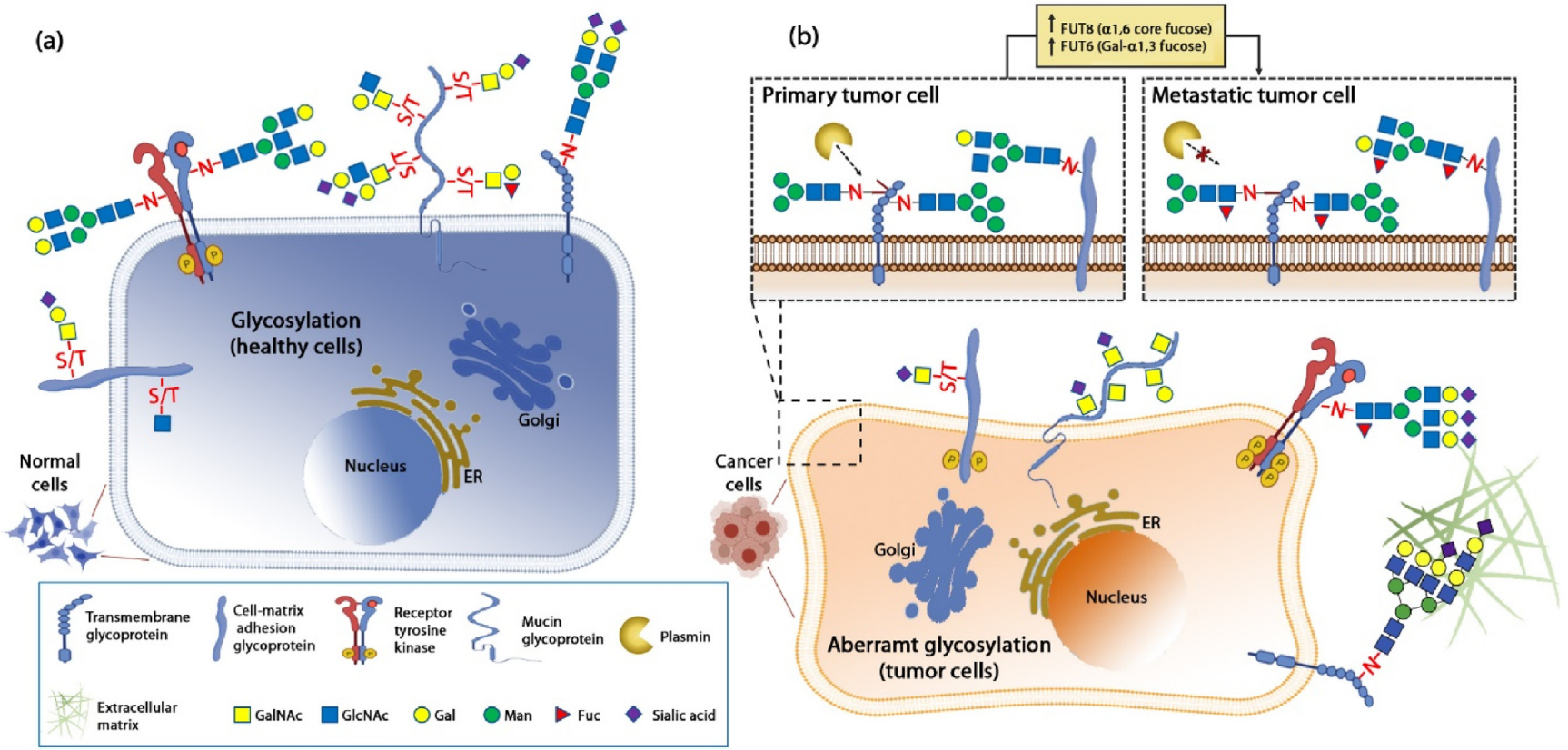

Fig. 1. Schematic diagram of abnormal protein glycosylation in tumor cells. (**a**) N-glycans and O-glycans are present on the surface glycoproteins of healthy cells. The process of glycosylation biosynthesis takes place in the endoplasmic reticulum (ER) and Golgi apparatus. Glycosylation occurs on transmembrane proteins, cell-matrix adhesion proteins, mucins, and receptor tyrosine kinases (RTKs). (**b**) Aberrant glycosylation of cancer cells by dysregulated glycoenzymes in the tumor microenvironment. Sialylated glycans and truncated O-glycans are synthesized on the surface glycoproteins of cancer cells. Mucin carries dense O-glycans of T, Tn, sT and sTn antigens. The metastatic cells upregulate fucosylation due to the increase of FUT genes, including FUT8 (α1,6 fucose) and FUT6 (α1,3 fucose). The core-fucosylation and branching-fucosylation are characteristics of metastatic cancer cells. Oncogenesis or metastatic tumors can alter the protein O-GlcNAcylation and hyperphosphorylation through the crosstalk between O-GlcNAcylation and phosphorylation.

