TOPIC HIGHLIGHT

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Potential role of metabolomics in diagnosis and surveillance of gastric cancer

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Abstract

Gastric cancer is one of the deadliest cancers worldwide, and is especially prevalent in Asian countries. With such high morbidity and mortality, early diagnosis is essential to achieving curative intent treatment and long term survival. Metabolomics is a new field of study that analyzes metabolites from biofluids and tissue samples. While metabolomics is still in its infancy, there are numerous potential applications in oncology, specifically early diagnosis. Only a few studies in the literature have examined metabolomics' role in gastric cancer. Various fatty acid, carbohydrate, nucleic acid, and amino acid metabolites have been identified that distinguish gastric cancer from normal tissue and benign gastric disease. However, findings from these few studies are at times conflicting. Most studies demonstrate some relationship of cancer cells to the Warburg Effect, in that glycolysis predominates with conversion of pyruvate to lactate. This is one of the most consistent findings across the literature. There is less consistency in metabolomic signature with respect to nucleic acids, lipids and amino acids. In spite of this, metabolomics holds some promise for cancer surveillance but further studies are necessary to achieve consistency and validation before it can be widely employed as a clinical tool.

Core tip: There are differences in metabolomic profiles of gastric cancer patients and healthy controls, as well as between different stages of gastric cancer. The transition from normal to malignant consistently shows upregulation in lactate and downregulation of glucose consistent with the Warburg effect. This trend is perpetuated as cells advance from non-invasive to invasive. Key tricarboxylic acid (TCA) cycle intermediates and amino acids are elevated as a result of anaplerotic reactions. Perpetuation of the TCA cycle generates energy for essential cell functions. There is less consistency between lipid and nucleic acid metabolites.

INTRODUCTION

The burden of gastric cancer is significant in Canada and worldwide. In 2013, the Canadian Cancer Society estimated there were 3300 new cases of gastric cancer which caused 3.3% of all male cancer related deaths, and 2.2% of all female cancer related deaths[1]. On a global scale, an

estimated 990000 people were diagnosed in 2008, with 60% of those cases occurring in East Asia[2]. With an estimated 736000 deaths worldwide[3], the fatality to case ratio is approximately 70%[4]. Despite these grim statistics, overall morbidity and mortality are declining due to changes in diet, treatment for *Helicobacter pylori* (*H. pylori*), early screening programs, improved surgical techniques and chemotherapy regimens.

Much of the mortality is attributable to delayed symptoms of gastric cancer. Early stage gastric cancer is asymptomatic: it takes an estimated 44 mo to progress to an advanced stage[5]. Commonly patients present with vague epigastric pain, unintentional weight loss, anemia from occult blood loss, or dysphagia if the tumour is proximal. Gastric cancers that do not penetrate into the muscularis propria are asymptomatic in up to 80% of cases; occasionally, patients experience epigastric pain or "dyspepsia". Dyspeptic symptoms occur in up to 40% of the population, so its value as a predictor of gastric cancer is limited. Furthermore, amongst those who have dyspepsia, previous studies have found that only 1%-2% of them will develop gastric cancer[6-8]. With such high morbidity and mortality, early diagnosis is key. This review will highlight current surveillance methods and summarize how metabolomics may have important applications in future cancer surveillance and diagnosis.

CURRENT SURVEILLANCE METHODS

There are currently several methods of detecting gastric cancer, but no uniform screening guidelines. In Japan, where there is a high incidence of gastric cancer, screening has been introduced for everyone forty years of age and over. Since 1962, Japanese have employed barium-meal photofluorography as a screening test. The initial exam consists of a series of 8 X-rays. If this is abnormal, a detailed exam with 11 X-rays is undertaken. Endoscopy is then used to analyzed suspicious lesions identified on barium exam[5]. Case control studies suggest a 40%-60% decrease in gastric cancer mortality with photofluorography screening. The sensitivity of photofluorography is 60%-80% and specificity is 80%-90%. Studies indicate that survival rates of the screened group are 74%-80% compared to 46%-56% in the non-screened group[9]. Currently gastro fluorography is a Grade B recommendation.

