REVIEW

The Current State of Proteomics in GI Oncology

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Abstract Proteomics refers to the study of the entire set of proteins in a given cell or tissue. With the extensive development of protein separation, mass spectrometry, and bioinformatics technologies, clinical proteomics has shown its potential as a powerful approach for biomarker discovery, particularly in the area of oncology. More than 130 exploratory studies have defined candidate markers in serum, gastrointestinal (GI) fluids, or cancer tissue. In this article, we introduce the commonly adopted proteomic technologies and describe results of a comprehensive review of studies that have applied these technologies to GI oncology, with a particular emphasis on developments in the last 3 years. We discuss reasons why the more than 130 studies to date have had little discernible clinical impact, and we outline steps that may allow proteomics to realize its promise for early detection of disease, monitoring of disease recurrence, and identification of targets for individualized therapy.

The term "proteome," coined in 1995, is analogous to "genome," and was initially used to describe "the entire complement of proteins expressed by a genome, cell, tissue or organism" [1]. The human genome contains about 23,000 protein-coding genes [2], but because of the occurrence of alternative splicing, the proteome is much larger, and probably consists of more than 100,000 distinct polypeptides [3]. The prevalence of post-translational modifications contributes additional diversity. Proteomic analysis is more challenging than genomic analysis, but is also more rewarding, because it captures regulatory effects at all levels of gene expression (i.e., transcriptional, translational, and post-translational). The goal of proteomics, in most cases, is the discovery of protein biomarkers, which are signatures of physiological or disease state. These can be used, alone or in combination, for screening and diagnosis, establishment of individual prognosis, prediction of individual response to therapy, and monitoring of disease progression [4, 5].

This review is in three parts. First, we present an overview of current proteomic technologies. Proteins and peptides are much more chemically diverse than nucleic acids, and the technologies required for proteome analysis are correspondingly more complex. It is helpful to introduce terminology and current technical approaches before considering clinical studies in detail. Second, we describe the use of proteomic technologies in GI oncology. This compilation is based on a MEDLINE search of the

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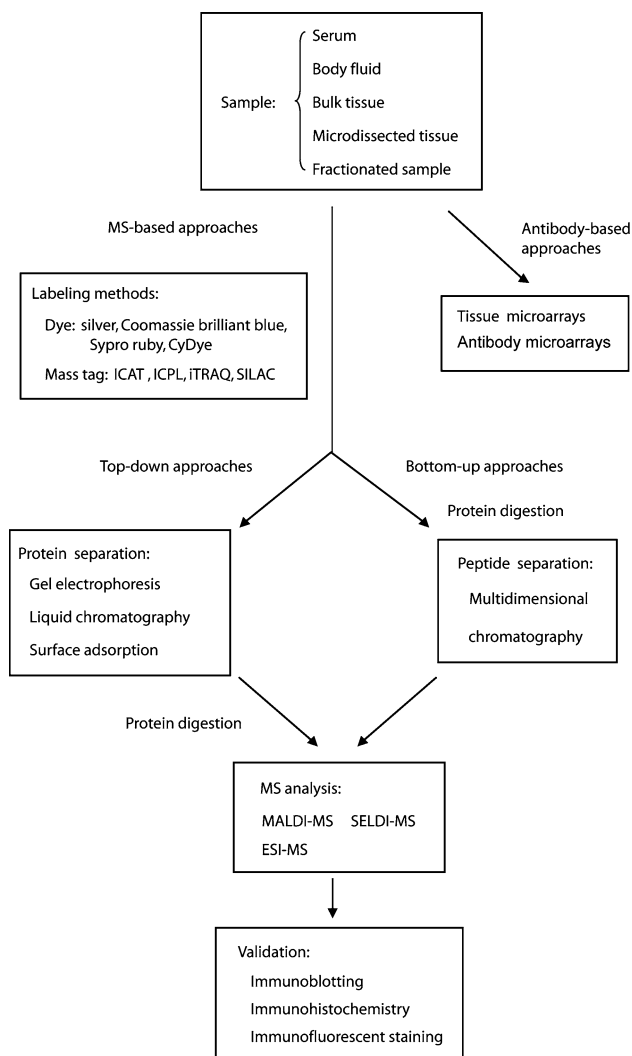Department of Pathology, Medical College of Georgia, Augusta, GA, USA

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literature through August 2008. In addition to summarizing our results in table form, we provide an overview and briefly summarize a few notable findings in the main text. Third, we discuss prospects for application of proteomic findings in GI oncology, including the limitations of current studies and a discussion of steps that are needed to advance the field to the next level.
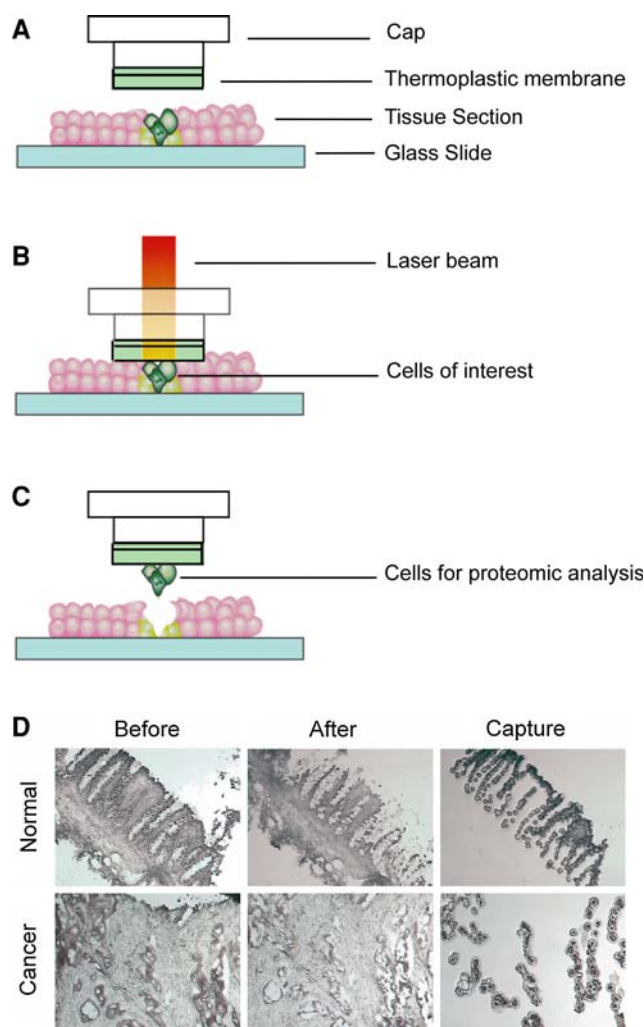
Proteomic Technologies

Figure 1 provides an overview of currently available analytical strategies, including types of samples and profiling methods.

Types of Samples

Samples used for clinical proteomics come from three sources: serum, other accessible body fluids (e.g., saliva, gastric juice, pancreatic juice, or bile), and tissue. Serum is advantageous for screening and early detection of disease because collection is minimally invasive. It presents challenges for analysis, however, because cancer biomarkers are likely to be much more dilute in serum than in the tissue of origin. An additional complication is that a majority of serum peptides likely represent fragments of larger proteins degraded by various proteases [6]. GI fluids are advantageous because they are relatively organ-specific, and proteins of interest may be present at higher

Fig. 1 Strategies for proteomic analysis of clinical samples. Samples may include serum, other body fluids, or tissue. Profiling may be antibody-based or MS-based. A variety of labeling and protein separation techniques may be used prior to the MS. Top-down and bottom-up approaches differ in the order in which steps are performed. In many proteomic studies, key findings are validated by independent means (see text for details and definition of additional terms)

Fig. 2 LCM. **a** Thermoplastic membrane is placed over a tissue section, **b** infra-red laser pulse is used to heat a 7.5–30 μm diameter spot, briefly melting the membrane and capturing cells of interest. Heating and cooling of the membrane apparently has no adverse effect [7]. **c** Cells of interest become attached to the membrane and can be lifted from the slide for downstream analysis. **d** Application of LCM on colonic epithelium and colon cancer tissue slides

concentrations than those in the serum. Because collection is difficult and somewhat invasive, analysis of GI fluids is an option that is primarily applicable in symptomatic patients. Tissue samples are advantageous because tissue is the ultimate source of biomarkers present in serum and other fluids. Although collection is invasive, tissue extraction provides access to a variety of intracellular regulatory proteins, such as regulatory kinases or transcription factors, which would not routinely be present in serum or GI fluids. Tissue studies thus may provide more insight into disease mechanisms than can be obtained by analysis of samples from non-tissue sources.

Tissue proteomics may use material from bulk dissection or laser capture microdissection (LCM). The latter, illustrated in Fig. 2, allows analysis of specific cell types (e.g., cancer cells free of stroma) [7]. Other methods of sample fractionation have also been used to enrich for cancer cells, for example passage through a narrow gauge needle to detach tumor cells from stroma [8]. Tissue proteomics can also be performed using imaging mass spectrometry (IMS), where tissue sections are analyzed directly by mass spectrometry, circumventing the need for microdissection or protein extraction (recently reviewed in [9]).

Use of archival tissue is complicated by covalent protein modifications introduced by common methods of fixation and staining. Although several recent reports describe analysis of peptides recovered from formalin-fixed, paraffin-embedded tissues [10–13], the most common method of sample preservation for proteomic analysis is freezing, which necessitates dedicated sample collection. Alternative techniques based on alcohol or other chemical fixatives have also shown promise [14, 15].

