Gastric cancer: Metabolic and metabolomics perspectives (Review)

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Abstract. Gastric cancer is one of the most malignant tumors worldwide and remains a major health threat in Asia-Pacific regions, while its pathological mechanism is generally unknown. Recent research has advanced the understanding of the relationship between metabolic reprogramming and carcinogenesis. In particular, metabolic regulation and cancer research are being further brought into sharp focus with the emergence of metabolomics. Not only can metabolomics provide global information on metabolic profiles of specific tumors, but it can also act as a promising tool to discover biomarkers regarding diagnosis, metastatic surveillance and chemotherapeutic sensitivity prediction. Meanwhile, metabolism-based anticancer therapies will be further discovered. Up to now, accumulative studies have highlighted the application of metabolomics in gastric cancer research regarding different aspects; therefore we summarized the current available results of how metabolic changes are linked to gastric carcinogenesis, and how metabolomics holds promise for the diagnosis, metastatic surveillance, treatment and prognosis prediction of gastric cancer.

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1. Introduction

Gastric cancer remains third in ranking in cancer death worldwide, although its overall incidence is declining in recent years (1). In the past decades, studies aimed at *Helicobacter pylori* infection (2,3), hereditary susceptibility (4) and environmental factors (5) have made a great breakthrough in investigating its precise pathogenesis. Recently, application of various '-omics' technologies opened a new field to investigate the mechanisms behind this disease.

With the emergency of metabolomics, major progress has been made in the understanding of the relationship between metabolic regulation and cancer. Warburg, in fact, showed a characteristic metabolic pattern of tumors in the 1920s, that is, tumor cells consume a large amount of glucose for glycolysis even under the condition of sufficient oxygen (Warburg effect) (6). Extensive research also indicates that metabolic reprogramming is one of the hallmarks of cancer (7), and intricately linked to oncogenesis (8-10) and cancer immune escape (11-13). On the other hand, study methods combined conventional oncology research and metabolomics are more likely to provide deeper insights in this field. The procedure of these methods is illustrated in Fig. 1, and more detailed information can be found in literature (14-16).

Several excellent reviews have been published on metabolomics application in different diseases (17-19) especially cancer research (20-23). Hence, this report presents fresh and profound insights into metabolic changes in gastric cancer and possible mechanism behind these alterations is further discussed. Then, we focus on some studies including our data targeted on biomarkers involving diagnosis, metastasis and prognosis, and treatment in this disease. Finally, future directions are presented.

2. Metabolic alteration in gastric cancer

Up to now, several studies aimed at identifiable metabolic changes in macroenvironment-blood (24-29) (Table I) and urine (30-34) (Table II) or microenvironment-carcinoma tissues (35-41) (Table III) and gastric juice (42-44) (Table IV) have been done to map globally metabolic profiles and interpret its possible mechanism in the process of gastric carcinogenesis. Typical changes in metabolites of this disease are illustrated in Fig. 2.