Endoscopy is another tool used in gastric cancer surveillance. Its sensitivity ranges from 77%-84%[9]. It can identify superficial flat and non-ulcerative lesions that barium studies can miss[5]. In a Japanese study, detection of gastric cancer by endoscopy was 2.7 to 4.6 fold higher than with barium swallow. Endoscopy is versatile, as it allows clinicians to biopsy tissue, and perform endoscopic ultrasound to determine depth of invasion (tumour or T stage), should there be a lesion in the stomach. Despite these abilities, endoscopy has limitations in that it depends heavily on skills of the endoscopist and on availability of gastroscopy. Also it can be difficult to visualize early stage gastric cancers; the sensitivity is estimated to be 50%-60%. No studies have compared survival of gastric cancer patients between screened and non-screened groups. Therefore endoscopy has significant limitations as a screening technique, but currently it is still the best test available.

Since the 1990s, serum pepsinogen has been incorporated into gastric screening programs. Pepsinogen I and II are proenzymes of pepsin, which originate in gastric mucosa. These markers reflect morphological and functional status of the gastric mucosa and can act as a marker for chronic atrophic gastritis (CAG). CAG is regarded as a precursor of gastric cancer, especially the intestinal type[10]. In Japan, a serum pepsinogen (PG) test based on serum PG I level and PG I/II ratio have been used for screening. As mucosal atrophy increases, the level of PG I and thus the PG I/II ratio decreases[11]. Recent studies show that PG testing is useful at detecting early gastric cancers, especially in combination with barium X-ray. If either one or both of the two screening methods are positive, patients are referred for upper endoscopy. Cutoff values for serum PG tests are ≤ 50 ug/L and PG I/II ratio ≤ 3.0. These values detected gastric cancer in 0.28% of cases compared to 0.1% with barium X-ray. Early stage gastric cancer accounted for 100% of cancers detected by PG, 83% of cancers detected by barium X-ray, and 81% of cancers detected by both PG and X-ray. Eighty-nine percent of cancers detected by PG were intramucosal, compared to only 50% detected by barium X-ray. In this study, pepsinogen testing seemed to be useful in detecting small cancers arising from atrophic gastric mucosa.

METABOLOMICS IN CANCER

Metabolomics is a relatively new area of study and the latest addition to the "omics" family of genomics, transcriptomics, and proteomics. The central dogma of molecular biology describes flow of biological information in a system from DNA to RNA to protein to metabolites. Different "omics" interventions play a part at different stages of this dogma to glimpse the inner workings of cell, tissue and organism. The metabolome of an organism consists of the entire collection of low molecular weight (< 1500 Daltons) metabolites[12]. Metabolites are required for maintenance, growth and normal functioning of a cell. Mapping the metabolomic profile provides a global picture of the organism at a specific point in time under a specific set of conditions. For any given disease state, a small genomic change can be amplified many times at the metabolite level and quantitatively measured. Metabolites in biological samples such as tissues, urine, saliva and blood plasma can be measured, and this allows researchers to identify specific metabolic pathways. Previous studies have demonstrated that metabolic activities of cancer cells are markedly different from that of healthy cells. Studying the metabolomic profile may help distinguish certain cancer biomarkers, and provide keys to early diagnosis.

Biofluids such as urine and blood are optimal samples to study, as they can be obtained through minimally invasive means. Profiles of these biofluids can be linked back

to their genetic origins to provide a view of disease pathways. As metabolites are "downstream" entities compared to genes, they reflect cellular conditions at the time of sampling and can be considered "endpoint markers" for disease. There are currently several technologies for analyzing the metabolome: nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), liquid and gas chromatography.

NMR utilizes a magnetic field. Spins of the atoms inside the tissue sample or fluid align themselves with respect to the magnetic fields. A radiofrequency pulse from the NMR machine elevates spins to a higher energy orientation. When the radiofrequency is turned off, spins undergo relaxation and release energy, returning to their original lower energy configurations. During this process, an NMR signal is emitted that can be detected by a computer system. A series of peaks are generated. Their positions are characteristic of certain known molecules. The NMR spectra of most metabolites have been identified and any new spectra can be identified in reference to available data. Liquid and gas chromatography are two separation techniques that rely on partitioning liquid or gas from a sample solution. Separation depends on the physical properties of the substance such as boiling point and solubility. As these chemicals are eluted off the column, they can be detected and quantified. Mass spectrometry is an analytical technique that identifies compounds based on their mass to charge ratio.

Each of the different analytical techniques has its benefits and drawbacks. A major advantage of NMR is its non-invasiveness and non-reliance on metabolite separation. Samples are not eluted off so they can be recovered for further analysis by chromatography or spectrometry. Sample preparation for NMR is simple, and lends itself well to metabolite profiling of intact biofluids like culture medium or semi-solid samples like cells or tissue. However, a major disadvantage of NMR is its low sensitivity compared to chromatographic techniques[13]. A cross-platform comparison of metabolomic methods by Büscher *et al*[14]. demonstrated that the three platforms of gas chromatography, liquid chromatography, and capillary electrophoresis were roughly equivalent in terms of sensitivity, and all superior to NMR.

