Tumor Biol.
DOI 10.1007/s13277-016-5043-9

REVIEW

What gastric cancer proteomic studies show about gastric carcinogenesis?

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Received: 6 January 2016 / Accepted: 28 March 2016
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Abstract Gastric cancer is a complex, heterogeneous, and multistep disease. Over the past decades, several studies have aimed to determine the molecular factors that lead to gastric cancer development and progression. After completing the human genome sequencing, proteomic technologies have presented rapid progress. Differently from the relative static state of genome, the cell proteome is dynamic and changes in pathologic conditions. Proteomic approaches have been used to determine proteome profiles and identify differentially expressed proteins between groups of samples, such as neoplastic and nonneoplastic samples or between samples of different cancer subtypes or stages. Therefore, proteomic technologies are a useful tool toward improving the knowledge of gastric cancer molecular pathogenesis and the understanding of tumor heterogeneity. This review aimed to summarize the proteins or protein families that are frequently identified by using high-throughput screening methods and which thus may have a key role in gastric carcinogenesis. The increased knowledge of gastric carcinogenesis will clearly help in the development of new anticancer treatments. Although the studies are still in their infancy, the reviewed proteins may be useful for gastric cancer diagnosis, prognosis, and patient management.

Keywords Gastric cancer · Proteomics · Protein expression · Mass spectrometry

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Gastric cancer (GC) is the third leading cause of cancer death in the world [1]. Generally, GC has a very poor prognosis and a higher fatality-to-case ratio than any other prevalent diseases [2], in part due to the absence of specific symptoms in the early tumor stages, the frequent detection of the disease at advanced stages, and the limited therapeutic options. Additionally, the current insufficient determination of GC prognosis based only on the pathologic stages of the patients and the lack of sensitive and specific biomarkers in clinical practice restrict the successful management of GC patients [3, 4].

Over the past decades, several studies have aimed to determine the molecular factors that lead to GC development and progression. GC is well known to be a complex, heterogeneous, and multistep disease that involves environmental factors, mainly *Helicobacter pylori* infection, and several molecular alterations, such as genetic instability, telomerase activation, inactivation of tumor suppressor genes, and activation of oncogenes (see review [5, 6]). Furthermore, different epigenetic alterations have been reported to activate oncogenes and inactivate tumor suppressor genes during gastric carcinogenesis (see review [7]).

Currently, high-throughput technologies, including microarray assay and next-generation sequencing, have provided

Published online: 28 April 2016

additional insights into molecular alterations at much higher sensitivity [3, 8–13]. Moreover, these new methodologies have contributed to the discovery of new molecular intracellular pathways and molecular subtypes of GC. Recently, a new molecular classification of GC was proposed according to the spectra of genetic alterations associated with relevant clinical features, in which tumors as classified as Epstein-Barr virus (EBV)-positive subtype, microsatellite instability subtype, genomically stable subtype, and chromosomally unstable subtype (for more details, see [5, 14]). To suggest this new classification, about 295 GC samples were characterized using six molecular platforms: array-based somatic copy number analysis, whole-exome sequencing, array-based DNA methylation profiling, mRNA sequencing, microRNA (miRNA) sequencing, and reverse-phase protein array (in which the expression of 191 proteins with validated primary antibody was investigated) [14].

Despite the great advancement in research on GC, this disease is still poorly understood. Furthermore, GC subtype heterogeneity and very little consistent genomic alterations have been observed across different individuals [12]. Following the genomic revolution, recent technological improvements have allowed protein analyses of complex protein mixtures [15]. Thus, proteomics has emerged as an additional tool to determine protein expression patterns and the underlying mechanisms of GC initiation, progression, and metastasis.

Differently from the relative static state of genome, the proteome is a dynamic entity that changes according to the physiologic state of the cell. Due to differences in the translation regulation and stability of proteins, the correlation between mRNA and protein expression is usually weak [16]. A single gene can code several protein isoforms, and the protein activity depends not only on the translation process but also on its location and posttranslational modifications (PTM). Moreover, the activity of some proteins depends on the appropriate interaction with other proteins. In this context, proteins are the real functional effectors of cellular processes and reflect the state of in vivo molecular pathways [16]. Compared with genetic analysis, the analysis of proteins may provide more information about the cellular function or dysfunction [17]. Therefore, proteomic studies are essential for the improvement of all knowledge on GC molecular pathogenesis acquired thus far, which may be translated into clinical application in the future.

Basic knowledge about proteomic technologies

Proteomics refers to a set of analytical approaches that allow the identification, characterization, and quantification of virtually all proteins expressed by a genome in a cell, tissue, or organism [17]. Compared with genomic and transcriptomic studies, GC proteomic studies are still in their infancy. Proteins are largely heterogeneous, with variations in size, charge, hydrophobicity, and biospecific interaction. Therefore, the analysis of proteome profiles is complex.

The success of a proteomic study depends on several factors, such as increased proteome coverage to allow the detection of low-abundance proteins and increased coverage of the protein sequence to allow a confident identification or quantification [18]. The use of different methods of protein extraction may change the proteome profile. Moreover, it is still necessary to reduce the sample complexity before a large-scale analysis of proteins can be carried out. The proteomic approaches usually involve electrophoresis [mainly two-dimensional electrophoresis (2DE)] and/or liquid chromatography (LC) to reduce the sample complexity, as well as mass spectrometry (MS) analysis to quantify and/or identify proteins (Fig. 1; for more details about MS analysis, see [17, 19]). It is important to highlight that a proteomic study may target to study all the proteins of a cell or tissue sample or may target specific classes of proteins, such as membrane proteins [20–24] and phosphorylated proteins [25].

MS is the basis of proteomic studies. This method can be used to determine the mass/charge of a peptide or protein, as well as to quantify these compounds. Additionally, tandem MS (MS/MS) can provide the amino acid sequence and may allow the characterization of posttranslation modifications. In most GC studies, proteins were identified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) or electrospray ionization (ESI) MS [17]. MALDI imaging was used in a study that aimed to identify proteins associated with GC patient survival [26]. This method is used to identify and/or quantify peptides and proteins present in thin tissue sections (for more details about this method, see [27]).

GC proteomic studies have adopted a bottom-up approach, in which the target proteins are digested, mainly by using trypsin. The digestion can be directly in a solution or, after protein separation, on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel. The protein identification is usually done based on the primary sequence available in the databases, such as UniProt, NCBI, and IPI. UniProt is the most commonly used in human studies.

In the 2DE approaches, proteins are separated in two steps before digestion and MS analysis. First, the proteins are separated by the isoelectric point with the use of isoelectric focusing and then by their molecular weight with the use of SDS-PAGE. The result is an SDS-PAGE gel with several small spots, each virtually representing a protein; however, one spot may contain two or more proteins that were not well separated due to their similar isoeletric points and weights.

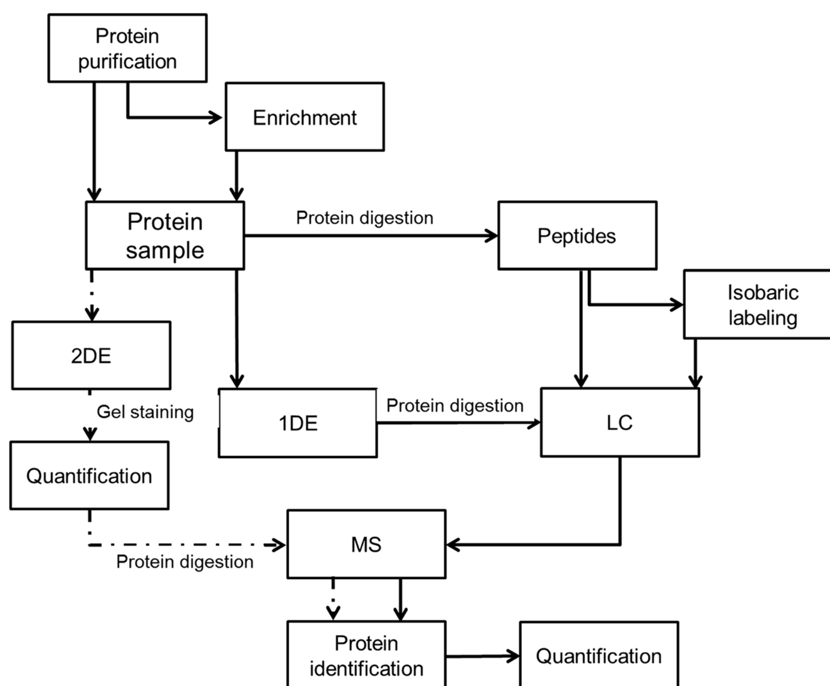

Fig. 1 Workflow of the main methods used in gastric cancer proteomic studies. First, the proteins of a sample are purified. In some studies, an enrichment step is done to select a specific group of proteins, such as membrane or phosphorylated proteins. The proteins of a sample can be separated by their isoelectric point and molecular weight through 2DE. The 2DE gels may be stained and the protein spots quantified; the selected proteins can be identified by MS. In another approach, the proteins may be separated by 1DE; the gel bands may be digested with trypsin, or the proteins in a solution may be directly digested with trypsin. Peptides are then separated by LC and identified by MS. MS analysis may also be used for protein quantification in a label-free approach or for quantification of peptides labeled with stable isotopes. 2DE, two-dimensional electrophoresis; 1DE, one-dimensional electrophoresis; LC, liquid chromatography; MS, mass spectrometry