Profiling Methods

Mass Spectrometry (MS)-Based Profiling

Many clinical studies are exploratory, that is, broad surveys of the proteome without prior knowledge of the proteins of interest. Profiling of tissue extracts can be performed using either a "top-down" or "bottom-up" approach (Fig. 1). Top-down approaches begin with one or more separation steps to resolve individual proteins, or classes of proteins, in a complex mixture. Because intact proteins are physically and chemically diverse, there is no single universally applicable separation method. Two-dimensional gel electrophoresis (2-DE), which separates proteins based on charge in one dimension and size in the other [16], can separate up to 5,000 distinct proteins simultaneously [17]. Proteins may be reacted with fluorescent CyDyes prior to electrophoresis, or the gel may be stained afterward. Limitations of 2-DE are that it cannot be fully automated,
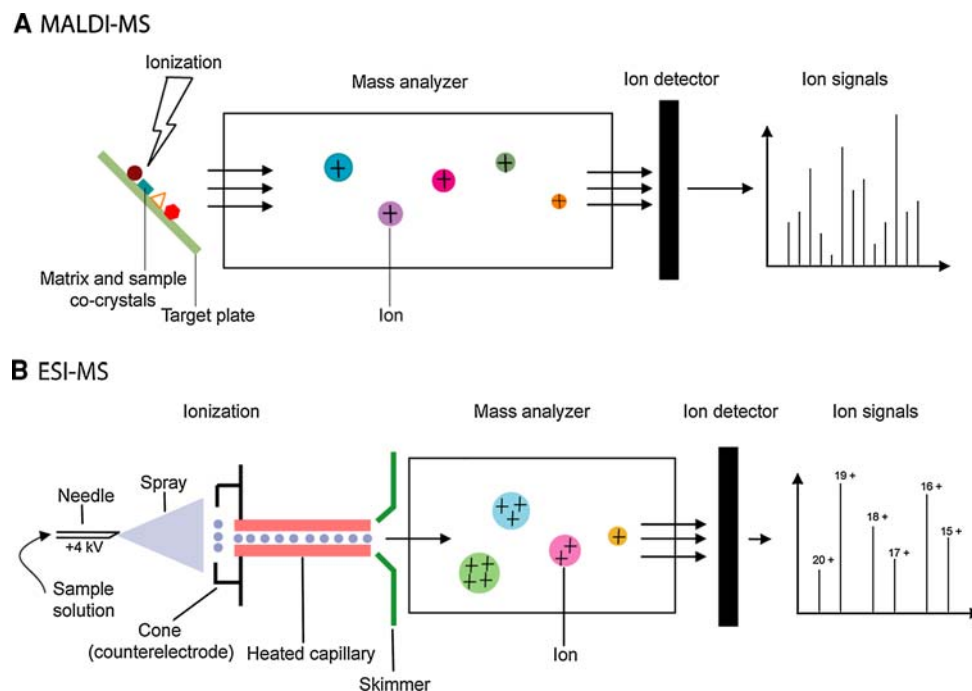
and tends not to resolve proteins that are large, hydrophobic, or strongly basic. Liquid chromatography (LC) provides an alternative to 2D gels; although it can be used for intact proteins, it has found wider application in the "bottom-up" approaches discussed below.

Surface adsorption is a specialized separation and concentration method used in surface enhanced laser desorption ionization (SELDI). A protein solution is incubated with a hydrophobic, charged, or other surface that is fabricated as part of a chip that can be introduced directly into a mass spectrometer, producing a complex mass spectrum that provides a "fingerprint" of a physiological state or disease. SELDI is easily applied to large numbers of clinical samples and is most commonly used for serum studies.

In a bottom-up approach, the order of the analytical steps differs [18, 19]. Proteins in the sample mixture are first digested to completion with a site-specific protease. Peptides (rather than intact proteins) are chromatographically separated by high-resolution ion exchange and reverse-phase LC. Products are again analyzed by MS in the final step [20]. Although throughput is limited, bottom-up approaches can identify very large numbers of proteomic features, and they can be applied to proteins that are difficult to solubilize and resolve when intact [21]. Top-down and bottom-up approaches are thus complementary and potentially provide somewhat different information.

Options for final MS analysis are similar in all approaches. A "soft ionization" procedure creates peptide ions in the gas phase, using mild conditions that maintain peptide bonds intact. In matrix-assisted laser desorption ionization (MALDI) (and its specialized variations, SELDI and IMS) a laser pulse is directed at a mixture of protein sample and an organic matrix (Fig. 3a) [22]. With electrospray ionization (ESI), the other common soft ionization method, a protein or peptide solution passes through a heated capillary, spraying droplets of solution into a vacuum chamber containing a strong electric field, where they then evaporate and ionize (Fig. 3b) [23]. The ions are passed through a mass analyzer, which separates them based on mass-to-charge (m/z) ratio.

Data is obtained in the form of a mass spectrum—a histogram with ion counts on the vertical axis and m/z on the horizontal axis (Fig. 3). In SELDI, pattern analysis may be applied to detect features of the spectrum that correlate with disease state, even without identification of proteins at the molecular level. In other MS procedures, the goal is molecular identification of proteins present in the sample. This is done by matching a pattern of peptides, or "peptide mass fingerprint," against a human protein database. Confidence in MS identifications is based on coverage (the fraction of the protein's total sequence represented among the identified peptides) and statistical criteria particular to the method used.

A MALDI-MS

B ESI-MS

Fig. 3 MALDI-MS and ESI-MS procedures. **a** In MALDI-MS, samples are co-crystallized with an organic matrix on a metal target plate. A pulsed laser irradiates the co-crystals, which causes rapid heating and desorption of ions into the gas phase. Ions go through the mass analyzer and the detector registers the numbers of ions at each individual mass-to-charge (m/z) value, then the peptide mass fingerprint is generated. MALDI-MS produces relatively simple spectra composed of ions with unit charge. **b** In ESI-MS, sample molecules are ionized directly in the analyte solution by passing through a heated capillary device, spraying droplets of solution into a vacuum chamber containing a high-strength electric field. The resulting ions pass through a mass analyzer and detector as in **a**. ESI-MS produces complex spectra with multiply charged ions

Tandem mass spectrometers, which have more than one mass analyzer connected in series, can perform MS/MS. This involves selection of an ion of interest based on m/z, partial fragmentation at peptide bonds (by collision with an inert gas), and passage of the products through a second mass analyzer, with the resulting fragmentation pattern providing amino acid sequence information for the precursor ion. Partial sequence data obtained by MS/MS provides a further basis for identification [24].

Imaging MS is performed by first coating a thin (10-μm) tissue section with organic matrix. The section is systematically moved underneath a laser beam and a mass spectrum is collected at each position. Software renders the data as a spatially resolved density map showing relative abundance of peptides or proteins of interest [9, 25].

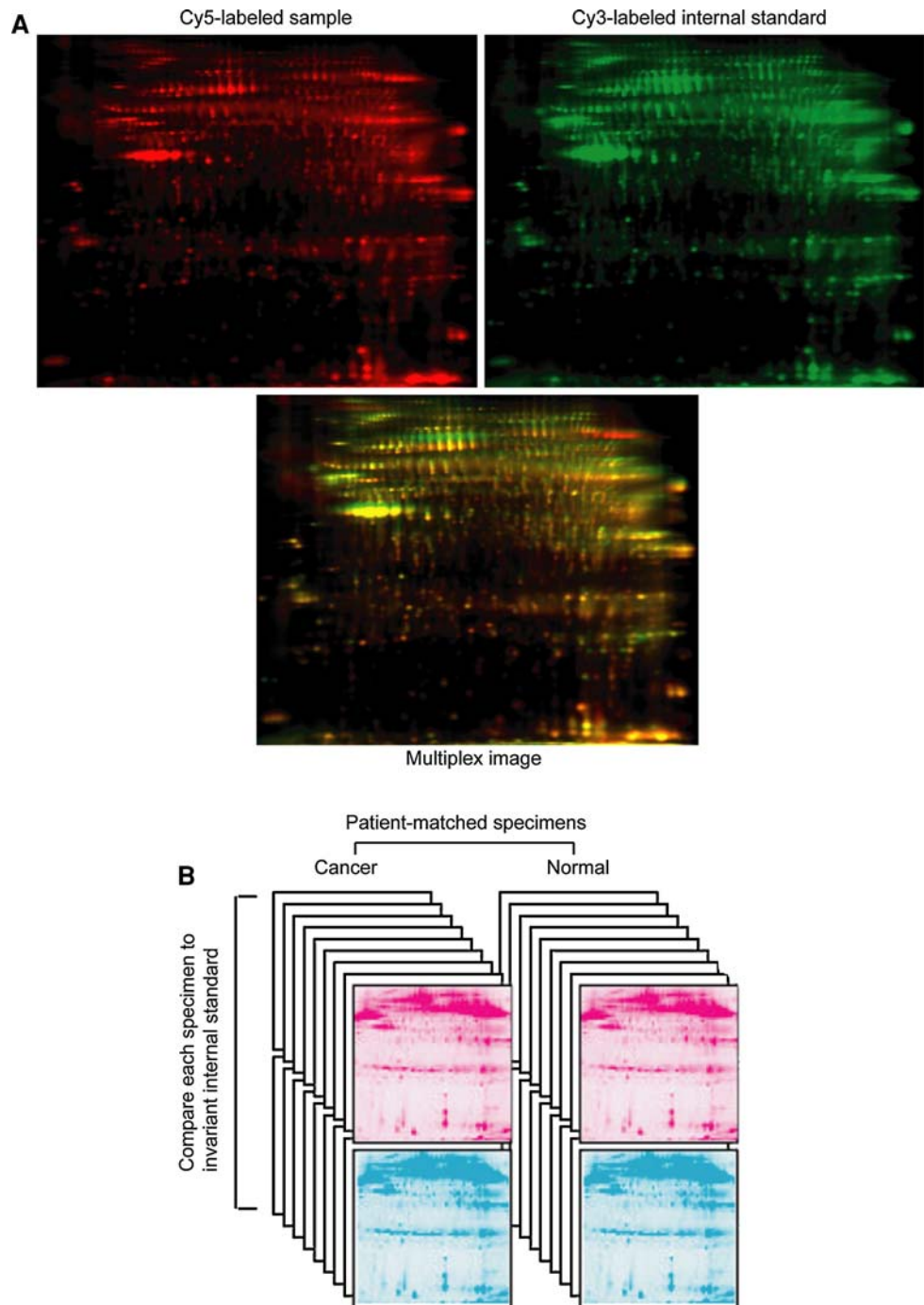### Quantification of Protein Abundance

Clinical laboratory studies require quantification of molecular species, rather than simple determination of presence or absence. Neither gel staining nor mass spectrometry provides a good indication of absolute quantity. Quantification thus relies on multiplex analysis, where samples from different sources are differentially labeled, mixed, and subjected to electrophoretic or chromatographic separation. The abundance of each protein or peptide is determined relative to the corresponding feature in the other sample. Clinical samples can be compared directly (e.g., diseased versus normal) or indirectly with reference to an invariant internal standard, consisting of a mixture of samples used in the experiment.

Two-dimensional difference gel electrophoresis (2D-DIGE) is the most common multiplex top-down approach [26, 27] (Fig. 4). Proteins are covalently labeled by reaction of cyanine dyes with cysteine or lysine residues. Spectrally distinct dyes are similar in molecular weight and do not change the protein charge. Thus, the same proteins in different samples, labeled in different colors, migrate to the same position in the gel. For each spot, the ratio of emission at different wavelengths provides a measure of relative abundance [28].