Metabolomics has been studied in other cancers[12,15] including breast[16], prostate[17], lung[17] colorectal[18], pancreatic[19] esophageal[20], ovarian[21], bladder[22] and renal cell carcinoma[23] but to date, very little has been studied in the area of gastric cancer. This review summarizes current available literature on gastric cancer metabolomics. As it is a relatively new field, there are only a few studies. Our findings are presented below.

NORMAL VERSUS MALIGNANT METABOLOMIC SIGNATURES

A few studies in the literature have compared metabolomic profiles of gastric cancer patients with healthy controls. The type of biofluid or tissue they use varies between studies. This review organizes metabolites from each study into four main classes of biomolecules: carbohydrates, amino acids, lipids, and nucleic acids. Table 1 summarizes metabolites from each study by biomolecular class.

CARBOHYDRATE METABOLISM

Hu *et al*[24] implanted human gastric cancer cells into 24 immune deficiency mice. They were randomly divided into a metastasis group, non-metastasis group and a normal group. Urine of these mice was collected and gas chromatography/mass spectrometry was employed to identify a metabolomic profile. Two diagnostic models for gastric cancer and metastasis were constructed by principal component analysis (PCA). PCA is a statistical technique that compresses multivariate data into fewer dimensions such that an overview of data is visualized in two or three planes (principal components). Data with the largest variation are summarized, scored and plotted as points on a graph. Points clustering together have more similar properties. PCA enables pattern recognition and classification of data into distinct groups. Ten metabolites were different between normal and cancer groups. Seven metabolites were different between metastasis and non-metastasis groups. On the PCA scores plot, the normal group and cancer group were scattered into different regions. Similarly the PCA plot showed differential scatter between non-metastasis and metastasis groups. Levels of tricarboxylic acid (TCA) intermediates such as butanedioic acid, malic acid, and citric acid were elevated in gastric cancer mice, as were lactic acid levels. This could be attributed to the "Warburg effect" in that glucose is often converted into lactic acid in cancer cells[25].

Hirayama *et al*[26] investigated metabolites in tumour tissue and compared this with adjacent normal tissue on twelve resected gastric cancer specimens. They quantified 95 metabolites involved in glycolysis, pentose phosphate pathway, TCA and urea cycles. Metabolites in normal stomach tissue and tumour tissue were not well separated on PCA plot, making two types of tissues less distinguishable. With regards to glycolysis and the TCA cycle, Hirayama found that glucose concentrations were much lower in tumour than in normal tissues. Also pyruvate was decreased, while lactate concentration was increased in tumour tissues indicating a higher reliance of cancer cells on anaerobic breakdown of pyruvate under hypoxic cell conditions. This lab group identified elevated levels of TCA intermediates specifically that of succinate, fumarate, and malate in malignant tissue. These findings correlated to ones from Hu *et al*[24].

Song *et al*[27] studied gastric cancer resections and compared the metabolomic profiles of the cancerous tissue matched to normal tissue at least 8 cm away on the specimen. This group noticed an increase in metabolites of aerobic glycolytic pathways namely alpha ketoglutarate and fumaric acid. Across all studies, lactate was the most consistently elevated carbohydrate pathway biomarker