After protein separation, the gels are stained. The size (area and intensity) of protein spots is directly correlated with the protein expression level. Thus, 2DE can be used for a quantitative comparison between groups of samples, for example, tumor and nontumor samples. Usually, the differentially expressed proteins are identified by MS analysis [17]. The gel images need to be well aligned to reduce the gel-to-gel variation and allow the compassion between samples. 2D-difference gel electrophoresis (2D-DIGE), which is designed for the analysis of multiple samples on one gel after labeling with different fluorescent dyes, simplifies the analysis and reduces gel-to-gel variations [28].

Samples can also be separated in SDS-PAGE (without previous isoelectric focusing), and the gel bands digested with trypsin. These gel bands present peptides of several proteins. Usually, the peptides are separated by LC and identified in a mass spectrometer. The purified proteins of a sample can also be directly digested with trypsin and separated by LC or 2D-LC in tandem with MS. These approaches are used for protein identification or quantification in a label-free method based on spectral counts, ion intensities, or chromatographic peak areas [29]. However, for more reliable quantification, an isotopic labeling method should be used. The most common labeling methods are those of stable isotope labeling, such as amino acids in cell culture (SILAC), isotope-coded affinity tags (ICAT), isobaric tags for relative and absolute quantification (iTRAQ), and tandem mass tags (TMT). Stable isotope quantitative proteomics allows the identification of equivalent peptides or peptide fragments by using the specific increase in mass due to mass tags with stable isotopes (for more details about isotopic labeling, see [30]). No study in this GC review used the SILAC approach, in which metabolic labeling of cell culture is done.

One study applied laser desorption ionization time-of-flight MS (SELDI-TOF-MS) by using a ProteinChip reader to identify peptides specific to GC [31]. SELDI-TOF is a technique that uses protein chips with different chromatographic surfaces to capture a specific set of proteins for analysis by MS. Although the SELDI-TOF methodology is fast and easy to use, the peaks are characterized only by their masses. Therefore, validation with sequence-based or antibody approaches before the direct use of SELDI-TOF in clinical practice would be interesting.

It is important to highlight that all the proteomic approaches have their advantages and disadvantages. As for the other "omics" studies (genomics, epigenomics, and transcriptomics), the use of different methods for validation or further evaluation of the obtained results is still necessary. Moreover, no proteomic technology can resolve the entire complexity of the human proteome. However, even if only a

part of the GC proteome is evaluated, such study of the protein profile of GC may help in understanding the mechanisms and underlying molecules that drive this malignancy.

Proteomic studies and the understanding of gastric carcinogenesis

The published GC proteomic studies did not present strong statistical power to identify a cancer biomarker and, thus, it was not the aim of this review. Here, we aimed to review the molecular aspect of GC as evaluated by proteomic approaches. Because the results obtained in biofluid samples are not necessarily correlated with the key molecular alterations required for GC development, we did not review the investigations involving this type of sample. Table 1 shows the most important proteomic studies that compared the protein profile between GC and gastric nonneoplastic samples. Tables 2 and 3 show proteins involved in the metastasis process and patient survival. Table 4 shows the proteomic studies concerning to drug resistance. Some proteomic studies evaluated a specific class of proteins, such as membrane proteins (Table 5), or PTM (Table 6) that may be involved in gastric carcinogenesis.

Some proteins have been reported in several GC proteomic studies, such as the upregulated heat-shock 27-kDa protein (HSP27; stress-related, chaperone) [32, 37, 40–42, 47, 55, 56], enolase-alpha (ENOA; glycolysis, energy metabolism) [33, 43, 44, 47, 49, 55, 56], nicotinamide *N*-methyltransferase (NNMT; methyltransferase, energy metabolism) [34, 37, 49, 56], annexin 2 (ANXA2; calcium-regulated membrane-binding protein) [40, 43, 44, 50, 52, 56] and transgelin (TGLN; muscle protein, epithelial cell differentiation) [32, 41, 42, 47, 56] proteins, and the downregulated gastrokine-1 (GKN1; mitogen) [33, 39, 41, 43, 44, 49, 55, 56] and carbonic anhydrase 2 (CA2; energy metabolism) [33, 39, 40, 56] proteins in GC samples. Conversely, downregulation of HSP27 [66] and ENOA [40, 56] has also been observed, highlighting the heterogeneity among gastric tumors. Moreover, the variations in the pre-analytical, analytical, and post-analytical steps can also contribute to modifications in the proteome profiles and therefore to the differences in the findings [17].

Heat-shock proteins

Our group found an increased expression of HSP27 and HSP20 in GC by using a proteomic approach; for further evaluation, we analyzed HSP27 by Western blot (WB) and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in a larger sample [56]. We observed that GC tumors might present both increased and decreased HSP27 expression. However, HSP27 seemed to be overexpressed in most of the GC samples. Our results were in agreement with several other proteomic studies [33, 43, 44, 47, 49, 55, 56] and supported a previous work that described the upregulation of HSP27 in GC and lymph node metastasis samples from Wistar rats treated with oral administration of *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) [62]. By using proteomic approaches, the upregulation of HSP60 [33, 40, 45] and HSP70 [33, 40] in GC cells has also been described. Moreover, HSP90 [23, 43, 50, 60, 61] and HSP105 [60] also seemed to have a role in gastric carcinogenesis.

HSPs are highly conserved molecular chaperones that are synthesized and expressed by the cell in response to stress conditions [67]. They play a crucial role in cell survival because they are responsible for many cytoprotective mechanisms [67]. The role of the identified HSP in gastric carcinogenesis is not fully understood. In our study, no association was observed between HSP27 expression and clinicopathologic characteristics. Hou et al. reported a decreased expression of HSP27 in a GC cell line derived from lymph node metastasis compared with GC cell lines derived from primary tumors [61]. Conversely, HSP27 has been previously associated with gastric tumor size, distant metastasis, lymph node state, and pStage in other populations [68, 69]. HSP60 [68, 70] and HSP90 [68, 71, 72] overexpression has also been previously associated with several prognosis factors. Chen et al. reported an increased expression of HSP60 and HSP90 in an invasive GC cell line compared with a noninvasive one [60]. Conversely, Hou et al. showed a reduced expression of HSP90 in a GC cell line derived from lymph node metastasis.

Further investigations are necessary to understand the role of HSPs in GC progression; nevertheless, these proteins may be an interesting target for anticancer therapy. Over the last two decades, HSP90 inhibitors have been tested in the clinic [73], where they seemed to have an effect on GC cells [74–76]. Multiple single-agent phase II studies have suggested that these drugs are unlikely to be superior to standard-of-care cytotoxic agents, specific signal transduction inhibitors, or monoclonal antibodies [73]. However, there is a real opportunity to combine heat-shock protein inhibitors with other anticancer agents to achieve tangible clinical benefits [73].