electrolytes. The proteins in saliva include mucin, amylase, defensin, cystatin, histatin, proline-rich protein, statherin, lactoperoxidase, lysozyme, lactoferrin, and immunoglobulin. Mass spectrometry (MS) analysis of exosomes and bullae in the saliva of lung cancer patients showed that 4% of saliva proteins were expressed in distal lung cells. Among them, BPIFA1 (BPI fold-containing family A member 1), CRNN (Cornulin), MUC5B (Mucin-5B), and IQGAP (Ras GTPase-activating-like protein) are dysregulated proteins (Sun et al., 2018), and most of these proteins are glycosylated. Changes in glycosylation are attributed to the differential expression of glycoenzymes and glycoprotein substrate in tumor environment. There are several glycosyltransferases in saliva, such as Glucosyltransferase B (GtfB) (Smith et al., 2007), α-1,3-fucosyltransferase (FUT5) (Gonzalez-Begne et al., 2011), α1,3-mannosyltransferase (ALG3), N-acetylgalactosaminide α-2,6-sialyltransferase 1 (ST6GALNAC1), and α-N-acetylneuraminide α-2,8-sialyltranserase 2 or 5 (ST8SIA2 or ST8SIA5) (*Human Protein Atlas*). Due to the presence of various glycoenzymes in saliva and salivary glands, microbe, phagocyte, mucin and agglutinin are highly decorated by glycans (Cross and Ruhl, 2018). Oral microbes bind glycoproteins such as mucins and agglutinin through O-glycans. These changes in glycosylation affect the function of oral microbes. In the process of tumorigenesis and metastasis, the tumor microenvironment alters the glycosylation of saliva glycoproteins, such as MUC5B, MUC7 (mucin-7) (Tenovuo and Levine, 1989), salivary agglutinin (SAG) (Madsen et al., 2010), β-2-microglobulin (Gussow et al., 1987), and proline-rich glycoprotein (PRG) (Tenovuo and Levine, 1989). Consequently, identifying TSG and its dependent regulatory factors is crucial for the discovery of biomarker.

Elucidating the saliva glycoproteins of healthy controls and lung adenocarcinomas (LADC) patients is the key to revealing TSG for biomarker discovery. To decipher protein glycosylation, it is necessary to analyze the glycans, glycosites, site occupancy, and glycan profile of glycosite in the 3D protein configuration. These can be achieved by the release of N-glycans by N-glycosidases and the removal of O-glycans by chemical β-elimination (Jensen et al., 2012; Yang et al., 2017a), while N-glycosites are determined by hydrophilic interaction liquid chromatography (HILIC) - tandem MS (MS/MS) of intact N-glycopeptides (Riley et al., 2019; Zacharias et al., 2016; Xiao et al., 2018). Complex O-glycosylation has been successfully studied by O-protease, which can cleave the N-terminus of O-glycosylated *Ser* or *Thr*, and EThcD (Electron-transfer and higher-energy collision dissociation) (Yang et al., 2018b, 2020; Malaker et al., 2019). In contrast, the linkages of labile sialic acids are differentially protected through ethyl esterification and reductive amination using an amine-containing compound (Reiding et al., 2014; Yang et al., 2017b). The derivatization of sialic acid on the solid phase not only stabilizes α-2,3 and α-2,6 linkages, but also make it easier to removes the reagents after the reaction (Yang et al., 2017b). By combining these analytical platforms and advanced MS technology, we can in-depth deconvolute TSGs by investigating protein glycosylation between healthy controls and patients.