Table 1 Marker metabolites between gastric cancer and healthy controls

Ref.	Animal *vs* human analytical platform	Sample type groups	Statistically significant metabolites identified ($P < 0.05$) (up or down indicate levels in cancer group)
Hu *et al*[24], 2011	Animal (mice) Gas chromatography, Mass Spectrometry	Urine Metastasis group ($n = 8$) Non-metastasis group ($n = 8$) Normal group ($n = 8$)	Carbohydrates: Anaerobic respiration: lactate up TCA cycle: citric acid up, malic acid up Nucleic acids: uric acid up Lipids: hexadecanoic acid up Others: butanoic acid, propanoic acid, glycerol, pyrimidine, glycerol all up
Hirayama *et al*[26], 2009	Human Capillary electrophoresis Mass spectrometry	Resected stomach specimens ($n = 12$) GC tissue Adjacent normal tissue	Carbohydrates: Anaerobic respiration: lactate up Glycolysis: glucose down TCA cycle: citric acid down, malic acid/fumarate up Amino acids: 19 elevated, except glutamine (no change) Nucleic acids: GMP up
Song *et al*[27], 2011	Human Gas chromatography, Mass spectrometry	Resected stomach specimens ($n = 30$) GC tissue Adjacent normal tissue	Carbohydrates: TCA cycle: fumarate up, alpha-ketoglutarate up Lipids: up: xylonic acid, octadecanoic acid Down: 9-hexadecanoic acid, *cis*-vaccenic acid, arachidonic acid, 9-octadecenamide, squalene Others: up: valeric acid, benzenepropanoic acid, 1-phenanthrene-carboxylic acid down: 3-hydroxybutanoic acid
Cai *et al*[28], 2010	Human Gas chromatography, Mass spectrometry	Resected stomach specimens ($n = 65$) GC tissue Adjacent normal tissue	Carbohydrates: Anaerobic respiration: lactate up Glycolysis: up: fructose, glyceraldehyde, pyruvate TCA cycle: up isocitric acid, fumarate down
Aa *et al*[29], 2012	Human Gas chromatography, mass spectrometry	Plasma and tissue Pre-op GC ($n = 17$) Post-op GC ($n = 15$) CSG ($n = 20$)	Tissue samples show larger variations between GC and CSG than plasma samples (up/down show change for tissue CG compared to tissue CSG) Carbohydrates: Anaerobic respiration: lactate up Glycolysis: glucose down, fructose-6-phosphate down TCA cycle: citrate up, malate up, fumarate up Other: maltose down, ribose down, glyceric acid-2,3-diphosphate down Amino acids: cysteine up Lipids: up: docosahexanoic acid, heptanoic acid, 9-Z-hexadecenoic acid, beta-hydroxybutyrate down: cholesterol Nucleic acids: uracil up, uridine down Others: up: 2-aminoadipate, monomethylphosphate down: inositol, ribitol, beta-*D*-methylglucopyranoside
Wu *et al*[31], 2010	Human Gas chromatography, Mass spectrometry	Resected stomach specimens ($n = 18$) GC tissue Adjacent normal tissue	Carbohydrates: up: galactofuranoside down: *L*-altrose, *L*-mannofuranose, *D*-ribofuranose Amino acids: up: *L*-valine, *L*-isoleucine, serine, *L*-glutamine Down: phosphoserine Others: up: heptanedioic acid, propanoic acid, phenanthrenol, butanetriol, acetamide, butenoic acid, oxazolethione, naphthalene down: myo-inositol
Song *et al*[32], 2012	Human Gas chromatography, Mass spectrometry	Serum GC ($n = 30$) Healthy ($n = 30$)	Carbohydrates: down: fumarate, 2-*O*-mesyl arabinose Amino acids: up: valine, sarcosine Down: glutamine, hexanedioic acid Lipids: down: 9, 12-octadecadienoic acid, 9-octadecenoic acid, trans-13-octadecenoic acid, nonhexacontanoic acid Cholesterol: up cholesta-3,5-diene, cholesterol, pentafluoropropionate, cholesterol, cholest-5- en-3-ol
Ikeda *et al*[36], 2012	Human Gas chromatography, Mass spectrometry	Serum GC ($n = 11$) Healthy ($n = 12$)	Carbohydrates: Glycolysis: down: pyruvate Others: up: 3-hydroxypropionic acid Down: 3-hydroxyisobutyric acid, octanoic acid, phosphoric acid
Kim *et al*[38], 2010	Animal (mice) ^{1}H-NMR	Urine GC ($n = 10$) Healthy ($n = 10$)	Carbohydrates: TCA cycle: Citrate up, 2-oxoglutarate up Others: up: 3-indoxylsulfate Down: taurine, trimethylamine, oxaloacetate, TMAO, hippurate
Miyagi *et al*[41], 2011	Human Liquid chromato-graphy, mass spectrometry electrospray ionization	Serum GC ($n = 199$) Healthy ($n = 985$)	Amino acids only: up: serine, glutamine, ornithine, proline down: asparagine, valine, methionine, tyrosine, histidine, tryptophan, phenylalanine, leucine

CSG: Chronic superficial gastritis; GC: Gastric cancer; GMP; Guanosine monophosphate; ^{1}H-NMR: Proton nuclear magnetic resonance spectroscopy; TCA: Tricarboxylic acid cycle; TMAO: Trimethylamine-*N*-oxide.