Proteins of cellular energetic metabolism

By using 2DE analysis, we described the presence of two spots for ENOA protein that presented a higher expression in GC compared with nonneoplastic gastric samples [77]; this finding was in agreement with another GC proteomic study [55]. One of the spots presented a higher expression in tumors with or without lymph node metastasis compared with nonneoplastic samples. However, the other spot differed only between tumors with lymph node metastasis and

Table 1 Proteomic studies that aimed to screen and understand proteins deregulated in gastric cancer

Study	Samples	Comparison	Proteomic approach	Identified proteins	Verification[a]/validation[b]	Candidate proteins[c]	Main finding
Identification of proteins deregulated in GC using clinical sample							
Ryu et al. [32]	Total protein of 11 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	7 upregulated and 7 downregulated proteins (fold change analysis)	–	–	The identified proteins may have a role in GC
He et al. [33]	Total protein of 10 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	12 upregulated and 9 downregulated proteins (fold change analysis and statistical analysis not specified)	Verification: WB, UV–vis spectrophotometer for ENOA, TPI, and PGM enzyme activity evaluation and IHC for AMP18	HSP60, 1α-AT, ENOA, AMP18, TPI, PGM	Co-up-regulations of heat-shock proteins and glycolytic enzymes were observed in tumor tissues, indicating self-protective efforts of cells and the growing energy requirement during malignant transformation. Diverse regulations also occurred with proteins involved in cell proliferation and differentiation. AMP18 was downregulated in GC
Jang et al. [34]	Total protein of 18 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	18 upregulated and 5 downregulated proteins (fold change analysis)	Verification: IHC and WB	CK19 and PHB	CK19 is upregulated and PHB was downregulated in GC
Wang et al. [35]	Total protein of 6 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	5 tumor-specific protein spots, including ANXA5, CA1, PHB, fibrin beta, and FGB	–	–	The identified proteins may have a role in GC
Ebert et al. [36]	Total protein of 10 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	5 upregulated and 13 downregulated proteins (fold change)	Validation: WB and IHC in tissue samples and ELISA in serum samples	CTSB	CTSB was upregulated in GC and its increased serum level was associated with advanced tumor stages and progressive disease
Melle et al. [31]	Total protein of gastric cell isolated by LCM from 21 GC (central tumor), 18 gastric tumor margins and 35 normal gastric epithelium	GC versus tumor margins versus normal gastric epithelium	ProteinChip arrays and MS	1 downregulated protein (statistical analysis not specified)	Verification: immune-deplete assay and IHC	PGC	Further studies were still required to investigate through which reduced PGC may play a role in GC biology
Nishigaki et al. [37]	Total protein of 14 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	9 upregulated and 13 downregulated proteins (fold change analysis)	Verification: WB and IHC	MAD1L1, HSP27, and CYR61	The two most notable groups included proteins involved in mitotic checkpoint and mitochondrial functions. The levels of expression of MAD1L1, HSP27, and CYR61 were altered in GC
Lim et al. [38]	Total protein of 152 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	NNMT	Verification: WB	NNMT	NNMT receives a posttranslational modification in cancer-specific manner
Yoshihara et al. [39]	Total protein of 5 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	8 differentially expressed proteins (fold change)	Verification: RT-PCR	CA1, CA2, FOV (Gastrokine-1), GST, MnSOD, HGM1	CA1, CA2, FOV, GST, MnSOD, and HGM1 were downregulated in GC
Cheng et al. [40]	Total protein of gastric cell isolated by LCM from 9 paired cardia GC and nonneoplastic gastric samples	GC cells versus nonneoplastic gastric cells	2DE and MS	15 upregulated and 8 downregulated proteins (fold change analysis)	Verification: WB and IHC	HSP27, HSP60, and PRX2	The identified proteins may have a role in GC. HSP27 and HSP60 were found consistently highly expressed in tumor tissues, whereas PRX2 was suppressed
Li et al. [41]	Total protein of 8 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS/MS	19 upregulated and 9 downregulated proteins (fold change analysis)	Verification: WB and RT-qPCR Validation: TMA	SM22	SM22 is unlikely to be a proper biomarker for GC. Instead, it can be considered a potential indicator for abnormal development of smooth muscles, blood vessels, or myofibroblasts triggered by tumorigenesis

Table 1 (continued)

Study	Samples	Comparison	Proteomic approach	Identified proteins	Verification[a]/validation[b]	Candidate proteins[c]	Main finding
Zhang et al. [42]	Total protein of 10 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS/MS	12 upregulated and 13 downregulated proteins (fold change analysis)	Verification: WB and RT-qPCR Validation: IHC and co-immunoprecipitation of MAWBP and MAWD with BGC823 cell line	TPM, ANXA1, MAWBP, and MAWD	Interactions of MAWBP and MAWD may have a key role in the gastric carcinogenesis
Lin et al. [43]	Total protein of 3 pair of GC (one with *H. pylori* infection) and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE, MS/MS	12 upregulated and 7 downregulated proteins (fold change analysis)	Validation: WB, IHC for ANXA4 and knockdown of ANXA4 using siRNA in SCM-1 cell line infect-ed with *H. pylori*	ANXA2 and ANXA4	ANXA4 was overexpressed in patients infected with *H. pylori*. ANXA4 was overexpressed in GC cell line after *H. pylori* infection
Liu et al. [44]	Total protein of 6 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS/MS	57 upregulated and 50 downregulated proteins (fold change analysis)	Validation: RT-PCR, WB, IHC for TTHY, fumarate and citrate quantification by HPLC assay and fumarate activity measurement by FUMH enzyme assay Analysis of HIF1 and VEGF, which were not identified by proteomic analysis, and in vitro and in vivo model to evaluate the effect of fumarate treatment were also performed	HYOU1, TTHY, KPYM, GRP78, FUMH, ALDOA, and LDHA	A group of metabolic proteins were deregulated, which suggested accumulation of triiodothyronine (T3; the major functional component of thyroid hormone) and overexpression of hypoxia-induced factor (HIF) in gastric carcinoma. T3-induced overexpression of HIF1-α was mediated by fumarate accumulation and could be enhanced by fumarate hydratase inactivation but inhibited by 2-oxoglutarate
Wu et al. [45]	Total protein of 3 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DIGE and MS or MS/MS	7 upregulated proteins and 16 downregulated proteins (fold change analysis)	–	–	DIGE is a useful technique for screening differentially expressed proteins in cancer tissues
Kim et al. [46]	Total protein of 63 GC samples and 43 nonneoplastic gastric samples obtained from endoscopic biopsy	GC versus nonneoplastic gastric samples	LC and MS/MS	Several signals overexpressed in the tumors include DEFA1, DEFA2, S100-A8, and two forms of S100-A9. Signals underexpressed in tumors were identified as LYZ, C-terminal fragment (res 149–175) of AGR2, and N-terminal fragment (res 1–70) of H2B.	–	–	The resulting protein profiles were able to accurately classify tumor and normal tissue, as well as distinguish early stage from more advanced stage cancer
Bai et al. [47]	Total protein of paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	26 upregulated and 6 downregulated proteins (fold change analysis)	Verification: WB Validation: TMA	AGR2, ENOA, GDI2, GRP78, GRP94, PPIA, PRDX1, PTEN, and VDAC1	ENOA, GRP78, GRP94, PPIA, PRDX1, and PTEN were identified as potential GC biomarkers
Aquino et al. [48]	Total protein of 4 paired GC and nonneoplastic gastric samples and 4 nonneoplastic gastric tissue obtained during upper endoscopy (control biopsies)	GC versus nonneoplastic gastric samples versus control biopsies	2D-LC and MS/MS; label-free approach (MudPit)	PGA, COL11A1, CP, CAST, CDH1, and ANXA1 were uniquely identified in the resection margins. TPD52 and PCNA were uniquely identified in the cancer samples. FN and FBLN2 were identified in the cancer biopsies and resection margins but not in the control biopsies	–	–	The resection margin plays a key role in Paget's "soil to seed" hypothesis, that is, cancer cells require a special microenvironment to nourish and that understanding it could ultimately lead to more effective treatments

Table 1 (continued)