6. Role of aberrant glycosylation in tumor signal transduction

Aberrant glycosylation defines malignancy of tumors, so dysregulated glycosylation can lead to changes in tumor signaling pathways. Normal cells contain high-mannose, sialic acids and extended O-glycans. Cancer cells have altered glycoenzymes and even mutated amino acids of enzymes/proteins, leading to unique protein glycosylation. As shown in Fig. 1, glycoproteins exhibit distinct glycosylation patterns, e.g., mucins with shorter O-glycans (T, Tn, sT or sTn). This may be due to the regulated O-glycosylation biosynthetic pathway. The extended and frequently branched O-glycans are initialized by adding GalNAc to *Ser* or *Thr* residues catalyzed by GALNTs, and then adding Gal or other monosaccharides to GalNAc. The enzymatic synthesis of the latter is catalyzed by C1GalT1 in the presence of *COSMC* (core 1 β3-Gal-T-specific molecular chaperone) (Ju and Cummings, 2002). However, this O-glycosylation pathway is bypassed in cancer by activating regulatory pathway. GALNT activity is also regulated by the subcellular relocation of these enzymes. When the proto-oncogene Src is activated, it will trigger the transport of GALNT from the Golgi to ER trafficking, resulting in a strong increase in the level of Tn in the cell. This process is called the GALA pathway, i.e., relocation of Src-dependent GALNT. When the GALA pathway is turned on in cancer, Tn or T antigen is synthesized (Chia et al., 2016). Recent studies have confirmed that the truncation of O-glycans directly induces the oncogenic characteristics of tumor cell growth and invasion (Radhakrishnan et al., 2014).

Cancer cells produce other features of glycans on the cell surface, such as sialylation, poly-LacNAc (Poly-N-acetyl-lactosamine), or fucosylation. Hypersialylation is a common characteristic of cancers in that glycans are modulated by their sialyltransferases and sialidases (Table 1). HER2 is one of RTKs, with increased α2,6 sialic acids, which can promote the progression of gastric cancer through Akt/ERK signal (Liu et al., 2018). In turn, the increase in RTK sialylation enhances the anticancer effect and chemosensitivity. This has been confirmed by the EGFR inhibitor (Gefitinib) in ST6Gal1-deficient colon cancer cells (Park et al., 2012). Poly-LacNAc is synthesized by alternate catalysis between 1,3-N-acetylglucosaminyltransferase 2 (B3GNT2) and β1,4-galactosyltransferases (B4GALT2), and it is often terminated by an α2,6 sialic acid (Stanley et al., 2015). Due to the elevated expression of FUT8, especially in metastatic or invasive tumor cells, core fucosylation of N-glycans is usually increased in the body fluids of cancer patients (Fig. 1b) (Höti et al., 2018; Wang et al., 2014). FUT8 also synthesizes the core-fucosylation of high-mannose in metastatic cancer cells (Lin et al., 1994; Magalhães et al., 2017). FUT6 is another fucosyltransferase that is elevated in cancer. It up-regulates sialyl-*Lewis* antigen and enhances cancer cell migration through the TGF-β-EMT pathway (Hirakawa et al., 2014).

According to the *Cancer Genome Atlas* (TCGA), many oncogenes play a vital role in tumorigenesis and cancer metastasis, including RTK-RAS, PI3K/Akt, Nrf2, Notch, Myc, Hippo, TGFβ, p63 and β-catenin/Wnt (Sanchez-Vega et al., 2018). A common carcinogenic driver is receptor tyrosine kinase (RTK), which is a high-affinity cell surface receptor for growth factors, cytokines, and hormones. Many RTKs are glycosylated, and changes in glycosylation affect the carcinogenic pathway. For example, deleting the *N134* glycosite of GPNMB (transmembrane glycoprotein NMB) can drastically reduce the binding affinity of GPNMB to EGFR mutations (one of RTKs). As a result, the deletion of the *N134* glycosite can block its downstream signal transduction, and ultimately inhibit metastasis of NSCLC (Han et al., 2021). Researchers have studied how glycosylation inhibitors affect the development and progression of tumors, such as tunicamycin (TUN) for N-glycosylation or BAG (benzyl N-acetyl-α-D-galactosamide) for O-glycosylation. The inhibition of epidermal growth factor receptor (EGFR) and insulin-like growth factor 1 receptor (IGF1R) by TUN treatment resulted in decreased receptor phosphorylation and tumor cell apoptosis (Pérez et al., 2020). TUN is also used as a drug to inhibit N-linked glycosylation, by blocking the addition of dolichol-linked GlcNAc precursor to nascent polypeptides, thereby preventing protein folding and transport through the ER (Heifetz et al., 1979; Moremen et al., 2012). BAG inhibition can reduce the rate of cell adhesion (Porowska et al., 2004), and it has also been reported that BAG can hinder the O-glycan sialylation of cancer cells, leading to enhanced metastatic ability (Vajaria et al., 2016b).