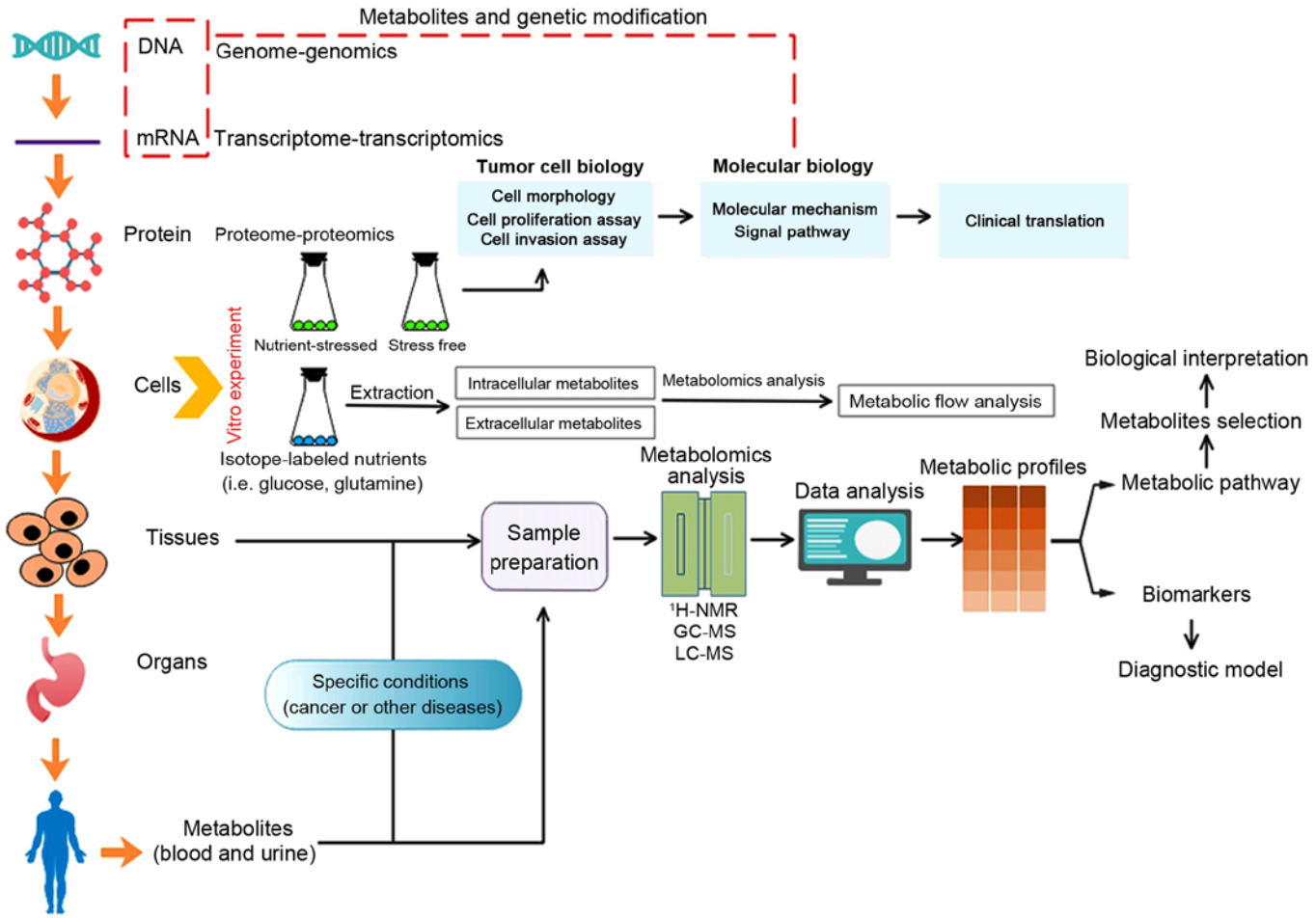

Figure 1. Metabolism and metabolomics in cancer research. Concerning tumor cell lines cultured *in vitro*, either conventional cell biology research or isotopic tracer experiment is available. Tumor cytobiological methods (cell morphology, cell proliferation assay and cell invasion assay) can be utilized to assess cytobiological behaviors under specific nutrient-stressed or stress-free condition, and further investigations targeted at precise mechanism and significance can be confirmed via molecular biology techniques. With regard to isotopic tracer experiment, the flow of nutrients and metabolites can be identified with isotopic tracer, then the significance of specific nutrients or metabolites and its potential divergent fates toward meeting the demands of either energetic utilization or synthesizing macromolecules in cancer cells can be identified (14). Metabolites that are different between tumor groups and control groups are able to be detected through metabolomics analysis (such as ^{1}H-NMR, ^{1}hydrogen-nuclear magnetic resonance; LC-MS, liquid chromatography-mass spectrometry; GC-MS, gas chromatography-mass spectrometry) and data analysis, metabolic biomarkers or metabolic pathway that is specific to certain cancers were discovered to benefit cancer research (15,16). Of note, combination of genomics, transcriptomics and proteomics plus metabolomics can further give us comprehensive understanding of cancers toward systematic biology.

Glucose metabolism. Cumulative evidence demonstrates that concentration of lactic acid shows a consistent increase in urine (30) or tissue (31,35,36,38) samples of gastric cancer groups, but glucose is considerably depleted compared with those healthy counterparts or non-malignant patients (like chronic superficial gastritis and chronic atrophic gastritis without intestinal metaplasia) (35,36). The high lactate level might be attributed to the special metabolism of most cancer cells, known as 'Warburg effect' we mentioned above (6). Scarce glucose might result from the overexpression of glucose transporters (42) and type II hexokinase (43), which are both confirmed in gastric cancer tissues. Higher fructose-6-phosphokinase (6-FPK) activity can also result in low glucose in gastric cancer tissues (44), as it regulates the output of glucose to glycolysis pathway. The glycolytic switch has been identified to be associated with oncogenic transformation and molecular signal transduction, such as hypoxia-inducible factor pathway, insulin signaling pathway and PI3K-Akt-mTOR pathway (45). Furthermore, over-expression of pyruvate kinase and lactate dehydrogenase is positively associated with tumor proliferation and poor prognosis, downregulation of them *in vitro* experiment can impair tumor invasion (38,46-49). On the other hand, such special microenvironment might be the requirement of rapid propagation of tumor cells. To our understanding, it has been reported that accumulated lactic acid moderates the activity of proteases that decompose extracellular matrix, which can produce some peptides and amino acids that are consumable for energy generation (44). Acidosis microenvironment is also ascribed to the formation of cancer blood vessels, meeting the plentiful supply of nutrients and leading to tumor invasion and metastasis (50). Moreover, tumor-derived lactate shows strongly negative effects on cytotoxic T-cell/NK cell function (11,51) and blocks differentiation of monocytes to dendritic cells (52), finally leading to tumor immune escape. However, such outcome demands further verification in gastric cancer.

Considering tricarboxylic acid cycle (TCA) intermediates, an increase of five metabolites (α-ketoglutaric acid, malic acid,

Table I. Metabolites in blood samples between cancer groups and non-cancer groups.

Year	Patients/ animal models	Samples	Sample size	Method	Major findings	Ref.
2011	Patients	Plasma	Healthy people (n=985) Gastric cancer (n=199)	HPLC-ESI-MS	i) Concentration of amino acids was significantly decreased in plasma of gastric cancer patients and this change occurs in early stage regardless of the subsequent progression and poor nutrition ii) Plasma-free amino acids have shared alteration among gastric cancer, lung cancer, colorectal cancer, breast cancer and prostate cancer, but specific profiles in gastric cancer were also detected	(24)
2011	Patients	Plasma	Chronic superficial gastritis (n=19) Chronic atrophic gastritis (n=10) Intestinal metaplasia (n=10) Dysplasia (n=15) Gastric cancer (n=22)	GC-TOFMS	i) 15 metabolites (increase, glutamate, asparagine, ornithine, pyroglutamate, 2-hydroxybutyrate, azelaic acid, 11-eicosenoic acid, 1-monohexadecanoylglycerol -γ-tocopherol, urate; decrease: creatinine, threonate) were different between chronic superficial gastritis and gastric cancer group ii) Metabolic phenotype of gastric cancer was greatly similar to intestinal metaplasia	(25)
2012	Patients	Serum	Healthy people (n=30) Gastric cancer (n=30)	GC-MS	i) 18 metabolites were different between gastric cancer and healthy control group, including fumaric acid, glutamine, valine, sarcosine, 9,12-octadecadienoic acid, 9-octadecenoic acid, trans-13-octadecenoic acid, nonahexacontanoic, cholesta-3,5-diene, cholesterol pentafluoropionate, cholesterol, Cholest-5-en-3-ol, 2-*O*-mesyl arabinose, hexadecanenitrile, benzeneacetonitrile, 2-amino-4-hydroxy-pteridinone, 1,2,4-benzenetricarboxylic acid and hexanedioic acid ii) Valine showed the greatest fold change and hexanedioic acid was the most depleted	(26)
2012	Patients	Serum	Healthy people (n=12) Gastric cancer (n=11)	GC-MS	i) Pyruvic acid, 3-hydroxypropionic acid, 3-hydroxyisobutyric acid, octanoic acid, phosphoric acid significantly changed in gastric cancer patients ii) Levels of 3-hydroxypropionic acid and pyruvic acid could discriminate gastric cancer from esophageal or colorectal cancer	(27)
2012	Patients	Plasma	Chronic superficial gastritis (n=20) Gastric cancer (n=17) Post-operation of gastric cancer (n=15)	GC-TOFMS	i) 15 discriminatory metabolites (increase, β-hydroxybutyrate, β-D-methylglucopyrano-side, heptanoic acid; decrease, succinate, malate, fumarate, citrate, serine, glycine, cysteine, and S-methyl-cysteine, docosahexaenoic acid, inositol-phosphate, octadecenoic acid, and 9-(Z)-hexadecenoic acid) were identified between gastric cancer and chronic superficial gastritis group ii) Surgical removal of the cancer tissues could change levels of many metabolites that characterized the metabolic phenotype of the cancer group	(28)
2016	Patients	Serum	Chronic superficial gastritis (n=17) Gastric cancer (n=32)	LC-MS	Gastric cancer displays upregulated kynurenine pathway of tryptophan metabolism: increase, indole-3-lactic acid, anthranilic acid, kynurenic acid; decrease, kynurenine, 3-indoxyl-sulfate	(29)

