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Review of mass spectrometry-based metabolomics in cancer research

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Abstract

Metabolomics, the systematic investigation of all metabolites present within a biological system, is used in biomarker development for many human diseases, including cancer. In this review we investigate the current role of mass spectrometry-based metabolomics in cancer research. A literature review was carried out within the databases PubMed, Embase and Web of Knowledge. We included 106 studies reporting on 21 different types of cancer in 7 different sample types. Metabolomics in cancer research is most often used for case-control comparisons. Secondary applications include translational areas, such as patient prognosis, therapy control and tumor classification or grading. Metabolomics is at a developmental stage with respect to epidemiology, with the majority of studies including <100 patients. Standardization is required especially concerning sample preparation and data analysis. In a second part of this review, we reconstructed a metabolic network of cancer patients by quantitatively extracting all reports of altered metabolites: Alterations in energy metabolism, membrane and fatty acid synthesis emerged, with tryptophan levels changed most frequently in various cancers. Metabolomics has the potential to evolve into a standard tool for future applications in epidemiology and translational cancer research, but further, large-scale studies including prospective validation are needed.

Keywords

Metabolomics; Biomarker; Early Detection; Cancer Prognosis; Mass Spectrometry; Epidemiology

Introduction

Metabolomics is a new promising “Omics-discipline” in systems biology, claiming to investigate the entire set of metabolites present in a biological system. It is an analytical approach to detect metabolites and determine their concentrations (1). Metabolomics is interdisciplinary, driven by basic sciences (analytical biochemistry, biology) and bioinformatics together with epidemiology and clinical research. The term “metabolome” was introduced in 1998 as the total metabolite content of a biological sample (2), whereas the terms for the discipline were coined subsequently: metabonomics in 1999 by Nicholson

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et al. (3); metabolomics in 2000 by Fiehn et al. (4). The numbers of metabolites present in the human metabolome is estimated to lie within the range of 10^4 to 10^5 , with the Human Metabolome Database currently containing about 15,000 entries (5). Variance in the fraction measured can be attributed largely to different analytical and detection methods for metabolites: Mass spectrometry (MS) based techniques have the specific advantages of being more sensitive and therefore superior in terms of metabolic coverage, compared to nuclear magnetic resonance (NMR) (6). Metabolomics experiments can be subdivided into targeted and untargeted analyses: Targeted studies aim to accurately determine concentrations of a limited and pre-defined subset of a few metabolites of a pre-defined class or pathway (commonly tens to hundred), whereas untargeted analyses use a more global approach to cover as many metabolites that can be detected by a given method (7, 8). The human metabolome can be interpreted as being the most downstream endpoint of cellular phenotype, influenced by changes in the proteome or genome (9, 10). It is hypothesized to carry more information on the actual phenotype than the latter two more upstream biochemical levels. Metabolomics is therefore a prime candidate for biomarker development.

Proteomics and genomics have been first developed as standard tools in cancer research and translational medicine, with regard to biomarker development for early detection, classification of tumors, measurement of therapy response and patient prognosis (11). Both proteomics and genomics have been extensively reviewed with regard to cancer research (12-14). In a recent review it was noted that metabolomics can be used as a promising approach in clinical applications (15). Metabolomic information is of high value, because metabolic reactions mirror the cell's function more directly (9).

This report aims to systematically review the results of recent metabolomic studies with respect to its use in epidemiology and translational oncology. In addition, we give a brief overview of the currently applied mass spectrometry-based methods and potential future applications.

Materials and methods

Identification of studies

Three databases (PubMed, Embase and Web of Knowledge) were searched for the keywords ("metabolomics" AND "cancer") or ("metabonomics" AND "cancer") in all fields (i.e. title, journal, abstract) from 1998 through November 2012. The Web of Knowledge search was further refined by excluding research areas of low relevance (see Figure 1 and Supplementary Data for search strategy).

Inclusion and exclusion criteria

Only primary research studies investigating either human tissues or body fluids were included. Any study using human *in vitro* models or animal models was excluded. Furthermore, we included only studies employing mass spectrometry; approaches using solely NMR were excluded because of the narrow metabolic coverage. We included only studies with more than 10 metabolites measured. A distinction was made between studies using an untargeted approach (comprehensive metabolomics or metabolic fingerprinting) or analyses focusing on metabolites of a component class, i.e. only lipids or amino acids (metabolic profiling) (8, 15). English language and an available abstract were further inclusion criteria.

Study selection

Studies with irrelevant titles were excluded within a pre-selection step. Duplicate findings were removed and studies underwent a first screen for the above inclusion criteria, based on their abstract. Full text articles of studies that passed all the inclusion criteria were further reviewed in detail. A flowchart of the study selection process is given in Figure 1.