Wnt signaling is one of the carcinogenic pathways affected by its glycosylation. The Wnt signal is initiated by the secreted Wnt protein, which binds to transmembrane receptors or ligands through the frizzled (Fz) genes (Polakis, 2000). Wnt can target downstream β-catenin through several promoters (such as DPAGT1). The activation of CHO cells by lithium chloride can increase the transcription level of DPAGT1 and increase the abundance of β-catenin (Sengupta et al., 2010). Both DPAGT1 and β-catenin are glycosylated. DPAGT1 participates in protein glycosylation by catalyzing the initial step of dolichol-linked oligosaccharide biosynthesis in the N-glycosylation. The differential glycosylation of β-catenin and LRP6 inhibits the Wnt signaling pathway and significantly

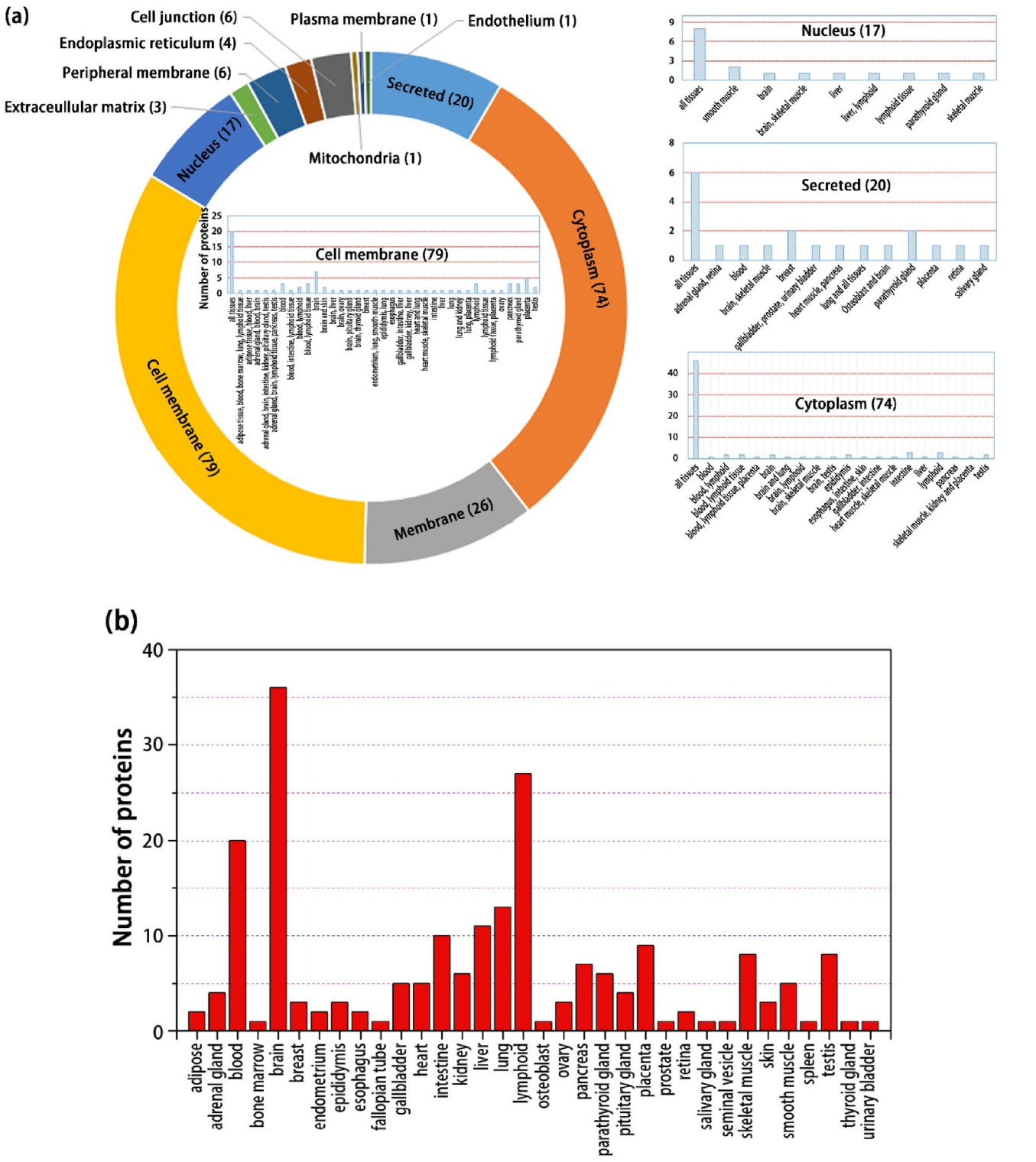

Fig. 2. List of 238 human receptor tyrosine kinases present in tissues according to the *Uniprot* Homo Protein Database. (**a**). The cellular location of receptor tyrosine kinases (RTKs) is mostly cell membrane (79), membrane (26), nucleus (17), cytoplasm (74), and secreted (20). There are 20 membrane RTKs are found in most human tissues, of which 7 are in the brain, 3 are in the blood, 3 are in the lymph tissue, and 3 are in the pancreas. Among these cell membrane proteins, FGR, AGTR2, DDR2, MAGI3, EFNB2 and TRPC6 are particularly abundant in the lung, including