HPLC-ESI-MS, high performance liquid chromatography-electrospray ionization-mass spectrometry; GC-TOMS, gas chromatography-time-of-flight mass spectrometry; GC-MS, gas chromatography-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry.

Table II. Metabolites in urine samples between cancer groups and non-cancer groups.

Year	Patients/ animal models	Sample size	Method	Major findings	Ref.
2011	SCID mice (male) SCG-7901 cell line	Gastric cancer (n=16) (metastasis group (=8 and non-metastasis (=8) Control group (n=8)	GC-MS	i) 10 metabolites were different between cancer group (metastasis and non-metastasis) and control group: lactic acid, malic acid, citric acid, glycerol, hexadecanoic acid, pyrimidine, uric acid, butanoic acid, propanoic, butanedioic acid ii) 7 metabolites were characteristic between metastasis and non-metastasis groups: alanine, L-proline, glycerol, butanoic acid, butanedioic acid, L-threonic acid, myo-inositol. iii) Changes in lactic acid and butanoic acid showed diagnostic value	(30)
2014	Patients	Training set/validation set Gastric cancer (n=50/23) Healthy people (n=50/31)	^{1}H-NMR	i) Altered metabolites in urine samples of gastric cancer are mainly related to amino acids and lipid metabolism; levels of 4-hydroxyphenylacetate, alamine, phenylacetylglycine, manmitol, glycolate and arginine are related to T stage of gastric cancer ii) Hypoxanthine is able to predict a recovery trend in postoperative groups of gastric cancer	(31)
2015	Patients	Gastric cancer (n=13) Healthy people (n=9)	LC-MS	16 metabolites were differently expressed between cancer and healthy group: succinic acid, malic acid, alanine, glycine, L-proline, hexadecanoic acid, pyrimidine, uric acid, glycocholic acid, hippurate, urea, 4-deoxythreonic acid, phenylacetylglycine, taurine, 2-oxoglutarate	(32)
2016	Patients	Gastric cancer (n=43) Healthy people (n=40) Barrett's esophagus (n=40)	^{1}H-NMR	i) Levels of sucrose, dimethylamine, 1-methylnicotinamide, 2-furoylgylycine, N-acetyl-serotonin, trans-aconitate, formate and serotonin greatly altered in urine samples of gastric cancer ii) Alanine, 2-hydroxyisobutyrate and 3-indoxylsulfate produced a discriminatory model regarding discriminating cancer and control group	(33)
2016	Patients	Gastric cancer (n=199) Healthy people (n=87)	GC-MS	i) 17 metabolites are largely different between cancer and control groups in training set: glycine, valine, isoleucine, serine, threonine, proline, methionine, tyrosine, tryptophan, ethyl 2-methylacetoacetate, levulinic acid, p-cresol, benzylmalonic acid, 4-hydroxybenzoic acid, hippuric acid, benzil, alamine ii) 14 of them show diagnostic value that is better than classic blood biomarkers on validation set, most of which were related to amino acid metabolism iii) Proline, p-cresol and 4-hydroxybenzoic acid produce outcome-prediction value by survival analysis	(34)

SCID, severe combined immune deficiency; ^{1}H-NMR, ^{1}hydrogen-nuclear magnetic resonance; LC-MS, liquid chromatography-mass spectrometry.

Table III. Metabolites in tissue samples between cancer groups and non-cancer groups.