Data extraction

After a detailed review of the full text articles, the following data were extracted from each study, if provided:

- Number of cancer patients, diseased controls, and healthy controls included
- Type of cancer
- Number of metabolites investigated (Distinction between untargeted metabolomics and targeted metabolic profiling)
- Type of biospecimen investigated
- Platform used for the analytical assessment of the metabolome
- Significantly altered metabolites in cancer patients compared to other groups
- Study type, grouped as follows: (a) case-control comparison, (b) therapy response, (c) patient prognosis, (d) method development, (e) tissue profiling or d) others

Data extraction was carried out by three independent researchers (DL, NH, RO) to avoid author bias. Based on the extracted data specific indicators were assigned to the studies:

N: At least $n > 100$ cancer patients were included in the study (with an unspecified number of total study subjects)

V: Two independent study populations (i.e. a discovery and validation set) were monitored

T: Patients were monitored over time (either prospectively or retrospectively)

T+: In addition to **T**, samples were collected repeatedly over time

To develop a metabolic map of cancer patients, each study that employed an untargeted profiling approach was screened for reported alterations of identified metabolites. Significantly altered metabolites were extracted and subjected to further analysis. A network was created with Cytoscape (16) using the MetScape v2.33 (17) plugin with “homo sapiens” as reference species. MetScape creates networks based on reaction from pathway information of the Kyoto Encyclopedia of Genes and Genomes (KEGG) (18). Constructed networks can be found in Figure 4. The Cytoscape file is available from the authors upon request.

Results

A) Descriptives

In total, 106 studies were reviewed in detail. The descriptive information, i.e. the cancer type studied, sample type used and type of study, are summarized in Figure 2.

Cancer types investigated—Colorectal Cancer (CRC) was investigated most often. Interestingly, cancers of the urogenital tract were ranked second, presumably because they were hypothesized to have more intensive and direct contact with the urinary matrix. In total, studies involving 21 different types of cancer were reviewed.

Sample types investigated—Conventional clinical sample types were studied most commonly (urine, followed by serum and plasma). Twenty-two studies extracted metabolites from tumor tissues, investigating the metabolome of cancer cells directly. Seven studies used less common samples with direct contact to tumor tissue, i.e. cerebrospinal fluid (glioblastoma) (19, 20), exhaled air (lung cancer) (21), saliva (oral cancer) (22), fecal extracts (23) or volatile signatures derived from skin (melanoma) (24). Wedge et al. compared plasma and serum and described a generally high overlap in metabolites (78%-87%) indicating that overall discriminatory abilities of the two biospecimens are comparable. They concluded that the choice for either plasma or serum is led clinically, rather than analytically (25).

Study populations—The number of patients investigated is shown in Figure 3. Only 15 out of 106 studies (14%) investigated samples from more than 100 cancer patients. Seventy-seven studies (73%) included healthy individuals as controls. Twenty-eight (26%) used additional controls, i.e. patients with benign tumors or other organ specific conditions (i.e. hepatitis for hepatocellular cancer (HCC) control) to enhance the specificity of the evaluated biomarkers. Studies investigating tumor tissue commonly used non-tumorous (“normal”) adjacent tissue specimens as matched control samples.

Instrumentation used—A general definition and overview of the current instrumentation used in mass spectrometry-based metabolomics can be found in the review by Dettmer et al. (26). Liquid (LC) and gas chromatography (GC) were equally often used for metabolite separation involving 61 and 59 studies respectively. Few studies employed other techniques, such as capillary electrophoresis (CE)-MS, direct injection-MS (27, 28) or matrix-assisted-laser-desorption-ionization (MALDI-MS) (29). Accurate mass methodologies such as time of flight (TOF) mass spectrometry dominate the untargeted metabolomic setups with 60 out of 106 studies (57%). Other instruments, i.e. GC-MS with single quadrupole, tandem LC-MS with triple-quadrupole (QqQ), or quadrupole-ion traps (QTRAP) are also frequently used. Figure 3B gives an overview of the analytical setups used in the reviewed studies.

B) Study types

Metabolomics for case-control comparison—The distribution of different study types is shown in Figure 2. The majority of studies, n=77 (73%) examined the use of blood or urine with the intent for future early diagnosis of cancer or a cross-sectional biomarker development. These studies used metabolomics to discriminate cancer cases from healthy individuals. Studies employing this common approach are not discussed in detail here. Most studies used sample sizes of pilot character and did not have independent validation. Eighteen out of 106 studies (17%) studied the metabolome of patients in a discovery and a separate validation set. Out of these studies only 7 had patient numbers >100 (see Table 1). In the following, we will discuss these higher-quality studies and will provide a brief overview of other studies with unusual biospecimen types and aims (tumor tissue profiling, therapy response and prognosis).

Three of the studies with separate validation investigated CRC (30-32). Nishiumi et al. and Ritchie et al. investigated serum of CRC patients in comparison to controls, and both achieved very good discrimination abilities with an area under the receiver operating characteristic curve (AUROC) of >0.90. Ritchie et al. further employed a targeted LC-MS approach focusing on polyunsaturated fatty acids (PUFAS) (30). Although levels of PUFAS were found to be lower in CRC, the discrimination abilities of only this targeted subset of markers was still adequate with an AUROC >0.85 (30). The analysis of urine of CRC patients by Cheng et al. revealed even better AUROC values >0.99 with significantly lower tryptophan downstream metabolites in CRC (tryptophan, kynurenine, 5-hydroxytryptophan,

indoleacetate, indole) (32). Budczies et al. characterized the metabolome of breast cancer tissue and normal breast tissue (33), using a more invasive approach. A metabolic map of the breast cancer metabolome showed marked increases in purine and glycerolipid metabolism (33). Maeda et al. measured the amino acid concentrations of a total of 303 patients (34). An AUROC of 0.82 indicated a limited performance of a targeted metabolomics study design, which used amino acids as the only markers (34). Chen et al. identified a previously unknown metabolite as 27-nor-5 β -cholestane-3,7,12,24,25 pentol glucuronide (CPG) as a biomarker for ovarian cancer (35). The authors showed in their validation in a cohort of over 600 individuals (>200 ovarian cancer patients) that the AUROC of the single metabolite alone was 0.74 with an accuracy comparable to the standard protein marker cancer antigen 12-5 (CA12-5) (35). However, all of these studies still require marker validation in prospectively collected (non-diseased) individuals to prove that they are able to detect cancers at a very early stage.