Isotope-coded affinity tag (ICAT) technology is the analogous method for the bottom-up approach. The ICAT reagent combines three moieties: a biotin group, a heavy or light isotope-tagged linker (e.g., containing ^2H vs. ^1H, or ^{13}C vs. ^{12}C), and a thiol-specific reactive group that reacts with cysteine in the protein sample (Fig. 5a) [29]. Two samples, pre-labeled with heavy- or light-isotope ICAT reagent, are mixed and proteolytically digested (Fig. 5b). Tagged peptides are isolated by avidin affinity
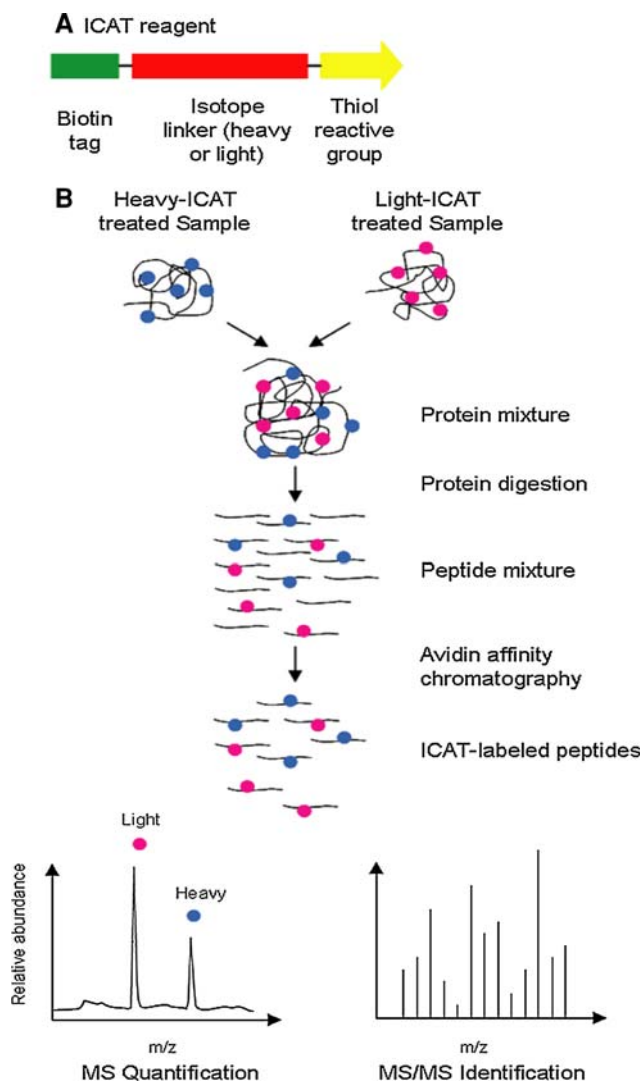
Fig. 4 Two-dimensional difference gel electrophoresis (2D-DIGE). **a** Representative gel images of proteins from analysis of a microdissected CRC specimen in our laboratory. *Red* represents Cy5-labeled sample proteins, and *green* represents Cy3-labeled pooled internal standard. In the multiplexed image, spots that are more abundant in the sample than in the standard appear *red*, spots that are less abundant in the sample appear *green*, and spots that are equal in the sample and the standard appear *yellow*. **b** Design of a clinical proteomics experiment. In this example, which is based on analysis of cancer-normal pairs, each patient contributes two samples: cancer and adjacent normal tissue. The number of gels equals the number of samples. For each spot in each gel, the ratio of emission at Cy5 and Cy3 wavelengths is measured. These "internal ratios" are used to compare the relative abundance of a given protein across the different specimens in the experiment

chromatography and analyzed by LC-MS [30]. The relative abundance of heavy and light isotope peaks for each peptide provides an accurate measure of the relative abundance of the peptide in different samples. A variation, isotope-coded protein label (ICPL) [31], is based on isotopic labeling of free amino groups in proteins, which are more abundant than thiols. Another variation, isobaric tags for relative and absolute quantification (iTRAQ) allows multiplexing of up to four samples simultaneously [32].

Antibody-Based Profiling

In contrast to MS-based methods, antibody-based profiling requires prior knowledge of proteins of interest. Tissue microarrays exemplify a broad class of technologies referred to as protein arrays where proteins or tissue samples are spotted on a surface and probed with antibody (Fig. 6a) [33, 34]. Often used for validation of biomarkers identified in MS-based methods, they have the same

A ICAT reagent

Biotin tag | Isotope linker (heavy or light) | Thiol reactive group

B

Heavy-ICAT treated Sample

Light-ICAT treated Sample

Protein mixture

Protein digestion

Peptide mixture

Avidin affinity chromatography

ICAT-labeled peptides

Light

Heavy

Relative abundance

m/z

MS Quantification

m/z

MS/MS Identification

Fig. 5 Schematic illustration of ICAT procedure. **a** ICAT reagent combines three moieties: a biotin tag, a heavy or light isotope-tagged linker, and a thiol-specific reactive group. **b** Samples, labeled with heavy- or light-isotope ICAT reagent are mixed and digested. Tagged peptides are isolated by avidin affinity chromatography and analyzed by LC-MS. The relative abundance of heavy and light isotope peaks for each peptide is then measured. Peptides of interest can be identified by MS/MS analysis

advantages and disadvantages as other forms of immuno-histochemistry (IHC). Interpretation of staining patterns can be subjective, and quantification is less precise than with other proteomic methods [35].

In another variation on array technology, a panel of antibodies is spotted on a surface and incubated with a solubilized mixture of proteins. After washing, the protein bound to each spot is quantified using a labeled secondary antibody or reagent (Fig. 6b) [36]. The technology of antibody arrays is just beginning to be applied in GI oncology [37, 38] and holds promise as a method for

simultaneous analysis of multiple biomarkers, or "proteomic signatures" in a clinical laboratory setting.

Use of Proteomic Technologies in GI Oncology

Methods

To identify relevant literature, we searched MEDLINE through August 2008 using entry terms including "proteomics," "biomarker discovery," "mass spectrometry," "gastrointestinal tumor," "serum," "human tissue," "gastric juice," "pancreatic juice," "bile," "GI secretions," "esophageal cancer," "gastric cancer," "small intestine tumor," "colorectal cancer," "pancreatic cancer," "hepatocellular carcinoma," and "cholangiocarcinoma" in different combinations. English-language abstracts of the retrieved articles were reviewed and categorized. In all but a few cases, full articles were obtained and reviewed. Additional citations were obtained from review articles and from the bibliographies of cited references.
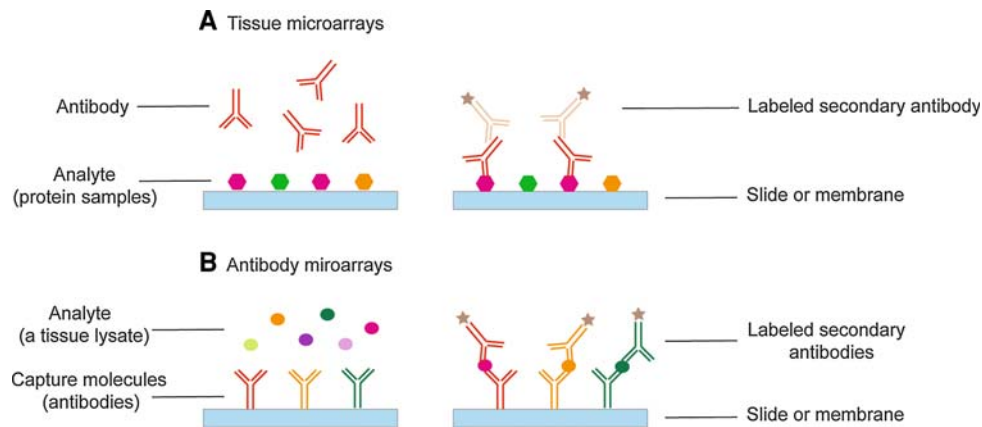
Serum Biomarkers

We identified 57 serum-based studies (Table 1). Of these, 54 used MS-based profiling, while three recent studies applied antibody-based profiling [37–39]. All but one of the MS-based studies used a "top-down" strategy, in the majority of cases SELDI-MS (38/54 studies).

Diseases of the Alimentary Tract

Eight studies analyzed sera from patients with esophageal cancer or related premalignant conditions such as dysplasia or basal cell hyperplasia. In three of these, anonymous SELDI m/z peaks were used in classification algorithms to discriminate between normal or disease states [40–42]. In a fourth SELDI study, m/z peaks were used to distinguish chemoradiation responders from non-responders [43]. Two 2-DE studies identified a small number of serum proteins that differed in pre- and post-surgery patients, with no overlap in the proteins identified in the two reports [44, 45]. Two other studies identified characteristic serum autoantibodies against peroxiredoxin VI and heat-shock protein 70, respectively, as potential diagnostic biomarkers [46, 47].

Five studies of gastric cancer have used the SELDI approach [48–52]. In each case, SELDI identified combinations of m/z peaks that correctly classified most cancer patients versus other subjects. In one study, relevant peaks were identified as stress-related proteins, including heat-shock protein 27, glucose-regulated protein, and protein disulfide isomerase [50]. Levels of these proteins declined following surgery, suggesting that they could be used in

Fig. 6 Protein microarray technology. a Tissue microarray. Multiple tissue sections (or protein extracts) are spotted onto an array, which is incubated with a specific antibody against the protein of interest. Samples that contain the protein of interest are then detected. b Antibody microarrays: A series of capture molecules (antibodies) are displayed on a slide or membrane that is exposed to analytes (a tissue lysate). The bound proteins are detected by labeled secondary antibodies

surveillance for recurrence [50]. Specificity and sensitivity using SELDI-based biomarkers were higher than those achieved for the same samples using two established markers, carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19–9, in combination [52]. A very recent study used antibody microarray technology to explore serum biomarkers of gastric cancer. Serum reactivity with IPO-38 antibody, which is directed against a small nuclear protein (possibly H2B), appeared useful both for diagnosis and for predicting survival in gastric cancer [38].