Year	Patients/ animal models	Sample size	Method	Major findings	Ref.
2009	Patients	12 pairs of matched tumor and normal gastric tissues	CE-TOFMS	i) Extremely low glucose, high lactate and glycolytic intermediate concentrations were found in both colon and stomach tumor tissues ii) Significant accumulation of all amino acids except glutamine in the tumor tissues	(35)
2010	Male SCID mice SCG-7901 cell line	Cancer group (Non-metastasis 8 Metastasis 8) Control group 6	GC-MS	i) 29 metabolites were differently expressed between metastasis and non-metastasis group: glucose, succinate, malicacid, lactate, alanine, glycine, valine, leucine, dimethylglycine, isoleucine, propanamide, butanedioic, proline, methionnine, serine, threonine, asparagine, glutamine, phosphoserine, glutamate, lysine, arginine, docosanoic, octadecanoic, pyrimidine, hypoxanthine, inositol, propanedioc, pyrrolidine ii) Serine and proline metabolisms were highlighted in metastatic group	(36)
2010	Patients	18 pairs of matched tumor and normal gastric tissues	GC-MS	i) L-glutamine, phosphoserine, L-valine, L-isoleucine, serine, heptanedioic acid, propanoic acid, phenanthrenol, butanetriol, acetamid, butenoic acid, oxazolethione, naphthalene, L-altrose, L-mannofuranose, galactofuranoside, myo-inositol, D-ribofuranose were detected differently between the malignant tissues and the adjacent non-malignant tissues of gastric mucosa ii) 5 of them were detected differently between the non-invasive tumors and the invasive tumors: higher levels of L-cysteine, L-tyrosine, hypoxanthine and lower levels of phenanthrenol, butanoic acid in the invasive group	(37)
2010	Patients	65 pairs of matched gastric cardiac cancer and adjacent normal tissues	GC-TOFMS	i) Dysregulation of pyruvic acid efflux was an important glucose metabolic signature in the development of gastric cardiac cancer ii) Transition from glycolysis to the Krebs cycle had an inhibitory effect on GCC progression, which could be served as a potential therapeutic target for gastric cardiac cancer	(38)
2011	Patients	30 pairs of matched tumor and normal gastric tissues	GC-MS	i) 15 differential metabolites were identified: α-ketoglutaric acid, fumaric acid, valric acid, 9-hexadecennoic acid, 3-hydroxybutanoic acid, hexadecanoic acid, octadecanoic acid, *cis*-vaccenic acid, arachidonic acid, 1-phenanthrene-carboxylic acid, 9-octadecenamide, squalene, xylonic acid, benzenepropanoic acid ii) Current models could not discriminate normal mucosa and different pathological stages of GC tissues based on their identified metabolic profiles	(39)
2012	Patients	Gastric cancer (n=17) Chronic superficial gastritis (n=20)	GC-TOFMS	Discriminating metabolites associated with glucose, amino acids, lipid and nucleotide metabolism were detected between two groups: increase, citrate, malate, fumarate, succinate), cysteine, 2-aminoadipate, 9-(Z)-hexadecenoic acid, docosahexaenoic acid, β-hydroxybutyrate, uracil, monomethylphosphate; decrease, glucose, maltose, ribose, β-D-methylglucopyranoside, fructose-6-phosphate, inositol and ribitol, glyceric acid-2,3-diphosphate, nonesterified cholesterol, uridine	(28) (31)
2014	Patients	30 pairs of matched tumor and normal gastric tissues	HR-MAS-NMR	Lipid metabolites were significantly lower, while some amino acids (such as isoleucine, glutamate, leucine, valine, alanine, lysine and phenylalanine), taurine and lactate were significantly higher in tumor tissues	

CE-TOFMS, capillary electrophoresis time-of-flight mass spectrometry; HR-MAS NMR, high-resolution magic angle spinning nuclear magnetic resonance.

Table IV. Metabolites in gastric juice between cancer groups and non-cancer groups.

Year	Patients/ animal models	Sample size	Method	Major findings	Ref.
2011	Patients	Benign gastric diseases (n=68) Gastric malignancies (n=33)	HPLC LC-MS	i) Aromatic amino acids in gastric juice can be used as diagnostic biomarkers to screen gastric malignancies, areas under receiver operating characteristic curves for tyrosine, phenylalanine and tryptophan were 0.838, 0.856 and 0.816, respectively ii) The sensitivity and specificity of gastric malignancy detection with phenylalanine reached 87.9% and 79.4%	(40)
2012	Patients	Non-neoplastic gastric disease (n=70) Early gastric cancer (n=49) Advanced gastric cancer (n=66)	HPLC	Levels of tyrosine, phenylalanine and tryptophan in gastric juice increased in the early phase of gastric carcinogenesis, which could function as a biomarker to screen this disease at early stage in the general population	(41)
2016	Patients	Chronic superficial gastritis (n=17) Gastric cancer (n=32)	LC-MS	Upregulated kynurenine pathway of tryptophan metabolism: levels of tryptophan, anthranilic acid, nicotinic acid, kynurenic acid, kynurenine and indole-3-lactic acid (P>0.05) were increased	(29)

HPLC, high performance liquid chromatography.

fumarate, succinate, citric acid) is noticed regardless of blood (26,28), urine (30,32) or tissue (28,35,36,39) samples in gastric cancer. There are some possible reasons that can explain this phenomenon. One account is that cancer cells still use a small portion of glucose for oxidative phosphorylation. Secondly, cancer cells might also utilize fumarate respiration to generate energy under special conditions of glucose deprivation and severe hypoxia in microenvironment (53), and succinate is one of the byproducts in this process except for originating from TCA. Hence, it provides a likely explanation for the accumulation of fumarate and succinate. Another reason is that some amino acids, such as glutamine, threonine, phenylalanine, tyrosine or proline, can be converted into these intermediates involving in TCA (Fig. 2). Additionally, elevated levels of citric acid can be used in the *de novo* fatty acid synthesis, but it is noted that citrate can also induce apoptosis in two gastric cancer cell lines *in vitro* experiment (54,55).

Amino acid metabolism. Availability of amino acids is pivotal for cellular protein biosynthesis and cytoskeleton formation, while it has been pointed out that amino acids especially those linking to TCA (Fig. 2) are an alternative energy source of cancer cell proliferation (56). By employing metabolomics technologies, levels of various amino acids (including serine, valine, phenylalanine, tryptophan, glycine, and proline) and their primary derivatives (such as kynurenine, kynurenic acid, anthranilic acid and nicotinic acid) are significantly higher in tissue specimens (31,36,37) and gastric content (29,40,41), but decreased concentration in some of them is observed in blood (24). The overexpression of L-type amino acid transporter 1 (LAT1) might be proposed to explain this dissimilarity (57). Free amino acids are greatly assimilated to cancer tissues via LAT1 from bloodstream, resulting in the low accumulation of amino acid in contrast to normal counterparts. Malnourishment may also be a contributing factor to these reduced levels of plasma amino acids. Apart from these, degradation of extracellular matrix mediated by the overexpressed matrix metalloproteinases (MMPs) and activated autophagic degradation of intracellular proteins are considered as the potential source of accumulative amino acids in tumor tissues (58-60).