Studies employing unusual biospecimen types—Abaffy et al. collected volatile signatures directly from skin of melanoma patients, whereas Gaspar et al. investigated exhaled air from lung cancer patients. Both samples have direct tumor contact and are still non-invasive. An AUROC of 0.94 (melanoma study) and a 100% discriminating ability (lung cancer study) show, that choosing such unusual biospecimens may be particularly useful (21, 24). It should be noted however, that sample sizes in the latter two studies were <20.

Investigation of tumor tissue—Twenty-one studies were categorized as tissue profiling studies, investigating the metabolome of tumor tissue and therefore cancer cells directly. These studies indicate a possible use of metabolomics in a pathological assessment: i.e. Brown et al. investigated 7 prostate and kidney cancer biopsies comparing metabolic profiles to regular histopathological examination (36). The authors noted that metabolomic analysis yielded additional information, distinguishing aggressive vs. mild forms of cancer (36). Two studies by Denkert and coworkers investigated metabolomic profiles of colorectal and ovarian cancer tissues (37, 38). Both studies showed a very high accuracy for the correct classification of tumors (88% and 95%, respectively). Two studies investigating lung (39) and gastric cancer tissue (40) were able to discriminate between tumors graded at an early stage (i.e. T1, T2) vs. late stage (i.e. T3, T4). Lin et al. used a similar approach for renal cell carcinoma (RCC) but in this case serum instead of tissue was investigated (41). These results indicate that the process of tumor grading can be aided by metabolomics, but discrimination between different tumor stages is not always successful (42, 43).

Metabolomics for therapy monitoring—Twelve studies (11%) monitored the impact of a therapeutic intervention (i.e. chemo/-radiotherapy or surgery) on the metabolome. Jiye and coworkers investigated the metabolome of chronic myeloid leukemia patients treated (n=33) / not treated (n=26) with the tyrosine kinase-inhibitor Imatinib (44). The authors showed that the metabolome of patients sensitive to chemotherapy changed, whereas the metabolome of resistant patients was similar to that of untreated controls. Saylor and coworkers investigated the effects of androgen deprivation therapy on prostate cancer patients. As expected, steroid levels were decreased, but also other metabolic effects of the therapy were observed: For example an increase in bile acids and a reduction in lipid oxidation (45). Wibom and coworkers used an unusual approach, measuring the metabolic changes in catheter-microdialysates from glioblastoma patients undergoing radiation therapy. The authors interpreted an increase in glutamate and glutamine after treatment as predictive for tumor proliferation and cell damage. Furthermore, a cross-link towards epigenetic mechanisms such as global DNA-hypomethylation was provided: S-methyl-cysteine, an end-product of demethylation of methylated nucleosides, decreased with

treatment. The authors concluded that the demethylation process could be hampered by radiation, proposing a mechanistic explanation as to why combining radiotherapy with Temozolomide (an alkylating drug) works so efficiently (20). Cai et al. monitored metabolic changes in plasma of lung cancer patients treated with radiation, but did not identify possible biomarkers that could distinguish treated from untreated individuals (46). Some studies investigated the effect of surgical intervention on the metabolome (47-51). Most authors report a restoration of the metabolome towards that of healthy controls after surgery, whereas only a few note a clustering into a third group (50, 51). In contrast, a study led by Silva et al. found no differences in pre- and post-surgical serum of patients with ovarian cancer (52).

Metabolomics for patient prognosis—Eight studies (8%) assessed associations of the metabolome with patient prognosis (i.e. cancer recurrence, metastasis and survival). The group of Farshidfar and coworkers used a retrospective approach, profiling the serum of CRC patients with locoregional recurrence, liver metastases, or extra hepatic metastases. These three groups were distinguished by metabolites from the galactose and glutamine/ glutamate metabolic pathway indicating an alteration of liver metabolism during metastasis (53). Locasale et al. prospectively followed a group of 10 glioblastoma patients of different stages, by investigating the metabolome of cerebrospinal fluid during lumbar puncture (19). The group was able to discriminate between newly diagnosed cancer patients and those with recurrent disease, indicating a possible role of tryptophan metabolites (19). Wedge et al. described that lower levels of glycerophosphatidylcholines, erythritol, and hexadecanoic acid in plasma and serum were associated with poor survival in lung cancer patients (25). However, no data was provided with respect to patients' tumor stage as a possible confounder, and only 29 patients were included (25). One of the few prospective studies by Ye et al. followed 19 HCC (stage II and III) patients prospectively and developed a classification model for recurrent disease, using lactic acid and acetic acid as potential biomarkers. The results of this small pilot study were promising with only 1 out of 7 recurring patients being misclassified (47). Tang et al. investigated serum of nasopharyngeal cancer patients (n=49). Patients were followed prospectively and a set of 4 metabolic markers (kynurenine, two n-acetylglucosamines, and hydroxyphenylpyruvate) increased gradually from hyperplasia towards cancer. This set of biomarkers was further validated within a subsequent targeted analysis. Furthermore, these markers correlated with tumor size and recurrence. Additionally, their levels normalized in patients who underwent radiation therapy (54). Asiago et al. developed a discrimination model for recurrence in breast cancer patients (n=56), where 86% sensitivity and 84% specificity was attained and 55% of the recurrent patients were detected 13 months prior to clinical diagnosis (55).