Study	Samples	Comparison	Proteomic approach	Identified proteins	Verification[a]/validation[b]	Candidate proteins[c]	Main finding
Kočevar et al. [49]	Total protein of 12 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS/MS	19 upregulated and 11 downregulated proteins (fold change analysis)	Validation: immunoblotting	SEPT2, UBE2N, TALDO1, GKN1, MRPL12, PACAP, GSTM3, and TPT1	SEPT2, UBE2N, and TALDO1 were upregulated and GKN1, MRPL12, PACAP, and GSTM3 were downregulated in GC samples. A higher rate of MRPL12 overexpression was found in the antrum and a higher rate of TPT1 overexpression was found in the cardia/gastroesophageal border. A higher rate of TALDO1 overexpression was found in pN0 and pN3 tumors
Zhang et al. [50]	Total protein of gastric cell isolated by LCM from 15 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	LC and MS/MS	42 upregulated and 39 downregulated proteins (fold change analysis)	Verification: WB Validation: TMA	ANXA1	The upregulated ANXA1 expression may be associated with carcinogenesis, progression, invasion, and lymph node and distant metastasis of GA. This protein could be considered a biomarker of clinical prognostic prediction and of targeted therapy of GC
Kikuchi et al. [51]	Total protein of isolated fibroblasts (primary cultured stromal cells) from 107 GC and paired nonneoplastic samples from patients with T2-T4b GC and without distant metastasis or tumor cell on peritoneal lavage cytology	GC versus nonneoplastic gastric samples	LC and MS/MS	20 upregulated proteins (fold change and statistical analysis not specified)	Verification: RNA microarray, RT-qPCR, and IHC	EPHA2	EPHA2 is upregulated in GC. EPHA2 may help to predict relapse after curative gastrectomy
Kočevar et al. [52]	Total protein of 12 paired GC and nonneoplastic gastric samples	Pool of GC versus pool of nonneoplastic gastric samples	2DE and MS/MS	15 upregulated and 9 downregulated proteins (fold change analysis)	Validation: immunoblotting	GAL4, HADHA, HADHB, and HNRNPM	HADHA was decreased, and HADHB, GAL4, and HNRNPM were increased in GC samples
Liu et al. [53]	Total protein of 9 paired GC and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples	2DE and MS	15 upregulated and 13 downregulated proteins (fold change analysis)	Verification: WB Validation: IHC, knockdown of S100A2 using siRNA in MGC-803 cell line and transfection of S100A2 expression vector in the MGC-803 cell line	S100A2	S100A2 is downregulated in GC. Loss of S100A2 expression seems to contribute to GC development and progression in vivo and in vitro
Identification of proteins deregulated in GC using cell lines							
Deng et al. [54]	Total protein of GC cell line (SGC7901) and normal gastric epithelial cell line (GES-1)	GC cell line versus normal gastric epithelial cell	iTRAQ and LC and MS/MS	7 upregulated and 5 downregulated proteins (fold change analysis)	Verification: WB Validation: IHC in clinical samples	SRI	Upregulation of SRI in GC cell line and GC samples. Overexpression of SRI was associated with depth invasion, TNM stage, and lymph node metastasis

2D two-dimensional, *2DE* two-dimensional electrophoresis, *ELISA* enzyme-linked immunosorbent assay, *GC* gastric cancer, *IHC* immunohistochemistry, *LC* liquid chromatography, *MS* mass spectrometry, *MS/MS* tandem MS, *RT-PCR* reverse transcription-polymerase chain reaction (semi-quantitative analysis), *RT-qPCR* reverse-transcription quantitative PCR (analysis in real-time equipment), *TMA* tissue microarray, *WB* Western blot

[a] Analysis of the same sample used in the proteome analysis

[b] Analysis of a larger number of samples than that applied in proteomic analysis or analysis of a different type of biological sample

[c] Proteins evaluated by using a different approach from proteomic analysis

Table 2 Proteomic studies that aimed to screen and understand proteins involved in the metastasis process

Study	Samples	Comparison	Proteomic approach	Identified proteins	Verification[a]/validation[b]	Candidate proteins[c]	Main finding
Identification of protein associated with GC development and metastasis using clinical samples							
Cai et al. [55]	Total protein of 65 paired cardia GC (42 moderate differentiation and 23 poor differentiation; 11 without lymph node metastasis and 64 with metastasis) and nonneoplastic gastric samples	Pools of GC versus nonneoplastic samples, in which 5 samples were mixed according to the status of tumor differentiation and lymph node metastasis	2DE and MS/MS	57 differentially expressed proteins (base in the ratio), including 11 glucose metabolic enzymes and 5 enzymes of Krebs cycle and oxidative phosphorylation. 44 protein spots associated with the degree of tumor differentiation 29 protein spots associated with tumor metastasis	Verification: RT-qPCR, WB and metabolome analysis Validation: knockdown of LDHA using siRNA in AGS GC cell line	ENO1, LDHA, LDHB, and ACO2	The level of several enzymes and their related metabolic intermediates involved in glucose metabolism were deregulated in GC. Either downregulation LDHA can force pyruvic acid into the Krebs cycle rather than glycolysis process in AGS cell line, which inhibits cell growth and cell migration
Leal et al. [56]	Total protein of 15 paired non-cardia GC (6 without lymph node metastasis and 9 with lymph node metastasis) and nonneoplastic gastric samples	GC versus nonneoplastic gastric samples; GC of patients with lymph node metastasis versus GC of patients with lymph node metastasis versus nonneoplastic gastric samples	2DE, LC, and MS/MS	26 upregulated and 56 downregulated proteins in GC samples (fold change and paired *T* test with 1000 bootstrapping samples); 9 upregulated and 54 downregulated proteins in tumors of patients without lymph node metastasis in relation to controls; 15 upregulated and 68 downregulated proteins in tumors with lymph node metastasis in relation to controls; 14 proteins differentially expressed between tumors of patients with and without lymph node metastasis (fold change and ANOVA with Games-Howell post-hoc analysis and with 1000 bootstrapping samples)	Validation: WB and RT-qPCR	ENO1 and HSP27	111 proteins involved in gastric carcinogenesis were identified. The computational analysis revealed several proteins involved in the energy production processes and reinforced the Warburg effect in GC. ENO1 and HSP27 were upregulated in some GC samples
Ichikawa et al. [57]	Total protein of gastric cell isolated by LCM from 22 paired GC (14 tumors of patients without lymph node metastasis and 8 tumors of patients with lymph node metastasis) and nonneoplastic gastric samples	GC of patients with lymph node metastasis versus GC of patients with lymph node metastasis versus nonneoplastic gastric samples	2DIGE and MS/MS	59 proteins differentially expressed (clustering analysis and Wilcoxon test)	Verification: WB Validation: knockdown of CAPG using siRNA in MKN28[29] and SH-10-TC GC cell lines	ACTR3, CNDP2, CAPG, and ALDH2	CAPG was upregulated in the GC of patients with lymph node metastasis, whereas it showed an equivalent expression level between nonneoplastic samples and GC of patients without lymph node metastasis. CAPG promoted tumor cell invasion, but not cell proliferation
Identification of proteins associated with metastatic processes using clinical samples							
Jung et al. [58]	Total protein of 4 GC of patients with lymph node metastasis and 3 GC samples of patients without lymph node metastasis	GC of patients with lymph node metastasis versus without lymph node metastasis	ICAT, LC and MS/MS	151 differentially expressed proteins (fold change analysis)	Verification: WB Validation: TMA	GAL2 (other proteins were verified in the 7 GC by WB)	Loss of GAL2 might play an important role in the aggressiveness of GC
Identification of proteins associated with metastatic processes using GC cell lines							
Takikawa et al. [59]	Total proteins of metastatic GC cells (MK-45-P) versus its parental cell line (MKN-45)	Metastatic GC cells versus its parental cell line	2DIGE and MS	8 upregulated and 5 downregulated proteins (fold change analysis)	–	–	The identified proteins may have a role in the metastasis process
Chen et al. [60]	Total protein of a metastatic GC cell line (TMC-1) and a noninvasive GC cell line (SC-M1)	Metastatic GC cells versus the noninvasive GC cell	ICAT, 2D-LC, and MS/MS	240 differentially expressed proteins (fold change analysis), including proteins functioning in cell-cell and cell-extracellular matrix (cell-ECM) adhesion, cell motility, proliferation, and tumor immunity	Verification: transcriptome profile by microarray, RT-PCR, immunocytostaining (for CTNNB1), and flow cytometry (for ITG)	CTNNB1, GAL1, IQGAF1, VIM, ITGA6, and ITGB4	CTNNB1, GAL1, IQGAF1, VIM, ITGA6, and ITGB4 were observed in the metastatic TMC-1 cells, whereas IQGAP1 was expressed at a lower level in TMC-1 cells
Hou et al. [61]	Total protein of 2 GC cell line derived from primary cancer (AGS, FU97) and 2 GC cell line derived from lymph node metastasis (AZ521, MKN7)	GC cell line derived from primary cancer versus cell line from lymph node metastasis	iTRAQ, 2D-LC and MS/MS	19 upregulated and 34 downregulated proteins (fold change analysis)	Verification: WB and immunocytochemistry Validation: IHC for CALD, knockdown of CALD using siRNA in AGS and FU97 GC cell lines and stable overexpression of CALD in	CALD, FSCN, and UCHL1.	CALD expression is reduced in GC. Reduced expression of CALD in gastric cells increases cell migration and invasion, while its overexpression reduces cell migration and invasion