Ten studies analyzed sera from colorectal cancer (CRC) patients. Nine used SELDI-MS; several showed that SELDI-MS biomarkers compare favorably with established tests, including fecal occult blood or CEA [53], or a triple combination of CEA, CA19–9, and CA 242 [54]. SELDI-MS markers can be used to classify different stages of CRC [55], and to differentiate between good and poor responders to neoadjuvant therapy [56]. As with other diseases, the majority of CRC SELDI-MS studies are based on anonymous peaks, although a few studies report identification of specific proteins associated with classifier peaks, including apolipoproteins A-I and C-I [57], complement C3a des-arg, alpha1-antitrypsin and transferrin [53], and serum amyloid A [58]. A 2-DE serum analysis found 28 spots differentially expressed between cancer and normal, among which clusterin, complement factor I and β-2-glycoprotein I were proposed as a potential panel of CRC biomarkers [59].

Although most studies focus on cancer, three used proteomic methods to identify biomarkers of benign diseases [60–62]. One of these studies showed the ability to distinguish patients with Crohn's disease or ulcerative colitis from control subjects who were either healthy or suffered from other inflammatory conditions based on four identified classifier proteins [60]. Another showed the ability to identify patients with large colon adenomas based on a set of anonymous m/z peaks [62]. Another used ICAT technology to identify proteins useful for differential diagnosis of familial adenomatous polyposis [61].

Diseases of the Pancreas and Hepatobiliary Tract

Ten studies profiled sera from pancreatic or biliary tract cancer patients. Three SELDI studies identified anonymous m/z peaks that correctly classified most pancreatic cancer [63, 64] or cholangiocarcinoma [65] patients. In one of these studies, SELDI-MS biomarkers, or SELDI-MS biomarkers in combination with CA19-9, were significantly more accurate than CA19-9 alone [63]. Serum CA 19-9 can also be sensitively detected with protein array technology [39]. Other studies, using 2-DE, identified proteins, or in one case autoantibodies, that are differentially present in sera from cancer patients versus control subjects [66–69]. In one of the first applications of antibody microarray to GI oncology, a recent study identified a signature consisting of 21 protein analytes that discriminates between short-surviving (<12 months) and long-surviving pancreatic cancer patients [37].

Twenty studies, primarily using SELDI-MS, characterized changes in the serum proteome of patients with hepatocellular carcinoma (HCC). HCC usually develops following a long history of chronic liver disease, and there is a need for markers of progression to cancer. All SELDI studies identified m/z peaks that accurately classified sera from patients with chronic hepatitis B or C infection, cirrhosis, and HCC (Table 1). Two studies specifically commented on prospects for use of SELDI-MS biomarkers for early detection: Kanmura et al. analyzed sera collected before the diagnosis of HCC by ultrasonography. They demonstrated the ability of SELDI-MS biomarkers to predict the diagnosis of HCC in 6/7 patients before HCC was clinically apparent [70]. Zinkin et al. demonstrated that SELDI-

Table 1 Serum proteomic surveys relevant to cancer and other diseases of the GI tract

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
Esophageal cancer	Monitoring	Serum	2-DE, MALDI-TOF MS	17 cancer (matched pre- and post-surgery)	5 identified proteins with differential expression in pre-surgery cancer	Zhang [45]
Esophageal cancer	Detection	Serum	2-DE, MALDI-TOF MS	6 cancer (before and after surgery), 6 normal	7 protein spots with significant difference before and after surgery	An [44]
Esophageal cancer	Prediction	Serum	SELDI -MS	Training set: 15 responder to preoperative chemoradiotherapy, 12 nonresponder; validation set: 15 cancer	4 m/z peaks that distinguish responder from nonresponder	Hayashida [43]
Esophageal cancer	Early detection	Serum	SELDI-MS	30 cancer, 27 dysplasia, 40 basal cell hyperplasia, 63 normal	4 m/z peaks that classify different disease states	Wang [41]
Esophageal cancer	Detection	Serum	2-DE, MALDI-TOF MS	30 esophageal cancer, 30 normal, 30 other cancers	Autoantibody against peroxiredoxin VI that classify esophageal cancer versus the others	Fujita [46]
Esophageal cancer	Detection	Serum	SELDI-MS	50 cancer, 11 normal	7 m/z peaks that classify cancer versus normal	Hammoud [40]
Esophageal cancer	Detection	Serum	SELDI-MS	36 cancer, 38 normal	31 m/z peaks with significant difference between cancer and normal, 4 protein peaks classify cancer versus normal	Wang [42]
Esophageal cancer	Detection	Serum	2-DE, MALDI-TOF MS	16 esophageal cancer, 13 normal, 36 other cancers	Autoantibody against Hsp70 increased in esophageal cancer versus the others	Fujita [47]
Gastric cancer	Detection	Serum	SELDI-MS	28 cancer, 9 stage I cancer, 11 non-cancer, 30 normal in a test set	Classifier ensemble correctly classified almost all gastric cancer and control patients in test sets	Ebert [48]
Gastric cancer	Detection	Serum	SELDI-MS	45 cancer, 40 gastritis, 42 normal	4 m/z peaks that correctly classify cancer, gastritis, normal	Liang [49]
Gastric cancer	Detection, monitoring	Serum	SELDI-MS	46 cancer, 40 normal	14 m/z peaks with differential expression in cancer, possible IDs	Ren [50]
Gastric cancer	Detection	Serum	SELDI-MS	60 cancer, 40 normal	17 m/z peaks with differential expression in cancer, 4 m/z peaks gave highest discrimination	Lim [51]
Gastric cancer	Detection	Serum	SELDI-MS	127 cancer, 9 benign gastric lesion, 9 colorectal cancer, 100 normal	3 m/z peaks correctly classify gastric cancer versus others	Su [52]

Table 1 continued

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
Gastric cancer	Detection, monitoring	Serum	Antibody microarray, immunoprecipitation, MALDI-TOF/TOF MS, immunoblotting, ELISA	Initial study of 3 gastric cancer, 2 colorectal cancer, 2 pancreatic cancer, 2 liver cancer, 2 breast cancer, and 2 normal; validation study of 94 gastric cancer and 41 normal	Up-regulation of IPO-38 was identified and validated in gastric cancer	Hao [38]
Colorectal cancer	Detection	Serum	SELDI-MS	73 cancer, 16 benign colorectal disease, 31 normal	9 m/z peaks with differential expression in different disease states	Zhao [110]
Colorectal cancer	Detection	Serum	SELDI-MS artificial neural network	55 cancer, 92 normal	4 m/z peaks that classify cancer versus normal	Chen [111]
Colorectal cancer	Detection	Serum	SELDI-MS, artificial neural network	62 cancer, 31 non-cancer (other disease or normal)	13 m/z peaks with differential expression in cancer, (6 of m/z peaks correspond to identified proteins).	Ward [53]
Colorectal cancer	Detection, monitoring	Serum	SELDI-MS	63 cancer, 46 non-cancer (benign disease or normal)	4 m/z peaks that classify cancer versus others, 2 m/z peaks that classify preoperative vs. postoperative samples, 2 m/z peaks that discriminate primary cancer from metastatic cancer	Zheng [54]
Colorectal cancer	Progression (stage)	Serum	SELDI-MS	76 cancer	7 models consisting different numbers of m/z peaks that classify different cancer stages	Xu [55]
Colorectal cancer	Detection	Serum	SELDI-MS	77 cancer, 80 normal	5 m/z peaks that classify cancer versus normal (3 of m/z peaks correspond to identified proteins)	Engwegen [57]
Colorectal cancer	Detection	Serum	SELDI-MS	Initial study of 74 cancer, 48 normal; validation study of 60 cancer, 39 normal	2 m/z peaks that classify cancer versus normal	Liu [112]
Colorectal cancer	Monitoring	Serum	SELDI-MS	4 cancer	16 m/z peaks (1 correspond to identified protein) with differential expression before, during, and after laparoscopic colon resection	Roelofsen [58]
Colorectal cancer	Detection	Serum	2-DE, MALDI-MS	5 cancer, 5 normal	28 protein spots with differential expression between cancer and normal	Rodríguez-Piñeiro [59]
Rectal cancer	Radiochemotherapy response	Serum	SELDI-MS	9 good responders, 11 poor responders	14 m/z peaks collectively differentiate good versus poor responders	Smith [56]

Table 1 continued

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
Inflammatory bowel disease	Differential diagnosis	Serum	SELDI-MS	30 Crohn's disease, 30 ulcerative colitis, 30 inflammatory controls, 30 normal	>20 discriminatory m/z peaks (4 identified molecularly)	Meuwis [60]
Colonic adenoma	Early detection	Serum	NanoESI-MS/MS	Training set: 37 large adenoma, 28 normal; validation set: 20 large adenoma, 50 normal	A model containing combinations of m/z features showed a sensitivity of 78%, a specificity of 53%, and an accuracy of 63%	Ransohoff [62]
Familial adenomatous polyposis (FAP)	Differential diagnosis	Serum	ICAT, LC-MS/MS	8 FAP, 2 hereditary nonpolyposis colorectal cancer, 3 sporadic colorectal cancer, 8 noncancer	6 proteins with differential expression in carpeting FAP, diffuse FAP and healthy control	Quaresima [61]
Pancreatic cancer	Detection	Serum	SELDI-MS	60 cancer, 60 nonmalignant pancreatic disease, 60 normal	2 m/z peaks that classify cancer versus normal, 3 classify cancer vs. all others	Koopmann [63]
Pancreatic cancer	Detection	Serum (immuno-depleted to remove most abundant proteins)	2D-DIGE, MALDI-TOF/TOF	Initial study of 3 cancer, 3 noncancer; validation study of 20 cancer/14 noncancer	24 unique proteins increased, 17 decreased. Apolipoprotein E, α-1-antichymotrypsin, and inter-α-trypsin inhibitor validated using independent patient set	Yu [66]
Pancreatic cancer	Detection	Serum	SELDI-MS	Initial study of 47 cancer, 53 normal; validation study of 27 cancer/27 normal	6 m/z peaks that classify cancer vs. normal	Yu [64]
Pancreatic cancer	Detection	Serum (immuno-subtraction to remove most abundant proteins)	2-DE, MALDI-TOF MS, LC-MS/MS	32 cancer, 30 normal	154 proteins with differential expression in cancer, 9 that classify cancer vs. normal	Bloomston [67]
Pancreatic cancer	Monitoring	Serum (12 most abundant proteins depleted)	2D-DIGE, LC-MS/MS	10 cancer (pre- and post- surgery samples)	32 proteins with differential expression between pre- and post- surgery samples, 16 identified	Lin [113]
Pancreatic cancer	Detection	Serum (most abundant proteins removed)	2-DE, MALDI-TOF MS, immunoblotting	16 pancreatic cancer, 16 gastric cancer, 16 other pancreatic disease, 16 normal	10 proteins with differential expression in different disease states	Sun [68]
Pancreatic cancer	Detection, differential diagnosis	Serum	2-DE, MALDI-TOF MS, LC-MS/MS, immunoblotting, IHC	70 pancreatic cancer, 40 normal, 30 non-pancreatic cancer, 15 chronic pancreatitis	8 proteins recognized by autoantibodies in cancer patients; the same proteins are overexpressed in cancer tissue	Tomaino [69]
Pancreatic cancer	Detection, prognosis	Serum	Antibody microarray	24 cancer, 20 normal	A protein signature consisting 21 proteins associated with poor prognosis	Ingvarsson [37]