C) Metabolomic methodologies are diverse

Analytical assessment—GC-MS is favored for its robustness and its low susceptibility to MS-matrix interferences such as ion-suppression (56). Additionally, well established electron impact databases facilitate the automated identification of metabolites. On the other hand, fewer metabolites are detected by GC-MS, as compared to LC-MS. This is mainly attributed to the derivatization process necessary for GC analysis and the limit in molecular weight of metabolites analyzed by this technique. Therefore, more metabolites are typically covered by LC-MS methods. As a drawback, LC-MS-specific electrospray ionization is more susceptible to matrix interferences such as adduct formation or ion suppression (56). Fourteen studies employed both GC and LC-MS analyses.

Pre-processing and data analysis—Pre-processing is essential in order to analyze and interpret metabolomics data. This step includes the import/conversion of raw data files, the detection of signals (peak picking), the assignment of single ions to the same metabolite

(deconvolution), the integration and alignment of chromatographic peaks and eventually different methods for baseline correction, normalization and smoothing (57). Extensive pre-processing software is available, both free-of-charge and commercially (57). Both, the process of pre-processing and available software have been extensively discussed previously (58-60). Appropriate data normalization techniques are critical and the type of procedure is mainly determined by the type of sample or methodology. A special concern arises for urinary metabolomics, where metabolite levels change depending on water intake and kidney function. Studies reviewed in this report generally normalize urine to creatinine levels (51, 61-66), but other types of normalization are also common (normalization to osmolarity or the mean/total peak area of the total ion chromatogram (TIC)). Because normalization methods may influence the results of a study (67, 68), we provide the type of normalization used in studies monitoring urinary metabolite levels in Table 1.

Data analysis includes both uni- and multivariate statistical approaches, such as principal component analysis (PCA), some type of discriminant analysis (PLS-DA) or clustering analysis, but also machine-learning techniques such as support vector machines. A good review of metabolomics data analysis was provided by Danielsson et al. (69).

Although guidelines for pre-processing and data analysis of large scale human metabolomics studies have been published recently (70, 71), there is still substantial diversity in the studies reviewed here, with more than 10 different software programs used for pre-processing and for data analysis. PCA, PLS-DA and clustering techniques are most common for studies reviewed here, but some authors favor support vector machines (28, 37, 38, 63, 72-77) or algorithms developed in-house (77). This heterogeneity reflects in part the state of metabolomics as a relatively young discipline, where fully standardized protocols are just beginning to be developed.

D) Patients with different types of cancer share similarities in alterations of their metabolomes

One hundred and ten studies investigated the metabolome of cancer patients in varying matrices. A comprehensive list of altered metabolites was extracted (see Supplementary Table 1). In total, 390 metabolites were reported as being significantly altered in cancer patients, compared to controls. Markers are more likely to be relevant, if the metabolite is repeatedly reported by multiple investigators. Supplementary Table 1 lists the metabolites as described in the publications. However one should be careful with some of these annotations which may only be tentative, in particular for uncommon metabolites for which chemical standards may not be available. Furthermore no standards exist thus far for reporting metabolite identification in metabolomics studies and some of the annotations proposed might be revised in future studies (78). Pathways most commonly different between cases and controls are presented in Figure 4 and the Supplementary Data. The Figures only represent the frequency of reported alterations and not their direction of change, because of the high heterogeneity within studies (i.e. different types of cancer, types of samples and instrumentation used). Therefore inconsistencies within the direction of change exist. Tryptophan for example, the most commonly reported metabolite, was found in lower concentrations in the serum of HCC, CRC and RCC patients (41, 79, 80). In contrast it was upregulated in urine of recurring HCC (47), breast cancer patients (62) and in bladder cancer tissues (81). In a recent report, Ng et al. also reviewed and discussed these types of discrepancies depending on cancer types (82). Using MetScape we identified the following pathways as potential hallmarks of the cancer metabolome.