Elevated amino acids in microenvironment are contributing factors in carcinogenesis. Most strikingly, it is indicated that many cancer cell lines cannot survive in the absence of glutamine (61), because it is required for anabolic growth of mammalian cells through its ability to control the master regulator of protein translation mTORC1 (62). Reprogramming of glutamine metabolism further contributes to the proliferative and metabolic responses regulated by oncogenic transcription factor c-MYC (63). In addition, it is also the nitrogen donor for several key metabolic enzymes and for the *de novo* synthesis of both purines and pyrimidines (Fig. 2). Serine also participates in the *de novo* synthesis of nucleotides by serving one carbon unit. Functional genomics further indicates that serine biosynthesis pathway is significant for breast cancer event, which can be attributable to the overexpression of phosphoglycerate dehydrogenase (PHGDH) that controls the flow of intermediates originated from glycolysis (64). Inhibition of PHGDH in cells can result in lower serine and decrease cellular proliferation *in vitro*. However, this remains

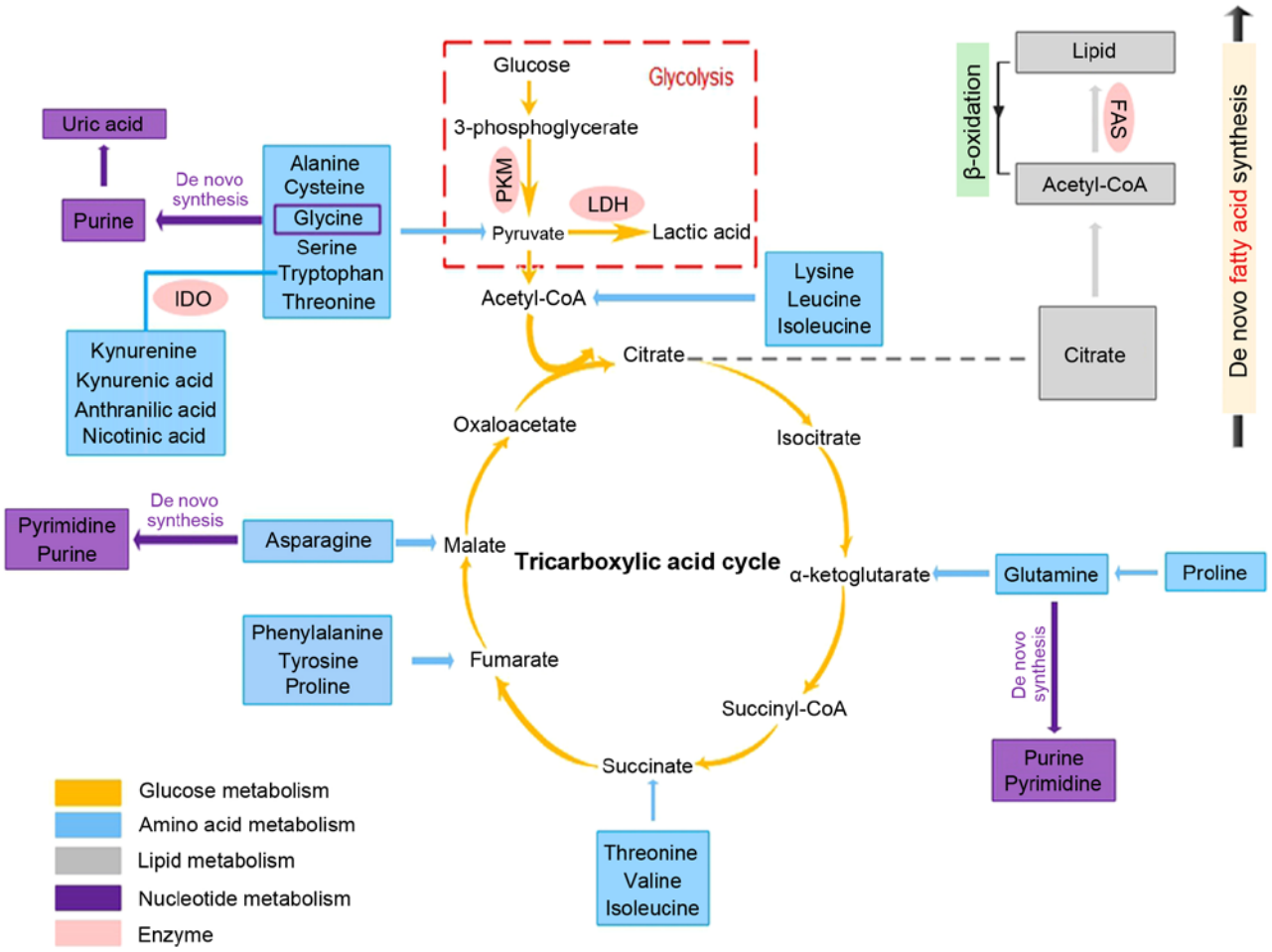

Figure 2. Metabolic regulation in gastric cancer. Altered metabolites in gastric can be categorized into four main biomolecules: carbohydrates, amino acids, lipids and nucleic acids. Activated glycolysis and impaired aerobic respiration shape the altered glucose metabolism in this disease. For amino acid metabolism, various amino acids (serine, valine, phenylalanine, tryptophan, glycine, and proline) and some primary derivatives (such as kynurenine, kynurenic acid, anthranilic acid and nicotinic acid) are significantly higher in tissue specimens and gastric content, but decreased concentration is observed in blood samples. Of note, glutamine is also the most greatly depleted. Increased rate of lipogenesis, upregulation of fatty acid β-oxidation and upregulated oxidative degradation are the typical characteristics of lipid metabolism in this disease. Accumulation of the end products of nucleotide catabolism is characterized by the higher levels of uric acid. Moreover, there is correlation between these four metabolisms. For instance, glycine, asparagine and glutamine are used as building blocks of purines.