(four of four[24,26,28,29] studies) between the cancer and control groups. Likewise glucose was the most consistently depleted (two of two[26,29] studies). Malate was the most consistently elevated TCA cycle biomarker (three of three[24,26,29] studies). Other carbohydrate pathway products showed inconsistencies.

AMINO ACID METABOLISM

Amino acids can be an alternative energy source, and can be generated through anaplerotic reactions, a process whereby intermediates in a metabolic pathway are replenished from biomolecules outside of the pathway. Glutamine is a prime example of an anaplerotic reaction. It is converted to glutamate and then into alpha-ketoglutarate, a TCA cycle intermediate[30].

Wu *et al*[31] investigated gastric cancer mucosa in conjunction with adjacent normal mucosa. Amino acids such as serine, phosphoserine, *L*-cysteine, *L*-tyrosine, glutamine, isoleucine and valine were elevated in gastric cancer specimens. These amino acids can be produced by diverting glycolytic intermediates down alternate biochemical pathways. Song *et al*[32] found that valine exhibited the greatest fold change in GC patients compared to controls. Overall, glutamine and valine were the most commonly recognized amino acids.

FATTY ACID METABOLISM

Cancerous cells are known to have dysregulation of fatty acid beta-oxidation and cell membrane synthesis. Hu *et al*[24] who studied human gastric cancer in mice models identified elevated levels of hexadecanoic acid and glycerol in cancerous compared to normal tissues. They interpreted this as upregulation of adipocyte lipolysis and elevated circulation levels of adipocyte hormone sensitive lipase. Song *et al*[27] found that squalene (an intermediate in cholesterol synthesis) was the most extensively depleted metabolite in gastric cancer specimens. Overall, there is great heterogeneity of lipids across studies.

NUCLEIC ACID METABOLISM

The literature on nucleic acid metabolites is conflicting. Several studies reports that uric acid, the final metabolic product of purines is upregulated[24,33]. Other purines such as hypoxanthine[31] and guanosine[26] are generally elevated. This is in contrast to Aa *et al*[29] which showed decreases in uridine, an RNA building block.

METABOLOMIC PROFILE AND STAGE

While it is interesting to see differences in metabolomic profile between normal and cancerous tissue, it is also useful to examine how the profile evolves along a gradient as it goes through the benign to dysplastic to cancerous sequence. In the 1980s, Correa[34] proposed a model of human intestinal-type gastric carcinogenesis from normal mucosa to chronic superficial gastritis (CSG), to CAG, to intestinal metaplasia (IM) to dysplasia (DYS) and then to intestinal-type GC[34]. Yu *et al*[33] employed gas chromatography and time-of-flight mass spectrometry to determine metabolite levels in plasma of 80 patients with the spectrum of disease described previously by Correa. They found that the metabolic phenotype of CSG is significantly different from GC, while that of IM is similar to GC. Knowing metabolites of each stage of the progression to GC, may be used as markers to indicate a risk for malignancy. Yu *et al*[33] also found that when they mapped metabolites identified in GC, it was not much different from postoperative GC specimens within a 4-6 wk window. Perhaps this is because it takes longer for metabolic derangements to resolve. Key metabolic differences between different histological stages are summarized on Table 2.

Yu also found significant differences in serum levels of proteins between GC and CSG patients. Levels of three amino acids- glutamate, cysteine, and glycine were upregulated. These amino acids are building blocks for glutathione synthesis, which is an important anti-oxidant. 2-hydroxybutyrate, which is postulated to be a by-product in glutathione synthesis was also elevated, as were asparagine and ornithine. Most other amino acids did not show an increase in this study, unlike previous studies on gastric cancer tissue[26]. This shows that metabolomic profiling in blood may be different than in tissue. Lipid synthesis was similar between CSG and GC, except 11-eicosanoic acid and azelaic acid, which were elevated in malignant samples. Postoperative GC patients had decreased levels of urate, the end product of purine catabolism. This suggests that growth and DNA proliferation is slowed once tumour is resected.

It is also interesting to note how metabolomic profile changes with increasing TNM stages. Song *et al*[27] did not notice any significant variation in metabolites as patients progressed through T stage. They postulated that either metabolic perturbations may not be directly associated with pathological stages, or that the platform of gas chromatography and mass spectrometry is not sufficiently sensitive to identify metabolite changes.