Pathways altered by cancer in the human metabolome—Seven out of the 10 most frequently reported metabolites altered in cancer were amino acids. This outlines the

importance of an altered protein synthesis in cancer metabolism. Interestingly, essential and aromatic amino acids such as tryptophan, phenylalanine, tyrosine and their downstream metabolites were frequently reported (41, 47, 62, 79-81) (see Supplementary Table 1). For example, many catabolites of tryptophan, i.e. several indole derivatives, nicotinuric acid and especially kynurenine were altered in various cancer types (54, 62, 73). Chen et al. concluded that the unusual increases of kynurenine and nicotinuric acid in urine of breast cancer patients might be a consequence of elevated estrogen levels (62), because of the hormonal regulation of tryptophan oxygenase, the initial enzyme catabolizing tryptophan (62). Conversely, Cheng et al. reported lower kynurenine concentrations in the urine of CRC patients, compared to controls (32) – this is consistent with opposing effects of estrogen on colorectal and breast carcinogenesis, as noted in the epidemiology of these diseases (83, 84). Recently kynurenine has been described as an endogenous ligand of the human aryl hydrocarbon receptor, providing a mechanism for important carcinogenic characteristics, such as tumor cell survival, motility and immune suppression (85). Downstream metabolites of phenylalanine, such as hippurate or *p*-cresol were frequently altered (32, 72, 80, 86). The latter metabolite has been controversial in the literature: Cheng et al. reported a decrease in urine of CRC patients compared to controls (32), whereas Qui et al. observed an opposite trend (80). The above mentioned metabolites are produced by the gut microbiota. A number of other metabolites most likely of microbial origin have also been described in various publications (Supplementary table 1). These metabolites may play a role in cancer etiology, especially regarding CRC pathogenesis, as discussed in more detail elsewhere (87-89). Other members of the pathway, such as phenylacetylglutamine, a downstream metabolite of phenylacetic acid and glutamine were higher in urine of CRC patients. Phenylacetylglutamine has been found to be cancer suppressive by initiating apoptosis. Its upregulation may reflect the organism's response to the presence of a tumor. (65)

Alanine, aspartate and glutamate are also frequently reported to be changed and link multiple pathways in cancer metabolism: i.e., they fulfill anaplerotic reactions for the tricarboxylic acid (TCA) cycle, providing an alternative energy source for cancer cells, which predominantly use energy produced by glycolysis rather than the TCA cycle and oxidative phosphorylation. This effect can be observed by the metabolic pathway analysis (lactate is in the top 10 metabolites altered) and has long been known as the Warburg effect (90).

Alterations in arginine and proline metabolism are furthermore linked to the urea cycle that has also been discussed in the review by Ng et al (82). Impaired amino acid metabolism is therefore not only a consequence of protein synthesis. An increased use of amino acids for energy production in cancers may explain the excretion of ammonia and therefore the impairment of the urea cycle. Because of the importance of amino acids in cancer metabolism, several targeted studies, measuring their exact concentrations, have been conducted (34, 91, 92). One large scale study pooled over 900 patients with different cancer types together. Despite this, an AUROC close to 0.75 indicates a limited value of amino acids as sole biomarkers (92).

The respective amines of aspartate and glutamate (asparagine and glutamine) are important amino-donors. Glutamine's role as an energy source for cancer cells, entering the TCA cycle via α -ketoglutarate has been previously discussed by several authors (82, 93). Both amines, being nitrogen-donors, can furthermore link amino acid to nucleoside synthesis (see Figure 4). Some studies also investigated this impaired nucleoside metabolism, focusing on several modified nucleosides excreted in urine of breast cancer patients (75, 94). A targeted study led by Yoo et al. showed that a decrease in downstream metabolites hypoxanthine and xanthine were suitable markers for non-Hodgkin Lymphoma with AUROC values of 0.85 and 0.83, respectively (29).

Another hallmark of cancer is impaired lipid metabolism: The chemical group of lysophosphatidylcholines and lysoethanolamines were prime markers of this pathway. Changes in fatty acid profiles and various carnitines were also frequently reported by multiple groups, indicating increased membrane synthesis and cellular turnover (95-99).

Myo-inositol, which was increased in various types of cancers (48, 64, 100), might provide a link to the phosphoinositide-3-kinase (PI3K) pathway in cancer. This indicates that well known cancer pathways, such as PI3K, not only show their effects in the proteome, but also in the metabolome.

Metabolic markers can be organ- and therefore cancer-specific—Metabolites that are exclusively synthesized within specific organs/tissues can be considered as good candidates for markers of the respective cancer, because of their specificity. This becomes evident in studies with a focus on HCC (liver) and adrenocortical cancer (adrenal glands):

Because of the liver's prime role in human metabolism, metabolic markers of liver disease, e.g. bilirubin, have a long clinical history. Therefore, most HCC studies included diseased controls (liver cirrhosis, hepatitis) for the development of biomarkers (23, 101-104). Liver-specific bile metabolites have been reported very frequently, impacting both bile acid and taurine metabolism. The decrease in bile acids is in line with a loss of the organ's function in HCC. Moreover, taurine is considered as an important antioxidant (105) highlighting the role of oxidative stress in cancer. Cao and coworkers also reported a decrease in bile acids and an increase in lysophosphatidylcholines (23). Tan et al. showed that an increase of three markers, namely taurocholic acid, lysophosphatidylcholine 22:5, and lysophosphoethanolamine 16:0, were enough to discriminate HCC from patients with hepatitis B or cirrhosis with a sensitivity of 87.5% and a specificity of 72.3% (103).