Table 1 continued

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
Pancreatic cancer	Methods development	Serum or plasma	Reverse-phase protein array	71 cancer, 30 chronic pancreatitis, 48 normal	Reverse-phase protein array showed superior sensitivity and comparable specificity to ELISA in distinguishing CA19-9 levels between cancer and normal	Grote [39]
Biliary tract cancer	Detection	Serum	SELDI-MS	20 cholangiocarcinoma, 20 benign billiary condition, 25 normal	A total 23 m/z peaks with differential expression in different disease states	Scarlett [65]
HBV-related liver disease	Detection	Serum	SELDI-MS	20 HCC, 25 liver cirrhosis, 25 normal	2 m/z peaks that classify cirrhotic cohorts vs. non-cirrhotic patients	Zhu [114]
HCV-related and other liver disease	Disease progression, differential diagnosis	Serum	SELDI-MS	57 HCC, 38 cirrhosis, 36 other liver disease, 39 no liver disease	38 m/z peaks distinguish disease states, improved accuracy if combined with known serum markers	Schwegler [115]
HCC	Differential diagnosis	Serum	SELDI-MS	44 HCC with cirrhosis, 38 liver cirrhosis	30 m/z peaks with differential expression in cancer, 6 that classify cancer vs. others (C-terminal part of the V10 fragment of vitronectin identified)	Paradis [116]
Liver cancer	Detection	Serum	SELDI-MS artificial neural network	Training group: 35 cancer, 14 cirrhosis, 21 normal; test group: 17 cancer, 8 cirrhosis, 11 normal	2 m/z peaks that classify cancer vs. normal; another 2 m/z peaks that classify cancer vs. cirrhosis	Wang [117]
HCC	Radiofrequency ablation treatment response	Serum	2-DE, MALDI-TOF/TOF	8 patients (sera compared before and after treatment)	4 identified proteins decreased, 7 identified proteins increased	Kawakami [118]
HCV-related liver disease	Disease progression, differential diagnosis	Serum	SELDI-MS artificial neural network	Training group: 60 HCC, 84 non-HCC; test group: 17 HCC, 21 non-HCC	17 m/z peaks that classify cancer vs. others (2 of m/z peaks correspond to identified proteins)	Ward [119]
HCC	Methods development, detection	Serum (low mw fraction enriched)	SELDI-MS, MALDI-TOF/TOF	20 HCC, 20 normal	45 proteins that classify cancer vs. normal (the most abundant peptide matches with des-Ala-fibrinopeptide A)	Orvisky [120]
HCV-related liver disease	Methods development, disease progress, differential diagnosis	Serum (fractionated)	SELDI-MS, 2-DE, nanoLC-MS/MS	55 HCC, 48 chronic hepatitis, 9 normal	1 protein, complement C3a that classify cancer vs. hepatitis	Lee [121]

Table 1 continued

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
HCC	Disease progression, prognosis	Serum	SELDI-MS	112 HCC	A total of 43 m/z peaks that classify patients corresponding to portal vein tumor thrombus, tumor size, tumor number, respectively	Huang [122]
HCC	Detection, disease progression, monitoring	Serum	SELDI-MS, LC-MS/MS	37 hepatitis C (27 developed HCC, 18 underwent radiofrequency ablation)	40 m/z peaks with differential expression in cancer ($\beta2$-microglobulin identified), 8 m/z peaks that classify pre- vs. post-treatment patients	Ward [123]
HCV-related liver disease	Detection, disease progression, differential diagnosis	Serum	SELDI-MS	34 HCC, 44 cirrhosis, 39 fibrosis	4 m/z peaks that classify cancer vs. others, 5 m/z peaks that classify cirrhosis vs. fibrosis (apolipoprotein C–I identified)	Göbel [124]
HCV-related liver disease	Detection, disease progression, differential diagnosis	Serum	SELDI-MS	Initial study of 35 HCC, 34 other liver disease; validation study of 36 HCC/33 other liver disease	6 m/z peaks that classify cancer vs. others	Kanmura [70]
HCC	Detection	Serum	2-DE, nano-HPLC-ESI-MS/MS	5 HCC, 5 normal	6 protein spots that classify HCC vs. normal	Yang [125]
HBV-related liver disease	Detection, disease progression, differential diagnosis	Serum	SELDI-MS	Training group: 39 HCC, 36 cirrhosis, 41 hepatitis, 105 normal; test group: 42 HCC, 18 cirrhosis, 34 hepatitis, 137 normal	A total of 8 m/z peaks that classify patients corresponding to different disease states	Cui [126]
HCC	Detection, monitoring	Serum	SELDI-MS	25 HCC without treatment, 25 HCC with chemotherapy, 50 normal	A total of 7 m/z peaks that classify patients corresponding to different disease states (all correspond to identified proteins)	Geng [127]
HCC	Detection	Serum	SELDI-MS, LC-MS/MS	41 HCC, 51 hepatitis C	11 m/z peaks with differential expression in cancer (1 m/z peak identified as cystatin C)	Zinkin [71]
HCC	Detection, disease progression, differential diagnosis	Serum	SELDI-MS	81 HCC, 36 cirrhosis, 43 chronic hepatitis B	7 m/z peaks classify HCC vs. others	Cui [128]

Table 1 continued

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
HCC (HBV-related)	Detection, disease progression, differential diagnosis	Serum	SELDI-MS, 2-DE, MALDI-TOF MS	50 HCC, 45 HBV infection, 30 normal	3 m/z peaks that classify HCC, HBV infection, normal (serum SAA identified)	He [129]
HCC (HBV-related)	Detection	Serum	2-DE, MALDI-TOF MS, immunoblotting, protein microarray	18 HBV-related HCC, 10 non-HBV-related HCC, 18 normal	13 HCC-associated antigens identified	Li [73]
HCC (HBV-related)	Detection, methods development	Plasma	Peptide-based 2-D liquid phase fractionation, 2-DE, nanoLC-MS/MS	1 HCC and normal	14 proteins with differential expression between HCC and normal	Lee [72]

MS biomarkers were more accurate than traditional markers in detecting small HCCs in Hepatitis C patients [71]. A 2-D liquid phase fractionation study, using chromatofocusing (similar to the first dimension of 2-DE but performed in solution) and reverse-phase LC, identified 14 proteins with differential expression in HCC, albeit based on a single patient per group [72]. Another recent study identified a characteristic autoantibody signature in HCC patients [73].

Prospects for Clinical Translation of Serum Biomarkers

When multiple studies of the same disease are compared, a major limitation of serum profiling becomes evident, which is the unsatisfactory reproducibility between studies. The majority of early serum studies used SELDI technology, resulting in identification of anonymous discriminatory m/z peaks. In only a very few cases were the same discriminatory peaks identified. This may well reflect technical differences in sample collection, processing, type of SELDI chip, or other variables. Inconsistency between studies, however, is a major barrier to clinical translation of SELDI biomarkers. In the minority of instances where m/z peaks have been identified at the molecular level, many of them correspond to high abundance, seemingly nonspecific molecules such as stress proteins, clotting factors, and other known serum components. Although tests based on these markers might be clinically useful, it is disappointing that markers have not been identified with a more obvious connection to biological mechanisms of cancer development. One reason for this, suggested by Diamandis [74], is that current SELDI-TOF technology is capable of detecting only those proteins present at a concentration greater than 1 μg/ml, which is approximately 1,000-fold greater than the concentrations of established serum tumor markers (e.g., CEA). Very recently, newer technologies such as ICAT and protein arrays have begun to be applied in serum studies, and it is possible that these may overcome some of the limitations of earlier methodologies.

A potentially difficult issue is that most serum studies relied on patients with advanced disease, where host-tumor (paraneoplastic) interactions are likely to be prominent. Serum biomarkers discovered thus far may not be applicable for early detection of cancer in the general, low-risk population, which is typically a stated goal in serum studies. A more immediate application of serum-based biomarkers may be for differential diagnosis in symptomatic patients or monitoring of disease progression and treatment responses following diagnosis. If issues of standardization and reproducibility can be overcome, accuracy in the various studies cited here (>80% sensitivity and specificity) seems well within the range that would be needed for clinical utility.

Biomarkers from GI Secretions

Biological fluids have a special role in proteomics as applied to GI oncology. The GI tract is unique among organ systems because of the amount and type of secretions. The normal adult produces about 7 l of GI fluids daily, including saliva, gastric juice, pancreatic juice, bile, and enteric secretions [75]. These are secreted and reabsorbed in balance. Fluids produced by the GI tract have less-complex compositions than serum, are relatively organ-specific, and are potentially good sources for biomarker discovery.

There have been two "top-down" 2-D gel-based proteomic profiling studies of gastric juice. These reported simple changes in proteomic pattern that differentiate cancer, precancerous conditions and benign disease, including loss of gastric digestive enzymes and appearance of α1-antitrypsin-related proteins [76, 77] (Table 2).

A SELDI-MS study by Rosty et al. [78] dramatically demonstrated the advantages of using pancreatic juice over serum for detection of pancreatic adenocarcinoma markers. They showed that hepatocarcinoma-intestine-pancreas/pancreatitis-associated-protein-1 (HIP/PAP-1) was present at 1,000-fold higher levels in pancreatic juice of cancer patients than in the serum of the same individuals. The fold difference in cancer patients versus other subjects was also much higher in pancreatic juice than in serum. Studies by Chen et al. [79, 80] used "bottom-up" ICAT and tandem MS-based proteomics to compare protein expression in pancreatic juice from cancer, chronic pancreatitis, and normal tissue. They identified 30 proteins specific to cancer, 27 specific to chronic pancreatitis, with nine in common. Three studies using "top-down" gel-based separations identified numerous potential cancer biomarkers, some of which were known and others of which were novel, including a HIP/PAP-1-related protein designated as PAP-2 [81–83].