On the other hand, Wu *et al*[31] identified that as cancers became more invasive (T3/T4 stage), there was a simultaneous increase in amino acids *L*-cysteine, hypoxanthine, *L*-tyrosine, as well as a decrease in levels of phenanthrenol and butanoic acid. Chen *et al*[35] found that proline was the most upregulated amino acid from non-metastatic to metastatic specimens (2.45 fold increase), while glutamine was the most downregulated amino acid (1.71 fold).

Apart from amino acids, other biomolecules show changes between stages. Ikeda *et al*[36] studied the sera of eleven GC patients and found that 3-hydropropionic acid and pyruvic acid, the terminal product of glycolysis, marked the greatest separation between healthy and cancer patients. In Stage I GC, there was a 1.5 fold increase in levels of 3-hydropropionic acid and 0.7 fold decrease

Table 2 Marker metabolites between stages of gastric cancer

Ref.	Animal *vs* human platform utilized	Sample type groups	Statistically significant metabolites identified ($P < 0.05$)
Hu *et al*[24], 2011	Animal (mice) Gas chromatography, mass spectrometry	Urine Metastasis group ($n = 8$) Non-metastasis group ($n = 8$) Normal group ($n = 8$)	Up/down for metastasis group compared to non-metastasis: Amino acids: alanine, L-proline, L-threonine all down Others: butanoic acid down, glycerol down, butanedioic acid up, myo-inositol up
Song *et al*[27], 2011	Humans Gas chromatography, mass spectrometry	Resected stomach specimens ($n = 30$) Gastric Cancer tissue, Adjacent normal tissue	Did not find metabolite differences between TNM stages
Wu *et al*[31], 2010	Human Gas chromatography, mass spectrometry	Resected stomach specimens ($n = 18$) Gastric Cancer tissue -T1/T2 stage ($n = 5$) -T3/T4 stage ($n = 13$)	Up/down for T3/T4 metabolites compared to T1/T2 Amino acids: L-cysteine, L-tyrosine both up Nucleic acids: hypoxanthine up Others: butanoic acid, phenanthrenol both down
Yu *et al*[33], 2011	Human Gas chromatography, mass spectrometry	Plasma ($n = 80$) CSG ($n = 19$) CAG ($n = 13$) IM ($n = 10$) DYS ($n = 22$) Pre-op GC ($n = 9$ pre-op) - 4-6 wk post-op ($n = 13$)	Up/down for pre-op GC compared to CSG: Carbohydrates: threonate down Amino acids: up: ornithine, pyroglutamate, glutamate, asparagine Lipids: 11-eicosenoic acid up Nucleic acids: urate up Other: up: 1-monohexadecanoyl-glycerol, gamma-tocopherol, 2-hydroxybutyrate, azelaic acid
Chen *et al*[35], 2010	Animal (mice) Gas chromatography, mass spectrometry	GC metastasis group ($n = 8$) GC non-metastasis group ($n = 8$)	Up/down for metastasis group compared to non-metastasis: Carbohydrates: Anaerobic respiration: lactate up Glycolysis: glucose down TCA cycle: malic acid up, succinate down Amino acids: up: alanine, glycine, valine, proline, serine, leucine, dimethylglycine, aspartic acid, phosphoserine, glutamate, lysine, arginine down: isoleucine, methionine, threonine, glutamine Nucleic acids: hypoxanthine down, pyrimidine up Others: up: propanedioic acid, pyrrolidine, inositol, docosanoic acid, octadecanoic acid down: propanamide, butanedioic acid
Ikeda *et al*[36], 2012	Human Gas chromatography, mass spectrometry	Serum GC ($n = 11$) Stages I -IV	3-hydroxypropionic acid up, pyruvic acid down (statistically significant only in Stage I GC)

CAG: Chronic atrophic gastritis; CSG: Chronic superficial gastritis; DYS: Dysplasia; GC: Gastric cancer; IM: Intestinal metaplasia; T1/T2/T3/T4: Tumour stage 1/2/3/4; TCA: Tricarboxylic acid cycle; TNM: Tumour node metastasis classification.

in pyruvic acid compared to healthy controls. Both values were only statistically significant in Stage I cancers. This may have some future utility in diagnosing GC early, but more studies validating similar findings will be necessary. Key metabolic differences between different stages are highlighted in Table 2.

METABOLOMIC PROFILE AND PROXIMAL GASTRIC CANCER

Over the last twenty to thirty years, there has been an increase in the numbers of proximal stomach tumours. As of 2011, gastroesophageal (GE) tumours affect 1.5 million people per year worldwide and contribute to 15% of cancer related deaths. The 5-year survival rate for localized tumours is 34%, while for all stages combined it is only 17%[37]. Given the poor prognosis of these proximal tumours, some recent metabolomic studies look at the unique profile of cardia and GE tumours in the hope of shedding light on early diagnostic possibilities.