Table 2 (continued)

Study	Samples	Comparison	Proteomic approach	Identified proteins	Verification[a]/validation[b]	Candidate proteins[c]	Main finding
					AZZ521 by transfection of a clone encoding *CALD1*		
Identification of proteins associated with metastatic process using animal model							
Chen et al. [62]	Total protein of normal gastric tissue, GC, and corresponding metastases of 3 Wistar rats treated with oral administration of N-methyl-N'-Nitro-N-Nitrosoguanidine (MNNG)	Matched normal gastric tissue versus GC versus corresponding metastases	2DE and MS	25 differentially expressed proteins (based in a semi-quantitative score); 11 proteins were up-regulated and 2 proteins were downregulated in GC compared with normal tissue; 12 proteins were upregulated and 8 proteins were down-regulated in the metastases compared with the primary tumor	Validation: IHC in human tissue	HSP27	In the nonneoplastic gastric mucosa, HSP27 was found in gastric glands of the corpus but not in nonregenerative, nontumorous foveolar epithelium. Cytoplasmic expression of HSP27 was found in all GC and lymph node metastases

2D two-dimensional, *2DE* two-dimensional electrophoresis, *2DIGE* two-dimensional difference gel electrophoresis, *ANOVA* one-way analysis of variance, *GC* gastric cancer, *ICAT* isotope-coded affinity tag, *IHC* immunohistochemistry, *ITRAQ* isobaric tags for relative and absolute quantitation, *LC* liquid chromatography, *LCM* laser capture microdissection, *MS* mass spectrometry, *MS/MS* tandem MS, *RT-PCR* reverse transcription-polymerase chain reaction (semi-quantitative analysis), *RT-qPCR* reverse-transcription quantitative PCR (analysis in real-time equipment), *TMA* tissue microarray, *WB* Western blot

[a] Analysis of the same sample used in the proteome analysis

[b] Analysis of a larger number of samples than that applied in proteomic analysis or analysis of a different type of biological sample

[c] Proteins evaluated by using a different approach from proteomic analysis

Table 3 Proteomic studies that aimed to screen and understand proteins involved in GC patient survival

Study	Samples	Comparison	Proteomic approach	Identified proteins	Verification[a]/validation[b]	Candidate proteins[c]	Main finding
Jia et al. [63]	Total protein of 8 GC samples of patients with good survival (early TNM stage and survival time >34 months) and 8 GC samples of patients poor survival (late stage and survival time <15 months)	GC of patients with good survival versus GC of patients poor survival	LC and MS/MS	117 proteins detected exclusively in GC samples of patients with good survival and 46 proteins exclusively in the group of poor survival	Validation: IHC	S100P	S100P was associated with tumor infiltration depth and tumor size. The survival rate of patients with S100P-positive cancers was highly significantly better than patients with cancers classified as S100P-negative.
Balluff et al. [26]	GC cells and nonneoplastic gastric cells from 63 tissue samples of patients with GC (fresh-frozen intestinal type GC samples cut on a cryostat)	GC cells versus nonneoplastic gastric cells; associations with patient survival	MALDI imaging and MS	150 to 200 peaks by case [corrected multiple testing using the Significance Analysis of Microarrays (SAM) package with a maximum false discovery rate of 0.1], including 7 peaks associated with patient survival	Validation: IHC	CRIP1, S100A6, and HNP1	Seven-protein signature (mass-peaks) predicts outcome in patients independently of clinical parameters; CRIP1 was associated with poor prognosis and survival

GC gastric cancer, *IHC* immunohistochemistry, *LC* liquid chromatography, *MALDI* matrix-assisted laser desorption ionization, *MS* mass spectrometry, *MS/MS* tandem MS

[a] Analysis of the same sample used in the proteome analysis

[b] Analysis of a larger number of samples than that applied in proteomic analysis, or analysis of a different type of biological sample

[c] Proteins evaluated by using a different approach from proteomic analysis

Table 4 Proteomic studies that aimed to screen and understand proteins involved in drug resistance

Study	Samples	Comparison	Proteomic approach	Identified proteins	Verification[a]/Validation[b]	Candidate proteins[c]	Main finding
Tseng et al. [64]	Total protein of SC-M1 cell line treated and nontreated with 5-fluorouracil	5-Fluorouracil-treated GC cell line versus nontreated GC cell line	2DE and MS/MS	6 upregulated and 12 downregulated proteins (fold change analysis)	Validation: IHC and ELISA in clinical samples, overexpression of 14-3-3β in GC cells by transfection of a plasmid with cloned 14-3-3β	14-3-3β	14-3-3β was upregulated in GC cells treated with 5-fluorouracil. 14-3-3β levels were elevated in tumor tissues and serum of cancer patients. Elevated serum 14-3-3β levels highly correlated with the number of lymph node metastases, tumor size, and a reduced survival rate. Overexpression of 14-3-3β enhanced the growth, invasiveness and migratory activities of tumor cells
Li et al. [65][d]	Cell surface glycoproteins of GC cell line SGC7901 and its multidrug-resistant variant cell lines (SGC7901/VCR and SGC7901/ADR)	GC cell line versus its multidrug-resistant variant cell lines	LC and MS/MS	56 cell membrane *N*-glycoproteins were identified, including 11 proteins associated with drug resistance in GC cell lines	Verification: WB Validation: transfection with wild-type and 99^{Asn}-mutated P-glycoprotein	99^{Asn}-Glycosylated P-glycoprotein	The glycoproteins identified may be biomarkers for multidrug resistance in GC. 99^{Asn}-glycosylated P-glycoprotein may increase the drug resistance

2DE two-dimensional electrophoresis, *ELISA* enzyme-linked immunosorbent assay, *GC* gastric cancer, *IHC* immunohistochemistry, *LC* liquid chromatography, *MS* mass spectrometry, *MS/MS* tandem MS, *WB* Western blot

[a] Analysis of the same sample used in the proteome analysis

[b] Analysis of a larger number of samples than that applied in proteomic analysis or analysis of a different type of biological sample

[c] Proteins evaluated by using a different approach from proteomic analysis

[d] Study also presented in Table 6

nonneoplastic tissues [56]. Capello et al. suggested that PTMs are important mechanisms in the regulation of ENOA function, localization, and immunogenicity. Therefore, one of the ENOA spots may have a role in the metastasis process in our Brazilian population [77].

However, inversely to the 2DE observation, the protein level of ENOA was decreased in most of the GC samples, as shown by WB. In our proteomic study, as in most of the other works, we selected for MS analysis only those differentially expressed spots that presented a 1.5-fold change between groups. Thus, the other spots for ENOA may present a slightly decreased expression but with a high impact in the mean of this protein expression. Our results reinforced that different spots may be differently regulated inside a heterogeneous gastric sample and that the two differentially expressed spots may have a role in gastric carcinogenesis.

ENOA directly regulates GKN1, which seems to be downregulated in GC [33, 39, 41, 43, 44, 49, 55, 56]. The silencing of ENOA resulted in growth inhibition and cell cycle arrest of GC cells, which is similar to the effect of GKN1 overexpression in cells, whereas ENOA overexpression blocked GKN1-induced growth inhibition and cell cycle arrest [78]. Moreover, ENOA has a role in the synthesis of pyruvate [79]. ENOA overexpression has been associated with tumor development through glycolysis and has been described in several tumor types (see review [77]).