MATK (cytoplasm), ROS1/LTK/AGER/SLC34A2 (membrane), and ANGPT4 (secreted). (**b**) The number of RTKs enriched and expressed in specific human tissues, including brain (36), lymphoid tissue (27), blood (20), lung (13), liver (11), intestine (10) and pancreas (7). Highly abundant RTKs in the lung include FGR, MATK, AGTR2, TRPC4, ROS1, DDR2, LTK, MAGI3, AGER, EFNB2, TRPC6, SLC34A2, and ROR1. (**c**) Glycosites of RTKs in human tissues. The glycosites of RTKs, from ABL2 to ZPR1 are listed in the red dashed line. *NetNglyc* predicts N-glycosylation (>0.1) and *ISOGlyP* predicts O-glycosylation (cutoff >3). The numbers in the doughnut chart represent the number of glycosites predicted by *NetNglyc* or *ISOGlyP*, or listed by *Uniprot*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

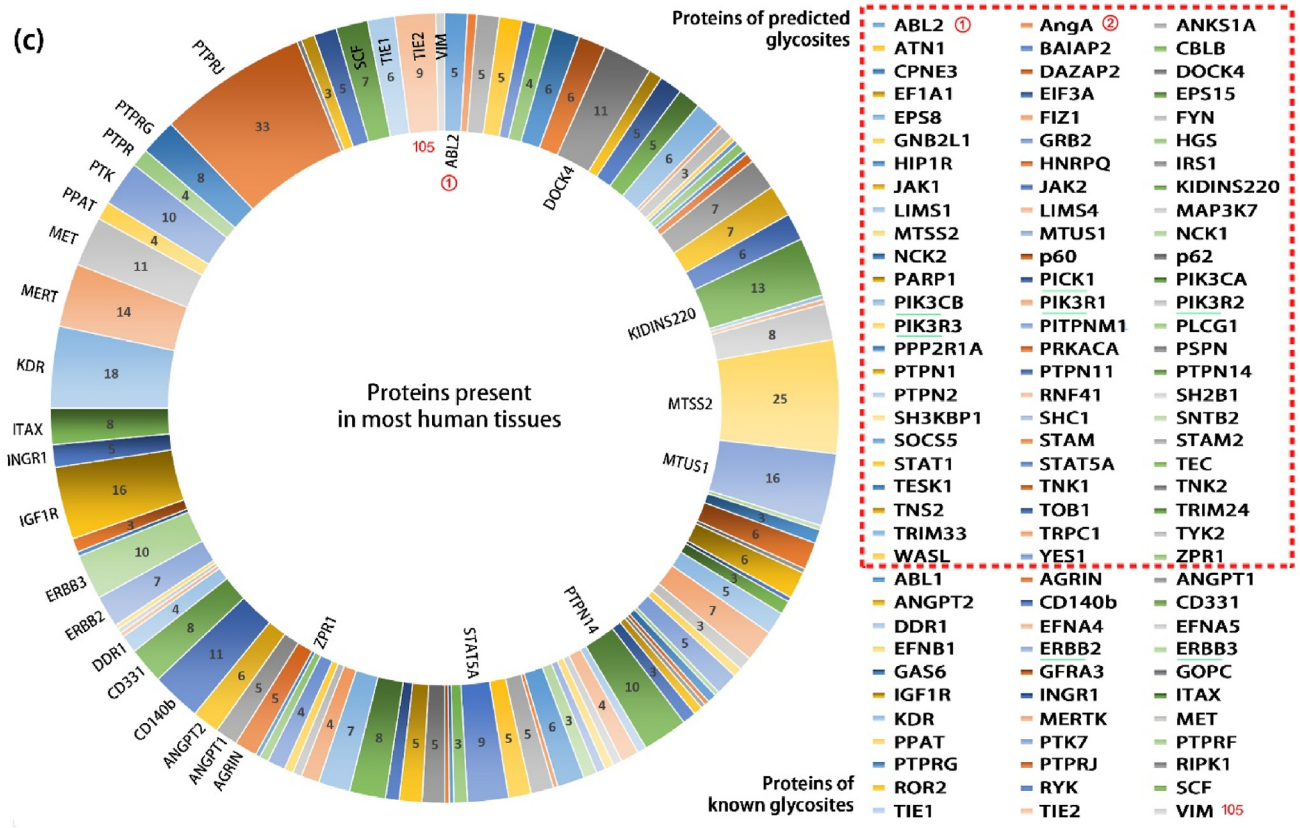

Fig. 2. (*continued*).

prevents the growth of tumor cells in prostate cancer, bladder cancer, rectal cancer, and liver cancer (Hernández-Maqueda et al., 2013). The dysregulation of DPAGT1 has a profound impact on the glycosylation of downstream effectors of the Wnt signal, such as the abnormal N-glycosylation of E-cadherin in oral cancer (Nita-Lazar et al., 2009). E-cadherin glycosylation affects the ability to develop mature adhesion junctions, thereby regulating its invasiveness to surrounding tissue (Diniz-Freitas et al., 2006).