unclear in gastric cancer. Tryptophan and its downstream metabolites (mainly including kynurenine, kynurenic acid, anthranilic acid, nicotinic acid) via kynurenine pathway are related to the pathogenesis and prognosis of various malignancies including gastric cancer (65,66). Kynurenine pathway catalyzed by indoleamine-2, 3-dioxygenase (IDO) plays a key role in adapting the tumor microenvironment to favor cancer progression because higher IDO expression is associated with an increase in immunosuppressive T-regulatory cell activity (67), and its immunosuppressive role inhibits T-cell mediated cytotoxicity and cell proliferation of gastric cell lines *in vitro* (68). Additionally, 3-hydroxyanthanilie acid (downstream metabolites in kynurenine pathway) also has suppressive effects on inflammation and immune response (69). Glycine used in living organism as building blocks of purines is strongly correlated with the rapid proliferation rates, and then antagonizing glycine uptake and its mitochondrial biosynthesis preferentially impair rapidly proliferating cells (70). The indirect anti-angiogenic impact of glycine is also identified *in vitro* (71,72) and *in vivo* (73,74), possibly because it might inhibit the proliferation of vascular endothelial cells, finally leading to angiogenesis (74). Elevated proline in tumor tissues might begin with the activation of MMPs and degradation of microenvironmentally extracellular matrix (ECM), subsequently the degradation of collagen catalyzed by proline dehydrogenase (PRODH) that can be regulated under conditions of nutrient stress linked to mTOR signaling system (75). Other elevated amino acids, such as tyrosine, valine and cysteine, can be converted into the TCA intermediates (except for citric acid, isocitrate, succinyl-CoA, oxaloacetate) to generate energy (Fig. 2).

Lipid metabolism. The notable feature of lipid metabolism in cancer cells is an increased rate of lipogenesis and the upregulation of mitochondrial fatty acid β-oxidation, gastric cancer shows a similar tendency and presents typical changes regarding various metabolites involving in lipid metabolism.

Fatty acids, such as hexadecenoic acid, docosahexaenoic acid, eptanoic acid and β-hydroxybutyrate, are significantly larger in gastric cancer tissues than in benign tissues (like

chronic superficial gastritis) (28). Octadecanoic acid is also found to be elevated in blood specimens obtained from gastric cancer patients (39). Of them, β-hydroxybutyrate is the common product of fatty acid degradation via β-oxidation, suggesting more intensive decomposition of fatty acids in microenvironment. The accelerated metabolism from lipids to fatty acids and finally ketone bodies consumes fat, which might explain the fact that patients become very thin in later stages of gastric cancer. This signature also has been identified by the xenograft animal models with gastric cancer showing elevated levels of glycerol and hexadecanoic acid (30), resulting from the high activation of adipocyte lipolysis in cancer cells as well as enhanced expression and function of adipocyte hormone-sensitive lipase in cancer cachexia (76). In contrast, some data show that unsaturated fatty acids such as 9-hexadecenoic acid, *cis*-vaccenic acid, arachidinic acid, hexadecanoic acid and 3-hydroxybutanoic acid are found to be significantly decreased in cancer tissue samples (26). Of note, the level of *O*-acetylcarnitine, which increases the β-oxidation of fatty acid, shows a declining trend as the early gastric cancer progresses into advanced stage (31). Accordingly, it seems that decreased *O*-acetylcarnitine might explain the impaired fatty acids β-oxidation in stage III/IV gastric cancer, which is characterized by the decline in unsaturated fatty acids we discussed above (9-hexadecenoic acid, *cis*-vaccenic acid, arachidinic acid, hexadecanoic acid and 3-hydroxybutanoic acid). However, this discrepancy between different research needs further elucidation with larger samples and different analytical methods. On the other hand, free fatty acids in plasma, including palmitic acid, stearic acid, 9-(Z)-hexadecenoic acid, oleic acid, linoleic acid, docosahexaenoic acid and arachidonic acid, are equivalent in both gastric cancer and gastric benign disorders (25). Therefore, it infers that free fatty acids in blood might be not utilized by tumor cells.

Upregulated lipid peroxides are also confirmed in this disease. Accumulation of 4-hydroxyphenylacetate resulting from the oxidative degradation of lipids was observed in the study of Jung *et al* (31). Elevation of azelaic acid in blood samples, which is the end product of linoleic acid when subjected to peroxide decomposition (77) and can serve as a marker of lipid peroxidation (78), as observed by Yu *et al* (25).

Based on that indicated above, these signatures show that cancer cells utilize massive fatty acids to meet the demand of cell membrane synthesis, mainly for lipid raft and lipid-modified signaling molecules (79); and a large fraction of their membrane lipids are biosynthesized *de novo* rather than scavenging from extracellular sources. In *de novo* lipogenesis, fatty acid synthase (FAS) catalyzes the synthesis of palmitate from acetyl-CoA or malonyl-CoA in the presence of NADPH as a redox equivalent. FAS expression is commonly low in non-proliferating cells that typically import lipids from the extracellular milieu. In contrast, actively proliferating cells, especially tumor cells, have increased demands for lipids, which is highly dependent on *de novo* synthesis. So FAS is frequently upregulated in many types of tumors (80-82) including gastric cancer (83,84); and increased FAS expression is linked to tumor proliferation, chemoresistance and poorer prognosis in cancers (85-88). Thus, this key enzyme implicated in lipogenesis has been studied as potential target in anti-neoplastic therapy (84). On the other hand, enhancement of fatty acid-β oxidation is also considered to be an important metabolic reprogramming in the early stage of some cancer types (89), as it produces more ATP and acetyl coenzyme A which in turn can accelerate the rate of citric acid oxidation and serve as the energy source (90). Furthermore, production of polyunsaturated fatty acids, to some extent, is also associated with tumor cell proliferation, apoptosis and angiogenesis (91,92).

Nucleotide metabolism. Tumor cells are in a state of such rapid proliferation and differentiation that frequent nucleotide synthesis and metabolism are upregulated significantly. Accumulation of the end products of nucleotide catabolism is characterized by the higher levels of uric acid or urate (25,30) in gastric cancer patients or animal models. Other purines compounds like hypoxanthine and guanosine were also increased (35,37), but Aa *et al* showed decreases in uridine (an RNA building block) (28). Nucleotides are also associated with energy metabolism, mainly in the form of ATP and GTP. Of tumor cells, adequate energy should be supplied to meet their proliferation. In this way, it is assumed that nucleotide phosphates should increase in cancer tissues compared with normal tissues. However, Hirayama and colleagues (35), identified that there was no noticeable difference between gastric cancer tissues and adjacent normal tissues with regard to most nucleotide phosphates (ATP, ADP, GTP, and GDP), total adenylate and energy charge. Accordingly, it infers that cancer cells gain growth superiority over their normal counterparts by switching metabolic patterns of energy to anaerobic glycolysis and possibly fumarate respiration that we have discussed above, instead of securing more ATP.