Proteomic studies have shown the deregulation of several metabolic proteins, particularly the downregulation of enzymes of the Krebs cycle and oxidative phosphorylation in GC [23, 44, 52, 55, 56]. These findings suggested that GC cells present a different metabolite profile compared with nonneoplastic gastric cells, which was confirmed by a GC metabolite profiling analysis [55]. Proteomic and metabolomic studies have suggested the Warburg effect in GC. Even under normal oxygen concentrations, tumor cells may shift from ATP generation through oxidative phosphorylation to ATP generation through glycolysis, converting most incoming glucose to lactate [80]. It has been proposed that highly active glycolysis provides a biosynthetic advantage to tumor cells. Glycolysis provides enough metabolic intermediates by avoiding glucose oxidation, which is essential for the synthesis of macromolecules, such as lipids, proteins, and nucleic acids, during cell division [81–83].

Cai et al. reported that either the downregulation of the lactate dehydrogenase (LDH) subunit LDHA or the overexpression of the pyruvate dehydrogenase (PDH) subunit PDHB can force pyruvic acid into the Krebs cycle instead of the glycolysis process in AGS GC cells, thus inhibiting cell growth and cell migration [55]. These results showed that forced transition from glycolysis to the Krebs cycle has an inhibitory effect on GC progression, providing potential therapeutic targets for this disease.

Table 5 Proteomic studies that aimed to identify membrane proteins that may have a role in gastric carcinogenesis

Study	Samples	Comparison	Proteomic approach	Identified proteins	Verification[a]/validation[b]	Candidate proteins[c]	Main finding
Guo et al. [20]	Membrane proteins of GC cell lines (AGS, Kato III, SNU1, SNU5, MKN7, and IM95)	Analysis of each cell line	Electrophoresis, LC, and MS/MS	Four classes of proteins implicated in invasive and metastatic behavior were well expressed in GC cells: integrins, other adhesion proteins, cell surface proteins known to be associated with tumor invasion, and tetraspanins. Metabolism-related cell surface proteins were also highly expressed by GC cells	Validation: flow cytometry and TMA in clinical samples	MET, EPHA2, FGFR2, and CD104/ITGB4	Intestinal GC had a much higher frequency of EPHA2, MET, and FGFR2 overexpression than diffuse GC
Yang et al. [21]	Cell membrane proteins of a GC cell line (MKN7) and a nonneoplastic gastric epithelial cell line (HFE145)	GC cell line versus nonneoplastic gastric cell line	iTRAQ, isoelectric focusing on immobilized pH gradient, LC, and MS/MS	87 differentially expressed plasma membrane or membrane-associated proteins (analysis using Paragon algorithm)	Validation: WB, immunofluorescence analysis in gastric cells and TMA in clinical samples (only for SLC3A2)	ErbB2 (Her2), PLCG1, SLC3A2 (CD98), LAMP-2, ICAM1, and SLC7A5 (LAT1),	SLC3A2 is upregulated in GC and it is glycosylated in GC cells
Leal et al. [22]	Membrane proteins of GC cell lines (ACP02 and ACP03). ACP03 cells have higher migration capability than ACP02 cell, which are more invasive	Analysis of the pool of cell lines	LC and MS/MS	2608 cell membrane proteins were identified, including 558 plasma membrane proteins	Verification: immunofluorescence, WB and RT-qPCR Validation: WB and RT-qPCR	ANXA2 and GAL3	ANXA2 was upregulated in some GC and its increased expression was associated with metastasis process *in vivo* and a higher invasive phenotype. GAL3 was downregulated in half of the studied tumors and its reduced expression was associated with distant metastasis and with a higher invasive phenotype in vitro
Goh et al. [23]	Membrane proteins of 11 GC cell lines (AGS, TMK-1, NUGC3, NUGC4, SNU484, MKN45, KatoIII, SGC7901, SNU5, SCH and HGC27) and a nonneoplastic gastric epithelial cell line (HFE145)	GC cell line versus nonneoplastic gastric epithelium	iTRAQ, LC and MS/MS	57 membrane proteins were upregulated in at least 6 of the 11 cell lines (fold change analysis)	Verification: WB Validation: knockdown of DLAT using siRNA in TMK-1 GC cell lines, growth and proliferation evaluation, pyruvate quantification	DLAT	DLAT was upregulated in some GC cell lines. Reduced expression of DLAT in gastric cells reduced cell proliferation and increased cellular pyruvate amounts. DLAT was involved in the aerobic glucose catabolic pathway in at least some GC cell lines
Gao et al. [24]	Membrane proteins of 12 GC (stage 1) and nonneoplastic gastric samples	Pool of GC samples versus pool of nonneoplastic gastric samples	TMT, LC, and MS/MS	58 upregulated and 24 downregulated plasma membrane proteins (fold change and ANOVA analysis with permutation based FDR)	Verification: WB Validation: TMA	CD36, CD9, SIGMAR1, and FLOT1 (by WB and IHC)	82 plasma membrane proteins involved in gastric carcinogenesis were identified, including CD36, CD9, SIGMAR1, and FLOT1. FLOT1 is a potential biomarker of GC

ANOVA one-way analysis of variance, *FDR* false discovery rates, *GC* gastric cancer, *IHC* immunohistochemistry, *ITRAQ* isobaric tags for relative and absolute quantitation, *LC* liquid chromatography, *MS* mass spectrometry, *MS/MS* tandem MS, *RT-qPCR* reverse-transcription quantitative PCR (analysis in real-time equipment), *TMA* tissue microarray, *TMT* isobaric labeling by tandem mass tag, *WB* Western blot

[a] Analysis of the same sample used in the proteome analysis

[b] Analysis of a larger number of samples than that applied in proteomic analysis or analysis of a different type of biological sample

[c] Proteins evaluated by using a different approach from proteomic analysis

Table 6 Proteomic studies that aimed to evaluate posttranslational modifications

Study	Samples	Comparison	Proteomic approach	Identified proteins	Verification[a]/validation[b]	Candidate proteins[c]	Main finding
Guo et al. [25]	Phosphoproteins of GC cell lines (KATO III, SNU1, SNU5, AGS, and YCC1)	Qualitative analysis ofphosphoprotein in each cell line	LC and MS/MS	Nonreduntant phosphorylation sites comprised 2144 phosphorylated serines, 673 phosphorylated threonines and 204 phosphorylated tyrosines; 15 phosphorylated protein kinases and 5 phosphorylated phosphatases were identified	Validation: transcriptome analysis and antibody arrays	–	Phosphoproteome and transcriptome analysis may help in the understanding of molecular signaling pathways in GC
Li et al. [65] (see Table 4)							

GC gastric cancer, *LC* liquid chromatography, *MS* mass spectrometry, *MS/MS* tandem MS, WB Western blot

[a] Analysis of the same sample used in the proteome analysis

[b] Analysis of a larger number of samples than that applied in proteomic analysis or analysis of a different type of biological sample

[c] Proteins evaluated by using a different approach from proteomic analysis

Annexins

Annexins are calcium-dependent and membrane-binding intracellular proteins. By forming networks on the membrane surface, annexins can function as organizers of membrane domains and membrane-recruitment platforms for the proteins with which they interact [84]. Annexins participate in several unrelated events, ranging from membrane dynamics to cell differentiation and migration [84].

Several proteomic studies have reported an increased ANXA2 expression in GC samples [40, 43, 44, 50, 52, 56]. Moreover, by using proteomics, we found that ANXA2 was elevated in tumors of patients with lymph node metastasis compared with control nonneoplastic gastric samples; however, there was no such difference between tumors of patients without lymph node metastasis compared with controls [56]. We also observed that an invasive GC cell line presented an increased ANXA2 expression compared with a less invasive cell line [22]. Increased ANXA2 expression has been previously associated with the tumor location, size, differentiation, histologic type, depth of invasion, vessel invasion, lymph node metastasis, distant metastasis, and stage [85, 86]. Moreover, ANXA2 knockdown by using siRNA in a GC cell line showed that ANXA2 overexpression is important for the GC cell motility and to maintain the malignancy of cancer cells [87]. These findings reinforce that proteomic approaches are reliable tools for the identification of key deregulated proteins in gastric carcinogenesis.