Cai *et al*[28] used a combined proteomics and metabo-lomics approach to investigate gastric cardia cancer. They found that there was a dysregulation of pyruvic acid ef-flux in development of cardia cancer. A transition from glycolysis to the Kreb's cycle was associated with cancer inhibition. Several biomarkers related to glucose metabo-lism were elevated in cardia cancer samples compared to non-cancerous cardia tissue. Five enzymes from glycolysis were upregulated while five enzymes involved in Kreb's cycle and oxidative phosphorylation were downregulated in malignant samples. Several intermediates in glucose metabolism were identified in higher concentrations in gastric cancer samples including fructose, glyceraldehyde, pyruvic acid and lactate. A higher level of pyruvic acid was transformed into lactic acid, rather than acetyl CoA following Krebs cycle. These results suggest that glycoly-sis followed by anaerobic respiration were the major bio-chemical pathways to metabolize glucose in cardia cells, whereas Krebs cycle and oxidative phosphorylation were impaired. This is consistent with previous studies validat-ing the Warburg effect.

A 2013 systematic review by Abbassi-Ghadi *et al*[37]

summarized metabolomic findings on gastroesophageal cancer. Twenty studies (11 tissue, 8 serum, 1 urine and 1 gastric content) were included. They classified metabolites into cellular respiration, proteins, lipids and nucleic acids. The most commonly recognized metabolites of the tricyclic acid cycle were lactate and fumarate. Valine, glutamine, and glutamate are the most commonly identified amino acid biomarkers. Most metabolites have shown contradictory results in terms of abundance between cancer and control groups, although there is a general trend of upregulation of amino acids. Amongst all tissues, glutamine is the most consistent biomarker of GE cancer as it is upregulated in serum, urine and tumour tissues.

Sulphur containing compounds, from either incomplete metabolism of methionine in the transamination pathway or by bacterial metabolism, were also upregulated in cancer patients. In terms of lipid metabolites, myo-inositol, and cell membrane constituents choline, and phosphocholine were elevated. Of the endogenous ketones acetone and beta-hydroxybutyrate, have been described as potential biomarkers of GE cancer. Nucleotide metabolites in esophageal cancer studies report increased levels of pyrimidines *via* gas chromatography-mass spectrometry and increased adenine and uridine with high resolution-magic angle spinning-NMR (HR-MAS-NMR).

DISCUSSION

This review demonstrates that there are significant inconsistencies in the relative abundance of metabolites between not only gastric cancer and controls, but also amongst various stages of cancer. Metabolites upregulated in one study may be downregulated in another. This may be attributable to analytical technique (GC/MS/NMR), sample choice (blood/urine/tissue), or type of subject (animal/human).

Of the four types of biomolecules, carbohydrates are most consistent in terms of type and quantity of metabolites. Glucose was consistently downregulated. This may be due to upregulation of glycolysis, high consumption by cancer cells and diminished delivery from structurally and functionally defective blood vessels. Lactate was consistently elevated across all studies. This observation is in keeping with the Warburg effect.

In 1924, Otto Warburg observed that most cancer cells produce energy by a high rate of glycolysis followed by lactic acid fermentation in the cytosol. This occurs even in the presence of sufficient oxygen to support mitochondrial oxidative phosphorylation *via* the TCA cycle. Scientists have called this phenomenon "anaerobic glycolysis". Healthy cells, in contrast, exhibit a lower rate of glycolysis followed by aerobic oxidation of pyruvate in mitochondria[25]. Metabolic differences observed by Warburg adapts cancer cells to the relatively hypoxic environment inside solid tumours. He originally postulated that there was a mitochondrial defect impairing aerobic oxidation; however, subsequent studies have shown that most cancer cells have normal mitochondria. Anaerobic

glycolysis is an inefficient way to produce energy in the form of adenosine triphosphate (ATP), and the reason as to why cancer cells predominantly utilize this method is still under study. Most studies have identified metabolites in glucose utilization and some kind of connection to the Warburg effect.