Other altered metabolisms. Except for the changed metabolisms mentioned above, other metabolite concentrations also show increased or decreased trend in the development of gastric cancer. Increased level of creatinine, a waste product of muscle metabolism, was detected in urine samples of tumor groups (33), which might be induced by lower total body skeletal mass among cachectic patients (93,94). Changes in inositol level of gastric malignancy patients are investigated in either tumor tissues (28,36,37) or urine samples (30), but its mechanism and significance are poorly understood.

3. Metabolomics in diagnosis, treatment and prognostic prediction of gastric cancer

Diagnosis. Early diagnosis is the key element determining the outcome of treatment in cancer research, but current application of cancer biomarkers, endoscopy and imaging is still not satisfactory. Serum biomarkers, like CEA and CA19-9, are not effective given their poor sensitivity or specificity. Inconsistent diagnostic efficacy at endoscopy that results from the variations in skill and experience of endoscopists and pathologist might lead to missed diagnosis at early phase, while positive results displayed on imaging examination (such as barium meal and computer tomography) are prone to advanced stage. Interestingly, utility of various -omics technologies open a new field to discover potential biomarkers for gastric cancer diagnosis, especially based on metabolomics.

Exploration of gastric cancer biomarkers in blood or urine is more appreciated because of its non-invasive priority. Yu *et al* demonstrated that metabolic profiles were quite different in gastric cancer patients with different pathological types in the Correa model, but intestinal metaplasia shared similar metabolic phenotype (threonate, glutamate and azelaic acid) in plasma with neoplastic groups (25,95). Ikeda *et al* also identified that there were obvious variations in serum metabolic profiles of gastrointestinal cancers (including esophageal, gastric and colorectal) in contrast to healthy volunteers (27). In particular, changes in the levels of 3-hydroxypropionic acid and pyruvic acid were sufficient to differentiate gastric cancer from esophageal and colorectal cancer, and showed high values for both sensitivity (84.6 and 70.0%) and specificity (71.4 and 90.0%) compared with conventional biomarkers (CA19-9 and CEA) (27). The diagnostic potential of serum metabolic profiles between gastric cancer and non-cancer groups was also confirmed by Song *et al*, and these alterations occurred at early stage of gastric carcinogenesis (26).

Recently, one urine metabolomics in gastric cancer found that 14 out of 17 metabolites detected from training set (94 urine samples) via GC-MS showed diagnostic value better than classic blood biomarkers on validation set (199 urine samples) (34). Six of them (L-alanine, L-isoleucine, L-serine, L-threonine, L-proline and L-methionine) revealed satisfactory diagnostic values with the area under the ROC of >0.75. Chan *et al* also revealed that gastric cancer has a unique urine metabolic profiles in contrast to benign gastric diseases and healthy patients, especially 2-hydroxyisobutyrate, 3-indoxylsulfate and alanine, producing a discriminatory model with the area under the curve (AUC) of 0.95 (33). Another study reported that metabolites altered in urinary data of gastric cancer patients was predicted with higher sensitivity than CA19-9 and CEA (31).

In tissue testing, Wu and colleagues indicated that 18 metabolites were detected differently between the malignant tissues and the adjacent non-malignant tissues of gastric mucosa with AUC value of 0.9629 (37), but tissue testing was not a non-invasive approach in contrast to blood or urine testing. Our data, on the other hand, showed that higher levels of tyrosine, phenylalanine and tryptophan in the gastric juice were detected in the early phase of gastric carcinogenesis (40), and the sensitivity and specificity for gastric cancer detection with phenylalanine was 87.9 and 79.4% respectively (41).

Metastasis and prognosis. Most gastric cancer-related deaths occur as a result of metastasis, even among patients undergoing gastrectomy. Unfortunately, no molecular markers for predicting metastasis and prognosis are accessible.

Based on metabolomics, Wu and colleagues showed that five metabolites (increased L-cysteine, hypoxanthine and L-tyrosine; decreased phenanthrenol and butanoic acid) were detected differently between non-invasive (T1 and T2) and invasive (T3 and T4) groups, furthermore, 4-hydroxyphenylacetate, alanine, phenylacetylglycine, mannitol, glycolate and arginine levels were significantly correlated with cancer T stage (37). By establishing animal models with gastric cancer cell line SGC-7901, Chen *et al* confirmed that metabolites correlated to proline and serine metabolism could distinguish metastatic from non-metastatic specimens with an AUC value of 1.0 (36). Study conducted by Hu *et al* suggested that decreased levels of alanine, glycerol, L-proline, butanoic acid and L-threonic acid as well as increased levels of butanediotic acid and myo-inositol could detect non-metastatic and metastatic groups (AUC=1.00) (30).

Significantly, Chen and coworkers recently evaluated the prognostic value of 17 urinary metabolites, which have been identified differently between gastric cancer group and normal group, by following up 82 out of 112 gastric cancer cases for 3-5 years after surgery (34). They discovered that patients with higher levels of proline, p-cresol and 4-hydroxybenzoic acid display poor prognosis with median survival time 16, 15 and 15 months, respectively. Furthermore, the concentration of p-cresol closely correlated with gastric cancer stage, which was gradually increased with the stage of the patients.

It is possible that changes in proline might be essential in tumor metastasis. As we have mentioned above, proline in tumor tissues might result from the degradation of collagen (73). This process mainly begins with the activation of MMPs and degradation of microenvironmental ECM, which partially accounts for the tumor invasion and metastasis (96). In this respective, elevated proline serving as metastatic biomarker for gastric cancer is possible, but further research is necessary.