The deregulated expression of ANXA1 in gastric carcinogenesis has also been described. However, contradictory results have been reported. Some proteomic studies on GC tissue samples and GC cell lines have found a decreased ANXA1 expression [23, 42, 55, 59], which was associated with poor patient survival [63]. Moreover, Chen et al. reported a reduced ANXA1 expression in a metastatic GC cell line compared with a noninvasive cell line [60]. In contrast, our group observed an elevated ANXA1 expression in GC samples, especially from patients with lymph node metastasis, compared with nonneoplastic gastric samples [56]. Zhang et al. also described the ANXA1 upregulation in GC and its association with GC progression, invasion, and metastasis [50]. Additionally, Hou et al. reported an increased ANXA1 expression in a GC cell line with lymph node metastasis compared with a GC cell line derived from a primary tumor [61]. Other nonproteomic studies have shown that ANXA1 expression was associated with prognosis factors, such as lymphatic invasion, venous invasion, lymph node metastasis, advanced disease stage, and peritoneal dissemination, as well as poorer overall survival in GC patients [88–90]. However, ANXA1 is also deregulated in precancerous gastric lesions, suggesting its involvement from the early stages of gastric carcinogenesis [91, 92].

Increased expression of ANXA3 [51], ANXA4 [40, 43, 50], ANXA5 [37, 56], and ANXA13 [24] has also been found in GC by applying proteomic approaches. ANXA3 expression has been previously associated with tumor volume and TNM stage and inversely correlated with patient prognosis [93]. Moreover, silencing of endogenous ANXA3 suppresses the proliferation, migration, and invasion of GC cells [94], thus reinforcing the role of these proteins in GC.

Other annexins may act as tumor suppressors in gastric carcinogenesis, such as ANXA6 [24], which is downregulated through promoter methylation in GC [95]. On the other hand, Yuan et al. found that the survival rate of patients with ANXA7-positive expression was lower than that of patients with negative expression [96]. However, Hsu et al. reported that loss of expression of ANXA7 was significantly related to distant metastasis [97]. Moreover, ANXA7 expression seemed to be higher in intestinal-type than in diffuse-type tumors [97]. Reduced ANXA10 expression has been observed in GC, and lack of ANXA10 expression has been previously associated with poor survival [98]. A functional analysis with the use of siRNA in GC cell lines showed that ANXA10 regulates GC cell proliferation [98]. Furthermore, the introduction of ANXA10-expressing plasmid into GC cells suppressed the cell growth and increased the apoptosis [99]. These results reinforce that ANXA10 may play a role as a tumor suppressor in GC. However, similarly to ANXA7, the expression pattern of ANXA10 seemed to differ between intestinal-type and diffuse-type GC [100]. In intestinal-type GC, ANXA10 overexpression has been associated with higher tumor stage and lower 5-year survival rate. Conversely, in diffuse-type GC, ANXA10 has been associated with earlier tumor stage and lymph node metastasis and better 5-year patient survival [100]. All these results highlight that several annexins have a role in GC development and progression.

S100 proteins

S100 proteins are a subgroup of the EF-hand Ca^{2+}-binding protein family, involved in several biological processes. Besides exhibiting some degree of cell specificity, S100 isoform expression depends on environmental factors to regulate important cellular functions such as proliferation and motility [101]. Moreover, secreted S100 proteins may have chemotactic and angiogenic activities, thereby further contributing to the tumourigenic process [101]. Additionally, several S100 proteins are known as binding targets of annexins involved in the regulation of actin cytoskeleton dynamic [102] and therefore contribute for the cancer development.

The S100 proteins may also have a role in GC [23, 26, 53, 63, 103–105]. The S100P has been previously associated with tumor infiltration depth, tumor size, and patient survival in a proteomic study [63]. This finding was supported by other GC studies [104, 105].

Liu et al. reported the downregulation of the S100 calcium-binding protein A2 (S100A2) in GC based on proteomic, WB, and immunohistochemistry (IHC) analyses [53]. Moreover, reduced S100A2 expression has been associated with poor differentiation, deeper tumor invasion, and lymph node metastasis. The relapse-free probability and overall survival rate also decreased with decreasing S100A2 expression. Other studies have previously shown a gradual loss of S100A2 expression from gastritis, intestinal metaplasia, and dysplasia to cancer tissue specimens; the S100A2 loss of expression was associated with poor prognosis [106, 107]. To further evaluate the role of S100A2 in gastric carcinogenesis, Liu et al. [53] induced the overexpression of S100A2 and silenced S100A2 in a GC cell line. The induced overexpression led to a reduced invasive ability of GC cells. Conversely, the silencing of S100A2 increased the invasion of GC cells. These findings suggest that S100S2 may modulate the signal pathway to regulate the invasive ability of GC cells.

Galectins

Galectins (GAL or LGAL) consist another important protein family that may have a role in gastric carcinogenesis. GAL contribute to many hallmarks of cancer, including sustained proliferative signaling, resistance to cell death signals, evasion of immune surveillance, induction of angiogenesis, and activation of the metastatic potential (see review [108]). However, the information about galectins remains limited in GC, as well as in other neoplasia.

It is important to highlight that the role of each GAL family members can vary in human cells despite they share a consensus amino acid sequence and betagalactosidecontaining sugar affinity. For example, GAL1 is a strong biomarker of hypoxia-induced cellular stress responses and seems to promote T cell apoptosis and inhibit various aspects of T cell effector functions. Conversely, GAL3 presents anti-apoptotic activity in protecting cancer cells from hypoxia stress, but it can behave as amplifiers of the inflammatory cascade (for more details about GAL family members, see review [109]).

Chen et al. found an increased GAL1 expression in a metastatic GC cell line compared with a noninvasive cell line [60]. Moreover, GAL1 expression has been associated with tumor size, differentiation grade, TNM stage, and lymph node metastasis, as well as with the overall survival rate of GC patients [110–112]. However, GAL1 upregulation is not specific to gastric neoplastic cells. Rather, GAL1 seems to be also overexpressed in precancerous gastric lesions [91, 92]. Kočevar et al. described the overexpression of another galectin, GAL4, in GC by using a proteomic approach [52].

Jung et al. observed that GAL2 expression level in GC was higher in patients without lymph node metastasis [58]. By applying IHC analysis, the authors confirmed that reduced GAL2 expression was associated with lymph node metastasis and advanced clinical stage. Therefore, GAL2 loss might play an important role in the aggressiveness of GC.

Our previous proteomic study also indicated a decreased GAL3 expression in GC [56]. In another study, we found that most of the GC samples had reduced GAL3 expression and that loss of expression of GAL3 was associated with distant metastasis [22]. Moreover, an invasive GC cell line presented reduced GAL3 expression compared with a less invasive cell line [22]. Our results corroborated the findings of Okada et al., in which reduced GAL3 expression was associated with poor prognosis of GC patients based on IHC analysis [113]. Decreased GAL3 expression has also been associated with poorer prognosis in several human cancers [114–119]. Several clinical trials with different galectin-targeting agents, especially GAL3 inhibitors, are ongoing [108]. Thus far, it remains unclear whether GC patients would benefit from this type of anticancer therapy.

14-3-3 protein family members

Some proteomic studies have shown the deregulated expression of 14-3-3 protein family members in gastric carcinogenesis [34, 56, 60, 64]. This family of proteins is highly conserved in eukaryotes. Although the exact 14-3-3 protein functions are not fully known, these proteins may act as a molecular scaffold, bringing together proteins that interact functionally and effecting phosphorylation-dependent cell regulation [120]. This protein family is involved in several biological processes and plays a regulatory role in apoptosis, mitogenic signal transduction, and cell cycle control (for reviews, see [121–123]). The regulation of apoptosis by 14-3-3 proteins is a complex process, and these proteins can have pro- and anti-apoptotic effects [124]. The role of 14-3-3 isoforms in the different cellular processes depends on their property as molecular chaperones by binding to various protein ligands.