7. Tissue specific receptor tyrosine kinase and its glycosylation

Receptor tyrosine kinases (RTKs) play a central role in cell proliferation and differentiation, cell survival, cell motility, invasion, and angiogenesis, thereby promoting tumorigenesis and cancer metastasis. For instance, oncogenic mutations in RTK or KRAS promote cancer cell progression (von Karstedt et al., 2015). There are approximately 90 unique tyrosine kinase genes in the human genome, of which 58 encode RTK. RTK has been shown to be not only a key regulator of normal cellular processes, but also plays a key role in the development and progression of many types of cancer. Mutations in RTKs cause the activation of a series of signal cascades that have multiple effects on protein expression (Robinson et al., 2000).

The *Uniprot* protein database lists more than 300 human RTKs, 238 of which are shown in the *Supplementary Information* Table S1. We tabulate accession ID, gene, protein, glycosite, cellular location, tissue specificity, biological process and molecular functions. Some RTKs are related to tumorigenesis and cancer cell metastasis, including ANGPT1, GNB2L1, INGR1, LIMS1, RIPK1, TNFRSF1B, EPHB2, HYAL2, and P53. Among them, ANGPT1 (Angiopoietin) and its receptor (TIE2) regulate the process of angiogenesis during tumorigenesis and metastasis, and TIE2 can activate JAK/STAT signals to enhance the expression of chemokines and cytokines (García et al., 2014). The inhibition of ANGPT1 by AMG 386 can suppress angiogenesis and tumorigenesis in mice, indicating its functions in cancer (Coxon et al., 2010). Additionally, kinase phosphorylation of RTKs play an important role in T-cell receptor signaling that regulates cancer development and progression (Lemmon and Schlessinger, 2010). The extracellular domain of RTK can also interact with T-cell receptors and modulate oncogenic signaling pathway with high specificity through its binding domain epitopes (Hatada et al., 1995).

Tyrosine kinases are widely distributed in different cellular components, some of which are particularly abundant in several human tissues (Fig. 2). The information of each protein is summarized according to the *Uniprot* database and *Human Protein Atlas*. Fig. 2a classifies the cellular components of 228 RTKs, many of which belong to the cell membrane (membrane) and cytoplasm. We further examined the tissue distribution of cell membrane RTKs. They contain 79 proteins, 20 of which are expressed in all human tissues. These proteins can make tumor cells proliferate and activate cell surface growth factor. For instance, the ANGPT-TIE pathway expressed in all tissues acts as an angiogenesis switch in tumors and participates in tumor metastasis and lymphangiogenesis, while ANGPT1 or ANGPT2 have been shown to promote tumorigenesis and tumor malignancy (Huang et al., 2010; Pari et al., 2020). Nucleus RTKs (17) are found in most tissues except the lung, and secretory RTKs (20) are detected in the lungs, salivary glands, and pancreas. Similarly, 46 of 74 cytoplasmic RTKs are expressed in all tissues.

The tissue-specific RTKs are shown in Fig. 2b. The main RTK-containing tissues are brain, lymphoid, blood, lung, liver, intestine and various glands. RTKs primarily expressed in the brain include CADM4, CD332, EFNB3, EPHA5, EPHB1, GAB1, HIP1, NRG1, NRG3, NTRK3, PLXNB1, SHC3, SIRPA, TIAM1, EPHA4 (Supporting *Information* Table S1), most of which are also in the salivary glands. The 13 RTKs enriched in lung tissue belong to cell membrane or membrane. The abundant MATK in the lung can bind phosphorylated ERBB2 through the SH2 domain of MATK, and their interaction is directly related to the growth inhibitory effect of breast cancer (Kim et al., 2002). The ROS1