Treatment. Chemosensitivity prediction that aims to maximize the therapeutic response and minimize adverse effects is a difficult task in the treatment of advanced tumors. One of classical approaches for predicting the activity of anticancer agents is cell culture testing, which is mainly based on clone formation, cell metabolic activity assays, proliferation and tumor growth *in vitro* experiments. However, it must be noted that these methods still fail to fully reproduce the tumor microenvironment, although current patient-derived primary cell culture or patient-derived tumor xenograft models are able to retain cellular heterogeneity of original tumors (97).

Lu *et al*, in particular, suggested that some conventional cytotoxic anticancer agents (vincristine, taxol, 5-fluorouracil, doxorubicin, cisplatin, camptothecin) lost their efficacy apparently when cultured PNAC-1 cells (pancreatic cancer) *in vitro* were deprived of glucose (98). Similarly, a recent study also identified that high glucose conditions promoted SGC-7901 proliferation *in vitro* and reduced chemosensitivity *in vivo* or *in vitro* (99). We could speculate that responses of gastric cancer against anticancer drugs in actual microenvironment *in vivo* might be considerably different from what we expect in culture condition. Therefore, utilizing metabolomics is considered to be a promising tool to assess the sensitivity of chemotherapy in virtual conditions and discovering therapeutic targets regarding specific tumor metabolism (20,21).

Wang *et al* applied high performance liquid chromatography coupled with a quadrupole time-of-flight mass spectrometer to predict chemotherapy response in a human xenograft model of gastric cancer administered with cisplatin plus 5-fluorouracil (5-FU) (100). Consequently, 1-acyl-lysophosphatidylcholine and polyunsaturated fatty acid were proposed to surveil gastric cancer chemosensitivity, since 1-acyl-lysophosphatidylcholine can regulate the activity of enzymes like phospholipase A2 (PLA2) and lysophosphatidylcholine acetyltransferases. PLA2 catalyzes the production

of arachidonic acid that is likely to promote cell cycle arrest and apoptosis dependent on ceramide pathway (101,102), while lysophosphatidylcholine acetyltransferases catalyzes phospholipid synthesis linked to tumor cell proliferation. Another study suggested that proline was reduced while glutamate increased dramatically, and PRODH (catalyzes the metabolic production of glutamate from proline proceeds) mRNA expression was upregulated 2-fold after 5-FU administration; but they were less affected in 5-FU-resistant cells (103). Thus PRODH might make it possible to be a marker for assessing intracellular dynamic responses to 5-FU. Additionally, Kim and colleagues utilized ^{1}H-NMR to investigate the metabolic changes in urine sample following Adriamycin (ADR) treatment for gastric adenocarcinoma in an animal model (104). This study revealed that levels of trimethylamine oxide, hippurate and taurine, which all decreased in tumor group without treatment, were increased dramatically after ADR disposal; while 2-oxoglutarate, 3-indoxylsulfate, trigonelline, trimethylamine and citrate recovered to those of normal group (104). Alterations in these metabolites might be ascribed to the pharmacological activity of ADR that activates apoptotic process of gastric cancer cells via ADR-induced genotoxic stress.

In another study, dysregulation of pyruvic acid efflux in gastric cardia cancer was observed with the combination of proteomics and metabolomics (38). Furthermore, Cai *et al* also found that downregulation of lactate dehydrogenase A (LDH-A) and overexpression of pyruvate dehydrogenase B (PDH-B) could force pyruvic acid into the Krebs cycle rather than the glycolysis process in gastric cancer cell line AGS, consequently inhibiting cell growth and migration (38). In view of the above, LDH or PDH might serve as a therapeutic target in gastric cancer treatment.

4. Current perspectives and future directions

As we indicated above, cumulative studies employing metabolomics have yielded initial and promising results in gastric cancer research. However, inconsistent results across studies can be observed, probably because of the different sensitivity of metabolomics methods (105), variety of experimental subjects (patients, animal models or *in vitro* cell culture), and the number of samples. Additionally, values of those biomarkers should be further validated with larger cohorts and normalized metabolomics analysis. Furthermore, it should be noted that investigations targeted at the mechanism of the altered metabolism and specific metabolic pathways in gastric cancer are relatively deficient at present, so it is difficult to draw a clear dividing line on metabolism for common cancers and this disease based on a handful of studies that looked also at the role of metabolomics. Overall, exploring the metabolic disorders and gastric carcinogenesis still has far to go.

On the other hand, metabolomics locate at the downstream of genomics, transcriptomics and proteomics, mapping the complete metabolic changes under specific conditions associated with pathogenic factors, host or environmental co-effectors. However, it is essential to combine metabolomics with other -omics methods to get a more integrated understanding of gastric carcinogenesis (Fig. 1). For instance, metabolomic genome-wide association studies (mGWAS) have their priority in quantifying metabolic data and uncovering genetic variants affecting metabolite levels (106). Impacts of the microbiome on the metabolome are also an area of increasing interest, because perturbation of gastrointestinal microbiota composition or function including *Helicobacter pylori* has been proved to play a role in gastric carcinogenesis (107,108). Furthermore, microbe-derived metabolites also produce effects on cancer cells, such as butanoic acid. Some research revealed that it can modulate immune response via the differentiation of colonic regulatory T cells (109) and inhibit colonic tumor cells (110,111), although the signaling mechanism was not clearly understood. Thus, it can explain the fact that some changed metabolites in gastric cancer such as butanoic acid (37), mannitol (37) and p-cresol (34) that are commonly thought of artificial substances, can originate from fermentation by microorganism in gastric flora. Given this, it is reasonable to presume that gastric flora might be incorporated into an in-depth study of the prominent disorders of metabolism in gastric cancer, but there is still a gap in further research.

5. Conclusions

Gastric cancer is one of the most malignant tumors worldwide, and remains a major global health threat. Though its pathogenesis is unknown, promising discoveries have been made with the emergence of -omics studies. Most strikingly, metabolomics provides us in-depth information on metabolic perturbation between healthy and neoplastic states in the stomach, and further help us discovery disease-specific biomarkers. As technology advances and our understanding of metabolic perturbation in gastric cancer grows, new diagnostic and therapeutic targets will undoubtedly emerge. Ultimately, these advances can be translated into clinical practice to realize the goal of truly personalized cancer treatment.

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