Our group [56] and Jang et al. [34] reported that 14-3-3ζ/Δ was overexpressed in GC samples. 14-3-3ζ is a potential pharmaceutical drug target. A dimeric 14-3-3 peptide inhibitor (Difopein) binds with high affinity to 14-3-3ζ, displaces 14-3-3–BCL2-associated agonist of cell death (BAD) interactions in NIH 3T3 fibroblast cells, and, thus, results in apoptosis [125]. It was demonstrated that 14-3-3 antagonists was also able to reduce the viability of A549 (lung), DU145 (prostate), and HeLa (cervix) cancer cell lines [125]. Thus, targeting 14-3-3ζ-ligand interactions may be a useful strategy as an anticancer treatment in GC cells with elevated 14-3-3ζ expression.

Chen et al. found that 14-3-3σ/τ protein expression was elevated in a metastatic GC cell line compared with a noninvasive GC cell line, suggesting that this protein may also have a role in invasiveness [60]. Although the more distant family member 14-3-3σ is a prominent tumor suppressor [126], its increased expression has been described in several tumors, including in GC [127]. In GC, 14-3-3σ immunoreactivity was correlated with clinical stage and tumor invasion [127]. Additionally, 14-3-3σ expression was able to predict poorer overall survival and progression-free survival [127].

Moreover, 14-3-3β was upregulated in tumors and serum of GC patients, as well as in GC cells treated with 5-fluorouracil, and thus may have a role in drug resistance [64]. Overexpression of 14-3-3β is able to enhance the growth, invasiveness, and migratory activities of tumor cells [64].

Conversely, we observed reduced 14-3-3ε protein expression in GC samples [128]. Low expression of 14-3-3ε has also been reported in other neoplasias [129–131], suggesting a tumor suppressor function. The reduced 14-3-3ε expression may be due in part to the loss of its locus (17p13), which is a common finding in GC of individuals from our population [132]. Interestingly, one of the MYC target proteins is CDC25, which is negatively regulated by 14-3-3ε [133–135]. Our research group previously reported that MYC mRNA and protein overexpression and its gene amplification are a common finding in GC samples, GC cell lines, and some preneoplastic gastric lesions from a Brazilian population, as well as in nonhuman primate models of gastric carcinogenesis [136–150]. We recently observed that the silencing of *MYC* by using siRNA in three GC cell lines led to CDC25 downregulation and 14-3-3ε upregulation (unpublished data). Therefore, 14-3-3 protein family members may play an important role in GC.

Prohibitins

Prohibitins (PHB) have been described as upregulated in GC [32, 33, 35, 151] by proteomic approaches and other methodologies. PHB overexpression has also been detected in some GC cell lines [23]. However, some studies have reported PHB downregulation in GC [34, 152]. Our group observed both reduced and increased PHB expression in GC [153]. Reduced PHB expression has been associated with tumor dedifferentiation and cancer initiation, whereas the T allele of the rs6917 polymorphism has been related to reduced *PHB* mRNA levels. Moreover, the increased *PHB* expression seemed to be regulated by the gain of gene copies. Thus, *PHB* copy number variation and differential expression of the rs6917 polymorphism may play a role in PHB regulation [153]. Our study highlighted that different genetic alterations may contribute to the protein expression heterogeneity.

Other interesting proteins identified by proteomic analysis

Other interesting proteins have been identified in GC proteomic studies and validated by using other methodologies; these proteins include intestinal cysteine-rich protein 1 (CRIP1), sorcin (SRI), EPH receptor A2 (EPHA2), macrophage-capping protein (CAPG), and caldesmon (CALD). CRIP1 and SRI have been associated with poor prognosis factors [26, 54]. SRI overexpression has also been previously associated with multidrug resistance in GC cells [154]. Moreover, EPHA2 may help to predict relapse after curative gastrectomy [51].

Ichikawa et al. described the CAPG overexpression in GC of patients with lymph node metastasis by using proteomic analysis and WB [57]. The knockdown of CAPG by siRNA in two GC cell lines showed that CAPG promoted tumor cell invasion but not cell proliferation. Therefore, GAPG may contribute to the metastasis process.

Hou et al. compared the proteome profile of GC cell lines derived from primary cancer with that of GC cell lines derived from lymph node metastasis [61]. CALD expression was reduced in the GC cell lines derived from lymph node metastasis. Then, the authors observed the CALD expression was reduced in GC samples by IHC. Moreover, in vitro functional studies have shown that the decreased expression of CALD in gastric cells increases cell migration and invasion, whereas its overexpression reduces cell migration and invasion. Thus, CALD may play a key role in GC progression.

Posttranslational modifications

PTMs are crucial to protein heterogeneity and contribute to the variations in protein stability, location, and function. As previously described in this study, ENOA may receive PTMs in GC. An elevated expression of NNMT has been detected in GC [34, 37, 49, 56]. Lim et al. observed a single spot of NNMT in gastric ulcer tissues, compared with four to five spots in GC tissues [38]. This study suggested that NNMT may also receive a PTM in a cancer-specific manner.

The study of PTM may provide insights into the regulation of gastric cell function. Some proteomic studies evaluated specific PTM in GC (Table 6). Guo et al. investigated the phosphoproteome of GC cell lines and identified several phosphorylated proteins potentially imply in critical roles in gastric carcinogenesis, including the phosphorylation of the tumor protein p53 [25]. In glioma stem cells, the phosphorylation of p53 and other checkpoint proteins has been associated with radioresistance [155]. Thus, phosphorylated p53 may explain the GC resistance to several nonsurgical treatments.

The analysis of the glycoproteome of GC cell lines has also contributed to the understanding of GC cell resistance to chemotherapy. Li et al. compared the cell surface glycoproteome of two multidrug-resistant cell lines with that of their parental drug-sensitive GC cell line [65]. The authors described 11 glycoproteins, including the glycosylated form of epidermal growth factor receptor (EGFR). Moreover, they reported that 99^{Asn}-glycosylated P-glycoprotein may increase the drug resistance. The identified glycoproteins may be possible biomarkers for predicting multidrug resistance or key regulators for targeted therapies.

It is important to highlight that MS technologies and sample fractionation methods have been constantly improving. Such progress will increase the knowledge of protein expression and its PTM in gastric physiology and pathologies and, consequently, the chances of discovering sensitive and specific GC biomarkers [17].

Proteins and the GC new molecular classification

The Cancer Genome Atlas Research Network evaluated the protein expression of 191 proteins by reverse-phase protein array in 295 GC samples [14]. Using this approach, the authors targeted specific proteins with validated primary antibodies. Key proteins of EBV-positive subtype, microsatellite instability subtype, genomically stable subtype, and chromosomally unstable subtype were described. The EBV-positive subtype presented caspase 7 (CASP7), proliferating cell nuclear antigen (PCNA), BCL2-associated X protein (BAX), spleen tyrosine kinase (SYK), and Src family tyrosine kinase LCK (LCK) elevated expression. In the microsatellite instability subtype, claudin 7 (CLDN7), von Hippel-Lindau tumor suppressor (VHL), and cyclin B1 (CCNB1) elevated expression were detected. KIT proto-oncogene receptor tyrosine kinase (KIT), v-myc avian myelocytomatosis viral oncogene homolog (MYC), v-akt murine thymoma viral oncogene homolog (AKT), and protein kinase C alpha (PRKCA) were highly elevated in the genomically stable subtype. On the other hand, chromosomally unstable subtype was marked by the elevated EGFR phosphorylation and p53 expression. These described proteins may help to elucidate the distinct pathways involved in the four GC subtypes and may serve as a valuable adjunct to histopathology [14].

Final considerations

This review highlighted the proteins or protein families that are frequently identified by using high-throughput screening methods and which may have a key role in gastric carcinogenesis. The attempt to understand the GC proteome, including dynamic PTM, may help in the elucidation of the complex mechanisms underlying the tumor phenotypes. The increased

knowledge of gastric carcinogenesis will help in the development of new anticancer treatments.

Although GC proteomic studies are still in their infancy, the proteins described in these studies may also contribute to the screening of possible novel biomarkers, which, after further investigations, may be useful in GC diagnosis, prognosis, and patient management. Additionally, GC proteomic studies reinforce the idea that several GC biomarkers are necessary in the clinical routine because the tumors are largely heterogeneous.

Acknowledgments This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; to MCS, FW, DQC, and MFL), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; to MCS and RRB), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; to COG) as grants and fellowship award.

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