Three studies characterized bile from patients with cholangiocarcinoma. Kristiansen et al. [84] used lectin chromatography to enrich for proteins of interest and deplete interfering proteins, facilitating analysis of the glycoproteome. Eighty-seven unique proteins were identified and 33 glycosylation sites were found. Two studies [85, 86] analyzed the proteomes of bile fluid from patients with malignant and benign bile tract obstruction using 2-DE; in one study, the pancreatic elastase/amylase ratio was confirmed to be a much more accurate marker than CEA or CA 19-9 [86].

Prospects for Clinical Translation of GI Secretions Biomarkers

Together, studies confirm the promise of GI secretions as a concentrated source of potentially useful biomarkers.

Accessibility of these fluids varies, with collection of gastric juice being considerably easier and less invasive than pancreatic juice or bile. Nevertheless, fluids are routinely collected in symptomatic patients and tests based on these fluids may therefore be practical.

Biomarkers from GI Tissue

Tissue biomarkers are useful when a sample of the diseased tissue is available as a result of biopsy or surgical resection. Biomarkers identified by proteomic profiling of tissue have the potential to be useful directly, for example in staging or prediction of response to therapy. Information gleaned from tissue studies also lays a foundation for development of clinical serum tests; for example, if proteomic profiling reveals that a particular protein is present at high concentration in tumor tissue, one might develop a more-sensitive assay (based, for example, on protein-chip or other approaches) to investigate the presence of the protein in serum from cancer patients.

Diseases of the Alimentary Tract

There have been ten studies of esophageal cancer, all using "top-down" analysis. Eight used 2-DE separation, one used chromatofocusing, and one used capillary high-performance LC (Table 3). One of the most comprehensive of all reported proteomic surveys of GI cancers, conducted by Hatakeyama et al. [87], used 2D-DIGE to analyze 129 microdissected tissue specimens, which identified 217 differentially expressed proteins at the molecular level. Thirty-three of these distinguished tumors with and without nodal metastasis. Extensive bioinformatic analysis identified clusters of similarly regulated proteins, and gene ontology analysis showed that differentially regulated proteins had structural, transporter, chaperone, oxidoreduction, transcription, and signal-transduction activities. Zhao et al. [88] performed an interesting comparison of protein and mRNA expression. Of 38 proteins that differed in cancer-metaplasia pairs, mRNA correlated with protein expression changes in some instances but differed markedly in many others, underscoring the value added by proteomic analysis.

There have been eight gastric cancer tissue studies, all based on "top-down" analyses. Greengauz-Roberts et al. [89] demonstrated the ability to profile very small amounts of tissue (5 µg protein) using LCM and reported 42 proteins with differential expression in gastric adenocarcinoma versus spasmolytic peptide expressing metaplasia. He et al. [90] identified an 18-kDa antrum mucosa protein that was dramatically down-regulated in cancer tissues and proposed a special role for this protein in pathogenesis of gastric cancer. GI stromal tumor is a rare, non-epithelial

Table 2 Proteomic surveys of GI-associated body fluids

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
Gastric cancer	Detection	Gastric juice	2-DE, MALDI-TOF MS, and LC-ESI-MS	30 cancer, 56 chronic atrophic gastritis, 13 premalignant, 5 normal	Loss of gastric digestive enzymes in majority of cancers, appearance of α1-antitrypsin and associated protease	Lee [77]
Gastric cancer	Early detection	Gastric juice	2-DE, MALDI-TOF MS, and LC-ESI-MS	21 advanced cancer, 6 early cancer, 33 gastric ulcer, 33 duodenal ulcer, 47 healthy	Samples classified into 3 2-DE patterns (basic, specific nonspecific) that discriminate between disease states	Hsu [76]
Pancreatic cancer	Detection	Pancreatic juice	SELDI-MS, ProteinChip immunoassay	28 cancer, 15 other pancreatic disease	1 protein, HIP/PAP-1 that classify cancer vs. other diseases	Rosty [78]
Pancreatic cancer	Methods development, detection	Pancreatic juice	1-DE, LC-MS/MS	3 cancer	A total of 170 proteins identified	Gronborg [81]
Pancreatic cancer and chronic pancreatitis	Detection, differential diagnosis	Pancreatic juice	ICAT, HPLC-ESI-MS/MS	1 cancer, 1 chronic pancreatitis, 10-11 normal	30 identified proteins with differential expression in cancer, 27 in chronic pancreatitis, 9 in common	Chen [79, 80]
Pancreatic cancer, benign pancreatic disease, cholelithiasis	Methods development, detection, differential diagnosis	Pancreatic juice	2-DE and MALDI-TOF MS	5 cancer, 6 benign pancreatic disease, 3 cholelithiasis	Proteomic patterns correlated with degree of obstruction of pancreatic duct; some spots are potential cancer biomarkers	Zhou [82]
Pancreatic cancer	Detection	Pancreatic juice	2D-DIGE, MALDI-TOF-MS/MS, immunoblotting, IHC	Initial study of 9 cancer and 9 non-cancer; validation study of 57 cancer and 14 non-cancer	24 proteins with differential expression in cancer; MMP-9, DJ-1 and A1BG were validated	Tian [83]
Biliary tract cancer	Methods development	Bile	1-DE, lectin affinity chromatography, and LC-MS/MS	1 cancer (cholangiocarcinoma)	Development of methods to enrich proteins of interest/depleted interfering proteins, analyze glycoproteome	Kristiansen [84]
Biliary tract cancer	Methods development, differential diagnosis	Bile	2-DE	1 cancer (cholangiocarcinoma), 1 cholelithiasis	Development of sample preparation methods, 16-23 unidentified proteins with differential expression in cancer	Chen [85]
Biliary tract cancer	Detection, differential diagnosis	Bile	2-DE, LC-MS/MS	Initial study of 9 cholangiocarcinoma and 9 gallstone; validation study of 22 cholangiocarcinoma, 28 gallstone	1 protein with differential expression in cancer	Chen [86]

malignancy of the GI tract, most commonly occurring in the stomach. Many cases are associated with mutation of the KIT protooncogene or platelet-derived growth factor receptor alpha. Two 2-DE studies identified proteins that were differentially expressed in patients in different mutation classes, or that discriminated patients with poor and good prognoses. Pfetin, a potassium channel protein, was identified as a powerful prognostic marker [91].

Eleven reports describe proteomic profiling of CRC or premalignant adenomas (Table 3). Seven studies using top-down 2-DE approaches identified numerous differentially expressed proteins including transcription regulators, signal transduction, and cytoskeletal proteins, molecular chaperones, protein synthesis factors, metabolic enzymes, apoptosis-associated proteins, and a proteoglycan (mimecan). A study of Pei et al. [92] is notable for identifying four proteins that differed specifically between primary tumors derived from node-positive versus node-negative patients. Two studies applied IMS technology on tissue sections without solubilization or protein-separation steps, an approach that is capable of providing spatially resolved images of in situ protein abundance in tumor areas versus normal areas [93, 94]. Another methodologically interesting study by Madoz-Gurpide et al. [95] selected 29 gene products for detailed investigation based on statistically significant up-regulation at the mRNA level and other criteria. They expressed these gene products in *E. coli*, prepared antibodies, and tested seven by IHC in a tissue microarray. They confirmed that six (ANXA3, BMP4, LCN2, SPARC, MMP7, and MMP11) were up-regulated at the protein level. Their unique, gene- and antibody-based approach avoids bias against interesting classes of proteins (i.e., very large, hydrophobic, or insoluble) that are readily overlooked in top-down proteomic approaches that rely on 2-DE as a first step.

Diseases of the Pancreas and Hepatobiliary Tract

Pancreatic cancer is second only to CRC as a cause of GI cancer deaths in the US. Unlike CRC, it is almost never detected until it has reached an incurable stage. Better detection, together with insights into disease mechanisms that might lead to better preventive or therapeutic options, would bring a large public health benefit. There have been seven "top-down" 2-DE studies (Table 3). The most comprehensive of these, by Lu et al. [96], identified 111 differentially expressed proteins at the molecular level. Proteins in this and other studies have structural, protease, metabolic, immune/inflammatory, transporter, RNA processing, transcription factor, signal transduction, cell adhesion, and other activities; some have been further validated by IHC. Studies by Chen et al. [97, 98] used "bottom-up" ICAT and tandem MS-based proteomics to identify 50

proteins as differentially expressed in cancer and 116 in pancreatitis, with considerable overlap between groups. Finally, A SELDI study [99] identified 33 anonymous m/z peaks that collectively distinguished pancreatic cancer, benign disease, and nonmalignant tissue. The same group applied a similar methodology to cholangiocarcinoma and identified 14 discriminatory, anonymous m/z peaks [65].

HCC is another GI cancer that has been widely studied using tissue profiling. As in the serum studies, progression of HBV or HCV-related disease to HCC has been the main focus. Of 19 studies, 16 used "top-down" 2-DE approaches, two used a "bottom-up" ICAT approach, and one used direct analysis of tissue slices by SELDI-TOF. Two of the most comprehensive studies combined LCM with ICAT and 2D-LC-MS/MS to compare the proteome of HCC with normal liver, identifying 149 differentially expressed proteins in one case, and 261 in another [100, 101]. Blanc et al. [102] identified 155 differentially regulated proteins in a 2-DE study, and Luk et al. [103], identified 90 in another. A 2D-DIGE study identified 127 differentially expressed proteins in cancer, and demonstrated in a validation study that a proteomic signature, based on clathrin heavy chain and formiminotransferase cyclodeaminase, could make substantial contributions to early diagnosis of HCC [104].

A methodologically interesting study by Emadali et al. [105] describes analysis of the hepatic tyrosine phosphoproteome using anti-phosphotyrosine antibodies to enrich for proteins of interest, followed by 1-DE and LC-ESI-MS/MS analysis. Although the study focused on ischemia/reperfusion (I/R) injury, the methodology could be readily extended to HCC. They found that the tyrosine kinase adaptor protein Nck-1 might play a role in I/R-induced actin reorganization.