Despite this, there are still elevated levels of certain TCA cycle intermediates, including malate (elevated in three of three studies[24,26,29]), citrate (elevated in three[24,29,38] of four[26] studies), and fumarate (elevated in three[26,27,29] of five[28,32] studies). While this may seem contradictory to the Warburg effect and cancer cell's preference for anaerobic reactions, these TCA cycle intermediates may be funneled in from anaplerotic reactions rather than elevated TCA cycle activity. Glutamine is one example of such. It is an essential nitrogen donor for several key metabolic enzymes and for the de novo synthesis of nucleic acids[39]. Glutamine is converted to alpha-keto-glutarate, which is a TCA intermediate; continuation of this cycle generates additional energy to produce building blocks for cells.

Amino acid metabolism demonstrated variations as well, but glutamine and valine were most commonly elevated across studies. Like glutamine, valine is essential as an anaplerotic substrate. Valine is a branched chain amino acid that can be oxidized into succinyl Co-A, another TCA cycle intermediate[40]. Other TCA intermediates include fumarate, citrate, and alpha-ketoglutarate, which are points in the cycle where amino acids can feed in.

Lipid metabolites have been inconsistent, although squalene, an intermediate in cholesterol synthesis, was downregulated. Cholesterol is an essential component of cell membranes. Squalene depletion may be a sign of excess demand for cell membrane synthesis. Although cancer cells are known to replicate quickly, it is interesting that nucleic acid metabolites do not show a consistent upregulation. Hirayama *et al*[26] inferred that cancer cells have a growth advantage over their normal counterparts, by utilizing alternative pathways such as anaerobic glycolysis, glutaminolysis, autophagic production of amino acids instead of securing more ATP and other building blocks for DNA synthesis.

For any given study, numerous metabolites were different between stages, but across studies, there were few consistencies. Similar to changes that occurred between normal to cancerous groups, a transition from non-metastatic to metastatic showed persistent elevations in lactate, malate and glutamate with a decrease in glucose[33,35]. This may indicate that the Warburg effect and anaplerotic reactions are still major contributors to the sustenance of metastatic cell lines.

The articles in this review have several limitations that may account for inconsistencies in metabolites. As previously mentioned, there are differences in analytical platform and different sensitivities for detection of such metabolites across different studies. Metabolomics is a relatively new field, and as such, the techniques are not yet standardized. Also several studies had a small sample

size ($n \leq 30$ per group). This increases the possibility of chance findings and diminishes power of the study. The examination of mice *vs* human metabolites could be another source of error. Although human gastric cell lines were implanted into mice, human physiology is still considerably more complex; this may account for differences between human and animals studies.

Some studies matched for age and gender between groups (Song *et al*[32]) but others (Ikeda *et al*[36]) just used twelve human volunteers. This introduces selection bias. The small sample size and lack of age and gender matching between cancer and normal groups could confound the metabolomic profile. Depending on the type of tissue or biofluid sampled, there may also be differences. Aa *et al*[29] noted dissimilarities in relative quantities between tissue and serum in their study between GC and CSG patients. For example, TCA intermediates, lactate, amino acids and free fatty acids were more abundant in tissues than in the patient matched sera. This suggests that metabolism is most intensive at the tissue level and becomes somewhat diluted in biofluids.

CONCLUSION

Gastric cancer is the one of the leading causes of cancer deaths worldwide, and is especially prevalent in Asian countries like Japan, China and South Korea. Current surveillance techniques such as barium photofluorography, endoscopy and serum pepsinogen testing are known to have limitations. As of late, metabolomics is a new area of study that has joined the armamentarium of diagnostic possibilities. Only a handful of studies have looked at the role of metabolomics in gastric cancer. Variations in fatty acid, carbohydrate, lipid and nucleic acid metabolites have been identified that distinguish cancerous from healthy individuals, as well as stage of gastric cancer. Aberrations in carbohydrate metabolism seem to be the most preserved feature of these metabolic studies, as well as elevation of key amino acids that contribute to carbohydrate pathways through anaplerotic reactions.

In spite of the differences identified, there are inconsistencies in metabolomic profiles between studies. This may be attributable to differences in sample type, as plasma compared to urine compared to stomach tissue may yield different metabolomic profiles, as well as sampling techniques, analytical platforms and subject type (animal or human). While these early studies on metabolomics show promise, this is a relatively new field in the pre-clinical phase. Our lab group is currently studying metabolic differences in urine between Stage I -III gastric cancer patients, benign gastric disease and healthy controls, as well as how *H. pylori* affects the metabolic signature. NMR spectroscopy will be employed. This future research will hopefully add to the growing body of knowledge and advance the clinical applicability of metabolomics in surveillance and diagnosis of gastric cancer.

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