Adrenocortical cancer is also characterized by specific metabolite patterns, because of the adrenal gland's function which influences the steroidal metabolome. Arlt and coworkers studied urinary steroid levels and showed that they are of high value in discriminating cancer patients from patients with adenomas. Sensitivity and specificity in the range of 90% could be attained (106). Similar observations have been made by Kotlowska et al. (107).

Metabolic alterations are only part of the systemic alterations present in cancer—One challenge of metabolomics is to elucidate the importance of altered metabolites in a biological system. Different theoretical options exist:

- a. A metabolite reflects a change in the genome, epigenome, transcriptome, or proteome. For example a mutation affecting an enzyme's activity may translate into a change in metabolite levels.
- b. A metabolite can be a driver, i.e. the altered metabolite levels may inhibit an enzyme or activate a receptor (see kynurenine (85)) or have epigenetic effects.

The question, whether or not a metabolite can be considered a) or b) is often difficult to answer and would most often require additional experiments. Ultimately, it is the entire biological system which is changed: Elevated levels of 2-hydroxyglutarate for example, are the consequence of mutated isocitrate dehydrogenase(s) (a), but the metabolite itself may promote epigenetically-driven pathogenesis (b) (108).

Another option to assess the functional role of metabolites is to embed them into pathways and their alterations. This question is not easily addressed by analyses of urine or plasma/serum levels, but tissue analyses are more promising. For example, Budczies et al. constructed a metabolic map of breast cancer, based on their tissue metabolomics data to reveal alterations in energy, amino acid and nucleotide metabolism (33).

Some of the included studies have used cross-omic approaches involving both metabolomics and proteomics (64, 81, 109). Two studies focused on changes in the metabolome based on altered protein levels (110, 111). For example Brockmöller and coworkers correlated the expression levels of the enzyme glycerol-3-phosphate acyltransferase with increased levels of phospholipids and phosphatidylcholines (110). This emphasizes, that increased membrane synthesis, accompanied by high cellular turnover is a hallmark of cancer and can be mirrored both on the metabolic as well as on the protein level. In the latter study is an example for a). In contrast, changes in the metabolome can also influence other cellular layers (b), for example the epigenome: pathways corresponding to epigenetic mechanisms, such as S-adenosylmethionine (SAM) and methylated nucleosides indicate connections between epigenomic and metabolic alterations in cancer. Henneges et al. investigated urinary nucleoside levels of breast cancer patients in a targeted fashion. They reported that methyltransferase activity was altered by monitoring pathophysiological patterns of methylated nucleosides and an increase of S-adenosylhomocysteine (SAH), the product of methylation reactions (75). Additionally, SAH is a potent inhibitor of methyltransferase activity. Its increased excretion suggests the ongoing methylation capacity of cancer cells. Woo et al. also found altered methylated nucleosides in the urine of breast, ovary and cervical cancer patients (112). Chen and coworkers reported only 1-methyladenosine together with two unknown components to be upregulated in HCC (113). A comprehensive study by Putluri and coworkers investigated tissue and urine from bladder cancer patients and revealed cross connections between elevated SAM and DNA methylation patterns (81). Furthermore the authors measured altered promotor-methylation of genes involved in xenobiotic metabolism (i.e. CYP1A1 and CYPB1) and their consequent silencing by epigenetic mechanisms. A very comprehensive study led by Sreekumar, analyzed urine, tissue, and plasma samples of prostate cancer patients (73). They could show that a single metabolite, namely sarcosine, was increased in prostate cancer progression and metastasis. The authors were further able to show that sarcosine is involved in the mechanism of a tumor's invasiveness, by performing cell culture and knockout experiments of the enzymes involved in sarcosine metabolism. In contrast, Struys et al. showed that serum sarcosine levels alone, were not enough to distinguish controls from patients with elevated prostate specific antigen (PSA) or prostate cancer (114). Moreover, Jentzmik et al. reported that urinary sarcosine did not improve the discrimination of prostate cancer patients from patients with no evidence of malignancy (115). They also reported no association with tumor stage or Gleason score. The relevance of sarcosine as a marker for prostate cancer is therefore controversial.

Discussion

The role of metabolomics in cancer research

Thirty-three studies with a >85% correct classification rate of cancer patients indicate, that metabolomics has the potential of identifying novel diagnostic markers. It is furthermore a high-throughput technique, which is fast and cost efficient. However as yet, metabolomics cannot be considered as a standard tool in clinical oncology. Once consistent marker sets have been discovered and replicated in independent populations, it will be critical to promote them to prospective settings for validation.

Secondary applications include tumor classification or grading, which can aid pathological assessment or patient prognosis. A few studies with low patient numbers showed promising results, by being able to differentiate recurrence or metastasis from primary disease (19, 47, 53, 55). Following patients over a longer period of time gives the additional opportunity to monitor changes in the metabolome during treatment. Whereas screening procedures are limited to minimal invasive sampling techniques (i.e. blood, urine) additional samples such as tumor tissue can be investigated from patients who undergo surgery. This will help to

better understand systematically the overall picture of metabolic changes in tumors. Together with studies targeting therapy control, metabolomics has the ability to become a valuable tool for a future personalized medicine approach.