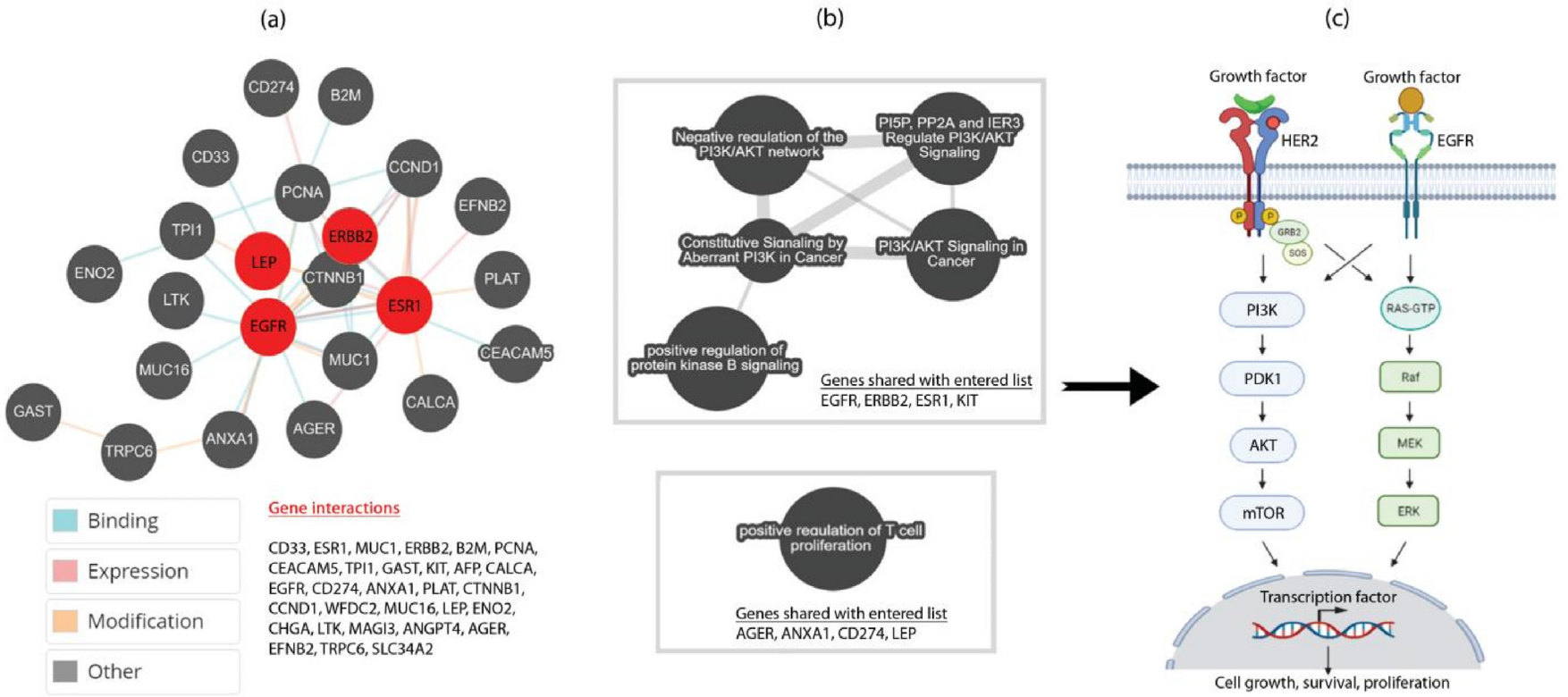

Fig. 3. Molecular interactions and signal transduction between lung tissue-specific proteins and salivary protein biomarkers. (**a**) Gene interactions of typical cancer biomarkers and their carcinogenic drivers. Thirty-one genes were analyzed by the *Pathway Commons* (www.pathwaycommons.org). The gene interaction includes protein binding, expression levels, and protein modification. (**b**) The network of pathways containing 31 genes. Pathway analysis showed that EGFR, ERBB2, ESR1 and KIT may negatively regulate PI3K/AKT signal, and PI5P, PP2A and IER3 also modulate PI3K/AKT signal. Genes including AGER, ANXA1, CD274 and LEP can regulate T-cell proliferation. (**c**) The canonical signal pathway, which is initiated by surface growth factor and transduces surface signal to downstream effectors. Mutation or up-regulation of these oncogenic drivers can promote cell growth, survival, and tumor cell proliferation.

proto-oncogene fusion protein is expressed in 1–2% of NSCLC, and its gene rearrangements produces a fusion protein in which the kinase domain of ROS1 becomes constitutively active and drives tumor proliferation (Davies and Doebele, 2013).