Identification of Site of Cancer Origin

Pathologists sometimes face the problem of identifying the original site of a metastatic cancer when no primary tumor has been identified. Bloom et al. [106] used 2-DE, MALDI-TOF, and LC-MS/MS to compare the proteomic profiles of 77 histologically similar adenocarcinomas arising from six different sites of origin. Using these data, a neural network could correctly classify a single held-out sample with an average predictive accuracy of 82%. These findings show that proteomic data can be used to construct an accurate classifier for tumors without knowledge of their primary site of origin.

Prospects for Clinical Translation of Tissue Biomarkers

Almost every tissue proteomics study provides quantitative expression values for at least a few hundred

Table 3 Proteomic studies of tissues relevant to GI cancers

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
Esophageal cancer	Detection	Microdissected tissue	2-DE and MALDI-TOF MS	24 cancer-normal pairs	20 proteins with differential expression in cancer (3 identified as annexin 1)	Xia [130]
Esophageal cancer	Detection, progression (grade)	Tissue	2-DE and MALDI-TOF MS	15 cancer-normal pairs, 2 tumor-pretumor pairs	20 proteins with differential expression in cancer	Qi [131]
Esophageal cancer	Methods development	Tissue	Capillary HPLC, MALDI-TOF MS	1 adenocarcinoma	20 proteins identified	Yoo [132]
Esophageal cancer	Detection, progression	Microdissected tissue	2D-DIGE, LC-MS/MS	72 cancer, 57 normal	217 proteins with differential expression in cancer, 33 proteins associate with nodal metastasis	Hatakeyama [87]
Esophageal cancer	Detection	Tissue	2D-DIGE, HPLC-ESI-MS/MS, immunoblotting, IHC	12 cancer-normal pairs	33 proteins with differential expression in cancer	Nishimori [133]
Esophageal cancer	Early detection	Tissue	Chromatofocusing, NPS-RP HPLC, ESI-TOF MS, capillary LC-MS/MS	6 adenocarcinoma-metaplasia pairs	38 identified proteins with differential expression in cancer	Zhao [88]
Esophageal cancer	Detection	Tissue	2-DE and MALDI-TOF MS	10 cancer-normal pairs	6 proteins with differential expression in cancer	Huang [134]
Esophageal cancer	Detection	Tissue	2-DE, MALDI-TOF MS, LC-ESI-IT MS	18 cancer-normal pairs	2 protein spots correspond to MnSOD with differential expression in cancer	Hu [135]
Esophageal cancer	Detection	Tissue	2-DE, MALDI-TOF MS, LC-ESI-IT MS, IHC, immunoblotting	Initial study of 41 cancer-normal pairs; validation study of 89 cancer	22 proteins with differential expression in cancer	Du [136]
Esophageal cancer	Detection, progression	Tissue	2-DE, MALDI-TOF/TOF MS, IHC	Initial study of 12 cancer-normal pairs; validation study of 442 cancer and 52 normal	22 proteins with differential expression in cancer	Fu [137]
Gastric cancer	Detection	Tissue	2-DE and MALDI-TOF MS	11 cancer-normal pairs	14 proteins with differential expression in cancer	Ryu [138]
Gastric cancer	Detection	Tissue	2-DE, MALDI-TOF MS, immunoblotting, IHC	10 cancer-normal pairs	21 identified proteins with differential expression in cancer	He [90]
Gastric cancer	Detection	Tissue	2-DE and MALDI-TOF MS	18 cancer-normal pairs	13 proteins with differential expression in cancer	Jang [139]
Gastric cancer	Methods development, progression	Microdissected tissue	2D-DIGE, MALDI-TOF MS	1 adenocarcinoma, 1 metaplasia	42 identified proteins with differential expression in cancer	Greengauz-Roberts [89]
Gastric cancer	Detection	Tissue	2-DE, MALDI-TOF MS, immunoblotting, IHC	Initial study of 10 cancer-normal pairs; validation study of 74 cancer-normal pairs	191 proteins with differential expression in cancer, cathepsin B over-expressed in cancer	Ebert [140]
Gastric cancer	Detection, differential diagnosis	Tissue	SELDI-MS, LC-MS/MS, IHC	74 samples of cancer and normal	1 m/z peak with differential expression in cancer (identified as pepsinogen C)	Melle [141]

Table 3 continued

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
Gastric cancer	Detection	Tissue	2-DE, MALDI-TOF MS, immunoblotting, IHC	56 cancer-normal pairs	50 proteins with differential expression in cancer, expression of chloride intracellular channel 1 was up-regulated in cancer	Chen [142]
Gastric cancer	Detection	Tissue	2-DE, MALDI-TOF/TOF MS, IHC, immunoblotting	Initial study of 10 cancer-normal pairs; validation study of 41 cancer-normal pairs, and 20 gastric ulcer	42 protein spots with differential expression between groups	Huang [143]
GI stromal tumor	Detection	Tissue	2-DE, MALDI-TOF MS	12 tumor (2 with PDGFRA mutations, 8 with KIT mutations and 2 lacking either mutation)	15 proteins with differential expression according to the mutation status	Kang [144]
GI stromal tumor	Prognosis	Tissue	2D-DIGE, nanoLC-MS/MS, immunoblotting, IHC	Initial study of 8 poor-prognosis tumor, 9 good-prognosis tumor; validation study of 210 tumors	25 proteins with differential expression between poor and good prognosis, pfetin was identified as a powerful prognostic marker	Suehara [91]
Colorectal cancer	Detection, progression	Fractionated tissue	2-DE, MALDI-MS MS, ESI-MS/MS	15 cancer (2 with metastasis, 7 with adenoma, 14 with normal tissue)	A total of 72 proteins with differential expression associate with disease progression	Roblick [8]
Colorectal cancer	Detection	Tissue	2D-DIGE, MALDI-TOF/TOF	6 cancer-normal pairs	52 proteins with differential expression in cancer	Friedman [145]
Colorectal cancer	Detection	Tissue	2D-DIGE, MALDI-TOF MS, immunoblotting, IHC	7 cancer-normal pairs	52 proteins with differential expression in cancer, 41 identified molecularly	Alfonso [146]
Colorectal cancer	Detection (validation)	Tissue	Antibody-based	6 cancer-normal pairs (immunoblotting); 97 tumor-normal pairs (tissue microarray)	Unique gene and antibody methodology; 6 of 7 characterized proteins showed mRNA-protein correlation	Madoz-Gurpide [95]
Colorectal cancer	Detection, progression	Microdissected tissue	SELDI-MS, 1-DE, LC-MS/MS, immunoblotting, IHC	39 cancer, 29 adenoma, 40 normal	1 m/z peak identified as HSP 10 with significantly high expression in cancer	Melle [147]
Colorectal cancer	Detection	Tissue	1-DE, 2-DE, MALDI-TOF MS	8 cancer-normal pairs	31 proteins with differential expression in cancer	Mazzanti [148]
Colorectal cancer	Detection, progression	Tissue	2-DE and MALDI-TOF MS	9 cancer-normal pairs, 9 polyp-normal pairs, 13 healthy	61 proteins with differential expression in healthy mucosa, 206 proteins that classify polyp mucosa vs. healthy mucosa, cytokeratins with differential expression in normal mucosa from cancer and polyp patients	Polley [149]
Colorectal cancer	Detection, progression	Microdissected tissue	Direct MALDI-TOF MS	71 cancer (14 with liver metastasis), 24 adenoma, 55 normal	256 m/z peaks with differential expression in different disease states	Li [93]
Colorectal cancer	Methods development	Tissue	Imaging MALDI-MS/MS	1 cancer (with liver metastasis)	A lot of signals with difference between cancer and normal areas, sphingomyelin identified	Shimma [94]

Table 3 continued

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
Colorectal cancer	Progression (node status)	Tissue	2-DE, MALDI-TOF MS, tissue microarray	5 node-positive cancer-normal pairs, 5 node-negative cancer-normal pairs; 80 validation samples.	25 proteins with differential expression in cancer, 4 with significant differences in node positive vs. negative.	Pei [92]
Colorectal cancer	Early detection, progression	Tissue	2-DE, MALDI-TOF MS, HPLC-ESI-MS/MS, immunoblotting	10 cancer-normal pairs (9 with concurrent adenoma)	A total of 27 proteins with differential expression in different disease states	Wang [150]
Pancreatic cancer	Methods development	Microdissected tissue	1-DE, 2-DE, MALDI-TOF/ TOF, IHC	Not stated for the initial study; validation study of 47 cancer-normal pairs	9 proteins with differential expression in cancer	Shekouh [151]
Pancreatic cancer	Detection	Tissue	2-DE, MALDI-TOF MS, IHC	Initial study of 12 cancer-normal pairs; validation study of 21 cancer-normal pairs	111 proteins with differential expression in cancer	Lu [96]
Pancreatic cancer	Detection	Tissue	2-DE, MALDI-MS, immunoblotting, IHC	6 cancer (2 with normal), 7 chronic pancreatitis, 6 normal	A total of 40 proteins with differential expression in different disease states, 9 specifically in cancer	Shen [152]
Pancreatic cancer	Methods development	Microdissected tissue	2D-DIGE, MALDI-TOF MS, LC-ESI-MS/MS	4 PanIN-2 grade, 8 normal	8 proteins with differential expression in PanIN-2 patients	Sitek [153]
Pancreatic cancer and chronic pancreatitis	Detection; differential diagnosis	Tissue	ICAT and HPLC-ESI-MS/ MS, tissue microarray	2 cancer, ? pancreatitis, 10 noncancer. 53 validation samples.	50 identified proteins with differential expression in cancer, 116 in chronic pancreatitis, with many in common	Chen [97, 98]
Pancreatic cancer	Detection, differential diagnosis	Tissue	SELDI-TOF MS	31 cancer, 19 benign pancreatic disease, 44 normal	A total of 33 m/z peaks with differential expression in different disease states	Scarlett [99]
Pancreatic cancer	Detection	Microdissected tissue	SELDI-TOF, 2-DE, IHC	9 cancer, 10 normal, validation study off 35 cancer, 37 normal	Two markers identified, HSP27 validated as serum biomarker	Mellle [154]
Pancreatic cancer	Detection	Tissue	2-DE, MALDI-TOF MS, IHC	Initial study of 8 cancer-normal pairs; validation study of 61 cancer and normal	A total of 48 proteins with differential expression in cancer	Qi [155]
Pancreatic cancer	Detection	Tissue	2-DE, MALDI-TOF/TOF MS, immunoblotting, IHC	Initial study of 8 cancer-normal pairs; validation study of 35 cancer-normal pairs (including 8 from initial study)	30 proteins with differential expression in cancer	Tian [156]
Pancreatic cancer	Detection	Tissue	2-DE, MALDI-TOF MS, immunoblotting	10 cancer-normal pairs	25 proteins with differential expression in cancer	Chung [157]
Biliary tract cancer	Detection	Tissue	SELDI-TOF MS	22 cholangiocarcinoma (13 with normal)	14 m/z peaks that classify cancer vs. normal	Scarlett [65]
HCC	Detection	Tissue	2-DE, MALDI-MS, immunoblotting, IHC	19 cancer-normal pairs	1 protein with differential expression in cancer	Park [158]
HCC	Detection	Tissue	2-DE, MALDI-MS	11 cancer-normal pairs	11 identified proteins with differential expression in cancer	Kim [159]