Standardization is needed for metabolomics to be used in translational cancer research

Method standardization is an important factor in metabolomics and has been extensively discussed by other authors (116, 117). The analytical platforms themselves (LC-MS or GC-MS) are robust. Problems are reported by several authors due to differences in sample pretreatment in different hospitals (50). If sample processing is not identical, systematically biased results are to be expected. For tissue metabolomics, contamination of tumor cells with surrounding normal tissue cells can furthermore confound the analysis. Therefore, techniques such as microdissection should be used, prior to sample homogenization (118). Fasting status of patients as a possible confounder is controversially discussed by different authors. Some do not note any problems for urinary metabolomics (50), whereas in serum, differences due to fasting status have been reported (39, 51). Ma and coworkers discussed decreases in valine and arachidonic acid as a result of prolonged fasting during surgery, by investigating serum levels pre- and post-surgery (119). Krug et al. studied the absolute metabolic changes due to various types of challenges such as fasting, meal frequency, and physical exercise which showed large intra-individual variation (120). It is important to develop biomarkers that are independent of fasting status, to make them truly cancer specific and suitable for the clinical situation, when fasting status cannot always be monitored.

Unidentified metabolites carry valuable information

Another important issue is the assessment of unidentified metabolites. Within the present work, unknown metabolites are frequently not included in the data analysis, which may be considered as a conservative approach. However, this could lead to the omission of important information, because unknowns are frequently found to be altered (37, 38, 121). Silva et al. were able to discover a new and unidentified metabolite with important discriminating abilities for ovarian cancer, using accurate mass spectrometry (52). Even if metabolites are not initially identified by library-based searches, they should still be reported as unknowns because they may contain valuable information and should be followed up.

Two validation strategies are needed, depending on the scientific question

1. Replication against statistical overfitting—Multivariate statistical approaches such as PCA or PLS-DA/ OPLS-DA are very frequently used to differentiate between groups, i.e. cancer vs. control. Due to the generally low sample numbers compared to the numbers of metabolites, overfitting is a common problem for supervised statistical methods. PLS-DA models were reported to be especially susceptible (38). It is therefore important to keep in mind that independent validation of putative markers is essential. This can be achieved by a replication study: Within the present work, only a minority of 18 out of 106 studies (17%) further validated their set of discovered biomarkers in a separate study population.

2. Prospective study designs for predictive markers—Many studies claim to aim for the early detection of cancer, but it is important to note that no study used a prospective study design, following a cohort of healthy individuals over time until the onset of cancer. This type of validation within a prospective screening is essential for early detection, as outlined in the five phases of screening for biomarkers approach by the Early Detection Research Network (ERDN) of the National Cancer Institute (122). A prospective design for patient prognosis was also implemented in only a few studies (19, 47, 54). Validation in prospectively collected samples will be essential for clinical translation.

Specificity of metabolic markers has to be improved: Cross-omics approaches might be a solution

Most of the altered metabolites are either not specific for one particular cancer or can be influenced by other metabolic conditions, such as comorbidities. e.g.: Lactate and gentisic acid are altered in alkaptonuria (123), various metabolites of the energy metabolism in diabetes, or urea in liver cirrhosis (124). Confounding due to comorbidities was investigated by Yu et al. The group used five different forms of gastric disease as controls for gastric cancer and was not able to differentiate between them (125). To achieve a higher specificity the implementation of diseased controls within the biomarker development is essential. For example, most authors investigating HCC included diseased controls in their datasets (23, 101-104). This has the advantage of reducing confounding factors due to inflammatory processes, frequently accompanied by HCC, such as hepatitis or cirrhosis. Some groups also included patients with benign tumors in their datasets (65, 79, 97, 106). This also enhances the possible specificity of the final validation metabolite set.

Another solution to increase specificity is to use multiple “omics” strategies in combination. Few authors compared their developed metabolomic markers with conventional biomarkers (102, 113, 126). Those who did, reported an improved sensitivity of metabolomic markers over conventional tumor markers, whereas Ikeda et al. reported higher specificity for proteomic tumor markers (126). Wang and coworkers showed that if metabolic biomarkers and the traditional tumor marker AFP are used together, a sensitivity of 96.4% and a specificity of 100% was reached (102). The group of Chen also reported that combined datasets (1-methyladenosine and AFP) perform better than one marker alone (113).

Strengths, limitations and conclusions

This review was limited to MS-based metabolomics. Therefore NMR analysis and non-MS methods were not included. Furthermore targeted studies measuring fewer than 10 metabolites were considered as being non-omics approaches and therefore excluded. In contrast the strength of this review is its comprehensive systematic investigation of 106 original research papers investigating 21 different types of cancers in 7 matrices. Therefore a representative overview of the MS-based metabolomics in cancer research is presented.

Based on this review, metabolomics should still be considered as a discipline at an early stage of development, but has the ability to become a valuable tool in epidemiology and clinical oncology. The results show that its role in cancer research may be primarily early detection and screening applications. In addition, initial studies in translational areas such as investigations of patient prognosis and therapy control indicate that metabolomics may evolve into a flexible tool in translational medicine. Moreover, additional tumor tissue profiling may be useful for precise tumor classification in a pathological setting. Nevertheless, standardization is needed regarding sample preparation, normalization, and data analysis. To enhance specificity and to further validate potential metabolic biomarkers as markers for early detection, large validation studies with a prospective study design are needed. Finally, cross-omic approaches, merging different disciplines in systems biology can help overcome drawbacks of a single discipline.