RTK is activated by the dimerization or oligomerization of the receptor, and the receptor is induced by a ligand that binds to the extracellular domain of RTK (Rodrigues et al., 2018; Du and Lovly, 2018). Recent studies have shown that glycosylation can regulate the ligand-dependent activation and signal transduction of RTK. For example, inhibition of N-linked glycosylation has been shown to significantly reduce RTK signaling (Chandler et al., 2019; Contessa et al., 2008). Therefore, RTK and ligand glycosylation will not only affect their binding, but also affect downstream tumor signal transduction. Fig. 2c shows the known and predicted glycosites of tyrosine kinases. The known glycosites come from literature and *Uniprot*, while the potential glycosites are predicted by NetNglyc for N-glycosylation or ISOGlyP for O-glycosylation. The doughnut chart lists the number of glycosites for each protein, as well as the predicted glycosites of proteins in the red dashed box. Importantly, mutations in RTKs can alter their glycosylation and regulate tumor signaling (Yang et al., 2021).

8. Interaction network of RTK proteins expressed in saliva and lung

We further examined 31 genes encoding proteins expressed in the lungs or found in saliva (Fig. 3). These genes are analyzed by the *Pathway Commons*, which provides detailed representations of various biological concepts, including biochemical reactions, gene regulatory networks, genetic interactions, transport and catalysis events, and protein physical interactions (Cerami et al., 2010). Fig. 3a shows the interaction network of these proteins, where CTNNB1, EGFR, ERBB2, ESR1 and MUC1 bind to each other and to other proteins. As shown in Fig. 3b, the gene network is generated by 4 shared genes (EGFR, ERBB2, ESR1 and KIT). Pathway analysis showed that the PI3K/AKT network is negatively regulated by phosphatases that dephosphorylate PIP3, thereby preventing the activation of AKT. On the other hand, PI3K/AKT signaling in cancer is often constitutively activated by a gain-of-function mutation in one of the two PI3K subunits - PI3KCA or PIK3R1. Therefore, in the absence of growth factor, the PI3K complexes with gain-of-function mutations will produce PIP3 and activate downstream AKT (Zhao and Vogt, 2008). Meanwhile, any process involving AGER, ANA1, CD274 and LEP can activate or increase the rate or extent of T cell proliferation.

The RTK signal pathway is summarized in Fig. 3c. Generally, growth factors located in the extracellular matrix bind to RTK (HER2, EGFR etc.), where the intracellular domain of RTK is phosphorylated and has tyrosine kinase activity (Yang et al., 2021). PI3K can regulate PDK1, downstream AKT and mTOR, thereby regulating transcription factors. The RAS-GTP signal contains several downstream oncogenes of Raf, MEK and ERK. These signaling pathways ultimately lead to tumor cell growth, survival and proliferation. The RAS-Raf-MEK-ERK pathway plays a vital role in tumorigenesis through small multi-faceted RNA and is the target for anti-cancer therapy (Hatley et al., 2010).

9. Concluding remarks

Aberrant glycosylation defines the malignancy of tumors and has unique characteristics that are different from the pathological and physiological state of normal cells. These differences are attributed to glycoenzyme variation in tumor microenvironment. Because the transition from normal cells to cancer cells is relatively slow and is often clinically silent (Al-Zhoughbi et al., 2014), the regulation of glycoenzyme occurs gradually. The initial glycosylation is expected to begin in the early stages of cancer development. Tumor biomarkers can be sought from clinical specimens of patients with early-stage tumors, and their structure can be compared with healthy and/or metastatic tumors. Biomarkers can be glycoenzymes, glycoproteins, or RTKs for diagnosis, prognosis, treatment assessment, and tumor recurrence. Changes in the pattern or structure of glycans have been seeking to discover cancer biomarkers (Adamczyk et al., 2012). The glycan structures known to be specific to cancer are Tn (*s*Tn) and T (*s*T) antigens, hypersialylation and hyperfucosylation. Most proteins for cancer diagnosis are glycosylated and detected by their abundance or attached glycans. However, there is still a need to develop glycoprotein biomarkers specific to cancer subtypes, because subtle changes in biomarker glycosylation may be intrinsically linked to specific cancers. Elucidating the glycosylation of these potential biomarkers through advanced MS and newly developed glycobiology techniques may be the next step in obtaining reliable cancer diagnostic biomarkers.

CRediT authorship contribution statement

Ziyuan Gao: Writing – original draft. **Mingming Xu:** Writing – original draft. **Shuang Yue:** Writing – original draft. **Huang Shan:** Writing – original draft. **Jun Xia:** Writing – review & editing. **Junhong Jiang:** Writing – review & editing. **Shuang Yang:** Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interests.

Acknowledgements

This work is supported by the start-up fund of Soochow University in China. We thank the Priority Academic Program Development of the Jiangsu Higher Education Institutes (PAPD).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crphar.2021.100079.

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