Table 3 continued

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
HCC	Technology development, detection	Microdissected tissue	"Bottom-up" (ICAT, 2D-LC-MS/MS)	Not stated (pooled samples).	149 identified proteins with differential expression in cancer	Li [100]
HCC (HBV-related)	Detection	Tissue	2-DE, MALDI-TOF MS, LC-MS/MS, immunoblotting	10 cancer-normal pairs	45 identified proteins with differential expression in cancer	Li [160]
HCC	Progression	Tissue	2-DE, MALDI-MS, immunoblotting, IHC	12 cancer (6 with metastasis)	16 proteins with differential expression in metastatic cancer	Song [161]
HCC (HCV-related)	Detection	Tissue	2-DE, MALDI-MS, LC-MS/MS, immunoblotting	4 cancer-normal pairs	155 proteins with differential expression in cancer	Blanc [102]
HCC	Detection	Tissue	2D-DIGE, nanoLC-MS/MS	8 cancer-normal pairs	30 proteins with differential expression in cancer	Lee [162]
HCC (HBV-related)	Detection	Microdissected tissue	2-DE, LC-ESI-MS/MS, immunoblotting, IHC	10 cancer-normal pairs	11 proteins with differential expression in cancer	Ai [163]
HCC	Detection	Tissue	2-DE, MALDI-MS/MS, immunoblotting, IHC	67 cancer-normal pairs, 12 normal livers	90 protein species with differential expression in different disease states	Luk [103]
HCC (HCV-related)	Detection	Tissue	2-DE, MALDI-TOF MS, immunoblotting, IHC	24 cancer-normal pairs	1 identified protein with differential expression in cancer	Kuramitsu [164]
HCC	Detection, progression	Microdissected tissue	2-DE, SELDI-MS, MALDI-MS	25 samples from central cancer, 23 from cancer margin, 28 non-cancer	3 proteins with differential expression in cancer	Melle [165]
HCC	Technology development, detection	Microdissected tissue	ICAT, 2D-LC-MS/MS	Not stated	261 proteins identified	Li [101]
HCC (HBV-related)	Detection	Tissue	2D-DIGE, MALDI-TOF/TOF MS, immunoblotting, IHC	12 cancer-normal pairs	71 identified proteins with differential expression in cancer	Sun [166]
HCC	Progression	Tissue	2-DE, MALDI-TOF MS, immunoblotting	5 cancer-cancer thrombus pairs	20 proteins with differential expression in primary cancer	Guo [167]
HCC	Early detection	Tissue	2-DE, MALDI-TOF/TOF MS, immunoblotting, IHC	6 Grade 1 HCC and normal pairs	15 protein spots with differential expression between Grade 1 HCC and normal	Zhang [168]
HCC (HBV-related)	Detection, monitoring	Tissue	2-DE, MALDI-TOF/TOF MS, immunoblotting, IHC	68 HBV-related cancer-normal pairs (35 recurrence free, 33 early recurrence), 16 normal liver	52 proteins with differential expression among different disease states, HSPA9 was up-regulated in advanced tumor stages	Yi [169]
HCC	Detection, prognosis, and monitoring	Tissue	2D-DIGE, LC-ESI-IT MS/MS	18 cancer-normal pairs	18, 25 and 27 protein spots associated with HCC, histological grade and AFP level, respectively	Teramoto [170]
HCC	Detection	Tissue	2D-DIGE, LC-MS/MS, immunoblotting, IHC	Initial study of 10 cancer-normal pairs; validation study of 103 cancer and 68 non-cancer	127 proteins with differential expression in cancer, 83 identified	Seimiya [104]

Table 3 continued

Disease	Purpose	Source of sample	Analytical technology	Study size	Principal findings	Citation
Ischemia/reperfusion injury	Methods development, ischemia/reperfusion injury.	Tissue (liver biopsy)	1-DE and LC-ESI-QTOF-MS/MS	34 transplanted livers (analysis performed on independent pools of 3 biopsies each)	7 identified proteins in ischemic tissue, 37 in reperfusion; Nck-1 specifically phosphorylated in ischemic phase	Emadali [105]
Various	Technology development, detection	Microdissected tissue	Proteohistography, SELDI-TOF MS	Not stated	2 m/z peaks with differential expression in fatty degeneration of liver cirrhosis	Ernst [171]
Various	Identification of site of origin for unknown primary	Tissue	2-DE, MALDI-TOF MS, and LC-MS/MS	77 adenocarcinomas representing 6 known sites of origin	227 discriminatory proteins in artificial neural networks; 69 identified molecularly	Bloom [106]

"features"—spots on a gel, m/z peaks, or linked sets of peptides identified in bottom-up analysis. Among these features, it is easy to find examples of proteins or other features that show significant differential expression characteristic of physiological or disease states. More than ten independent studies have appeared for some tumor types (i.e., CRC and HCC). However, lists of candidate biomarkers produced in studies of the same tumor type are often significantly different.

Some differences may be attributable to the distinctive patient population seen at individual centers or by the nature of the comparison sample (i.e., patient-matched normal tissue, patients with benign disease, or "healthy" control subjects). There are also sources of variability that are particular to proteomic analysis. Existing proteomic profiling technology samples no more than 1% of the total proteome, and different studies may sample a different 1% depending on details of the methodology. Additionally, in top-down studies, investigators choose only a fraction of the total features as "interesting" enough for molecular identification, and the criteria for selecting these features vary. There are also significant differences in sample preparation. Some studies (14/64) use microdissection or other techniques to enrich for tumor cells, whereas the majority use bulk specimens, where the tumor-cell specific proteomic signature may be partially obscured by intermixed host tissue.

Despite these sources of variability, some common themes are evident, notably quantitative changes in cytoskeletal proteins, stress proteins, and enzymes of intermediary metabolism. In addition to common and abundant proteins, most studies report a few proteins that seem "interesting" because they are potentially involved in processes that drive malignancy, rather than simply reflecting the malignant phenotype. These include transcription factors, signal transduction proteins, and tumor suppressors. Both types of markers are potentially valuable. Quantitative changes in abundant proteins may have value in establishing individual prognosis (e.g., changes in cytoskeletal proteins that are predictive of metastatic potential), whereas transcription factors and signaling proteins may provide novel therapeutic targets.

Application of Proteomic Findings in GI Oncology

The number of exploratory proteomic studies in GI oncology is astonishing: more than 130 to date. Given the multiplicity of studies, have we moved closer to the ultimate objectives of proteomic research? None of the discoveries cited here has yet made a significant impact on clinical care. Many barriers to clinical translation are evident: the disconnect inherent in use of late-stage patients to

discover markers of early disease, the lack of overlap in biomarkers identified in different studies of a same disease, and lack of standardization in sample collection and storage, protein-separation procedures, mass spectrometry, and statistical methodology. Rapid evolution of MS technology is a particularly significant contributor to lack of standardization, because studies performed at different times in different institutions almost inevitably involve different instrumentation.

Some progress has been made towards standardization, at least in areas of sample preparation and analysis, as the Early Detection Research Network of the US National Cancer Institute provides the Standard Operating Procedures and Assay Protocols for researchers via its Web site (http://edrn.nci.nih.gov). At present, however, the content of this resource remains limited.

Our review of published proteomic studies in GI oncology has suggested to us several additional steps that would be particularly valuable to the field:

– It is important that study design and patient selection reflect the intended application of the markers. Markers discovered in tissue-based studies are most likely to be used for establishing individual prognosis or predicting response to therapy when the presence of disease is already known. It is therefore desirable for these studies to focus on classifying disease subsets or establishing molecular correlates of response in existing trials. Markers discovered in serum-based studies are often intended for early diagnosis, in which case it is essential that future studies include subjects who are at risk but have not yet been diagnosed with disease.

– It would be helpful to incorporate uniformly rigorous statistical criteria in both design and analysis. There is a wide variation in the types and sophistication of statistical analysis employed in proteomic studies. It appears, in many cases, that study size is based on availability of samples or other resources, rather than explicit statistical reasoning. The use of "fold-change" remains prevalent as a criterion for ranking candidate biomarkers, although there is seldom explicit justification.

– It would be fruitful to maintain an up-to-date and searchable index of the lists of biomarkers obtained in different studies. Proteomic studies generate vast amounts of data; even a small study with 10–20 patients can generate tens of thousands of protein abundance values. In this review, we have relied primarily on the author's own assessment of their findings, as a comprehensive re-analysis of all of the primary data in the cited studies is beyond our scope.

– Finally, it is essential that future studies focus not only on identifying the disease-associated alterations in proteins but also on determining the cellular functions of the proteins identified, as well as the mechanistic networks in which they participate. The biomarkers identified experimentally should serve as entry points for investigating the mechanisms of carcinogenesis and tumor progression.

Despite the existing barriers to clinical translation, it is important not to lose sight of the ultimate promise of clinical proteomics in GI medicine. Standard diagnostic procedures for GI diseases are largely based on clinical data in combination with endoscopy, imaging examination, histopathology, and immunohistology. Yet, we often observe that individual patients sharing the same type of disease, with the same histopathologic diagnosis, at an identical stage, end up with different clinical outcomes with respect to survival and treatment response. This indicates that each patient's disease may have a unique constellation of molecular derangements [107].

In the future, clinical proteomics may provide a rational basis for individualized therapy. Patients with a GI malignancy could be identified early by screening serum or other GI fluids. A tissue biopsy could then be analyzed for a proteomic signature to establish prognosis, to select the best targets for individualized therapy, and to predict therapeutic responses and toxicities [108]. A recurrence could be detected early by serum analysis, providing an opportunity to alter the therapeutic regime. Using these new tools, GI malignancies could become manageable chronic diseases [109].

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