Supplementary Material

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Figure 1.
Schematic overview of the search strategy for this review

Figure 2.
Descriptive summary of the studies reviewed: Pie diagrams including numbers and percentages for study, sample and cancer types

Figure 3.

A: Number of studies plotted against the number of patients employed; B: Instrumentation used for metabolomics

Figure 4. Metabolic pathways altered in the metabolome of cancer patients. Red circles represent a reported alteration. Circle diameter is proportional to the report frequency in 106 metabolomics studies

Table 1

Metabolomic studies investigating human cancers. Normalization methods are listed for studies investigating urine. Studies are categorized as either targeted or untargeted. Aims are categorized as: a) case-control comparison, b) therapy monitoring, c) patient prognosis, d) method development, e) tissue profiling and f) others. V was assigned, if the putative markers were validated in an additional patient set; T was assigned, if patients were monitored over time (either pro- or retrospectively); T+: In addition to T, samples were collected repeatedly over time; N was assigned for more than 100 patients

Study	Cancer type	Number of subjects (cancer patients/ disease control/ control)	Sample (normalization)	Targeted or untargeted study designs	Method	Aim
<i>Studies investigating more than one cancer type</i>						
Brown 2012 (36)	RCC, Prostate	6 / 0 / 6	Tissue	Untargeted	GC-qMS, LC-IT	e)
Ikeda 2012 (126)	Esophageal	8 / 0 / 8	Serum	Untargeted	GC-qMS	a)
	Gastric	15 / 0 / 12				
Lin 2012 (127)	CRC	11 / 0 / 12	Serum	Untargeted	RP / HILIC LC-TOF	a)
	Bladder	12 / 0 / 12				
Danielsson 2011 (69)	RCC	20 / 28 / 20	Urine (total peak area)	Untargeted	LC-TOF	d)
	Prostate	20 / 28 / 20				
Tang 2011 (54)	Bladder	17 / 0 / 16	Serum	Untargeted	GC-qMS	a), b), c)
	Nasopharyngeal, throat	15 / 0 / 16				
Miyagi 2011 (92)	Lung	49 / 0 / 40	Plasma	Targeted (amino acids)	LC-QqQ	a)
	Gastric	37 / 0 / 40				
Patterson 2011 (95)	CRC	200 / 0 / 996	Urine (no normalization, headspace)	Untargeted + targeted (lipids)	LC-TOF, GC-MS	a)
	Breast	199 / 0 / 985				
Silva 2011 (128)	Prostate	199 / 0 / 995	Urine (no normalization, headspace)	Untargeted	Headspace GC-qMS	a), d)
	HCC	196 / 0 / 976				
Kelly 2011 (129)	AML	134 / 0 / 666	Tissue	Targeted (298 MRMs)	LC-IT	d), e)
	Leukemia	20 / 0 / 6				
Sugimoto 2010 (22)	CRC	22 / 0 / 6	Saliva	Untargeted	CE-TOF	a)
	Lymphoma	14 / 0 / 21				
	5 different sarcomas	12 / 0 / 7				
	Oral	7 / 0 / 7				
		5 / 0 / 0				
		69 / 11 / 87				

Study	Cancer type	Number of subjects (cancer patients/ disease control/ control)	Sample (normalization)	Targeted or untargeted study designs	Method	Aim
	Pancreatic	18 / 11 / 87				
	Breast	30 / 11 / 87				
Hirayama 2009 (118)	CRC, Gastric	16 / 0 / 0	Tissue	Untargeted	CE-TOF	e)
		12 / 0 / 0				
Woo 2009 (112)	Breast	10 / 0 / 22	Urine (creatinine)	Untargeted + targeted	GC-qMS, LC-IT	a)
	Ovarian	9 / 0 / 22				
	Cervical	12 / 0 / 22				
			<i>Studies investigating bladder cancer</i>			
Jobu 2012 (49)	Bladder	9 / 0 / 7	Urine (no normalization, headspace)	Untargeted	Headspace GC-qMS	a), b) T+
Huang 2011 (130)	Bladder	27 / 0 / 32	Urine (total peak area)	Untargeted	RP / HILIC LC-TOF	a)
Putlun 2011 (81)	Bladder	83 / 0 / 51	Tissue, Urine (osmolality)	Untargeted + targeted (55 MRMs)	LC-TOF	a), d), e)
Pasikantier 2010 (131)	Bladder	24 / 0 / 51	Urine (total peak area)	Untargeted	GC-TOF	a)
Issaq 2008 (132)	Bladder	41 / 0 / 48	Urine (mean peak area)	Untargeted	LC-TOF	a)
			<i>Studies investigating breast cancer</i>			
Budezies 2012 (33)	Breast	271 / 0 / 98	Tissue	Untargeted	GC-TOF	e) N, V
Gu 2011 (133)	Breast	27 / 0 / 30	Serum	Untargeted	DART-MS, (NMR)	a)
Asiago 2010 (55)	Breast	56 / 0 / 0	Serum	Untargeted	GCxGC-TOF, (NMR)	c) T+
Brockmüller 2012 (110)	Breast	186 / 0 / 0	Tissue	Untargeted + targeted (lipids)	GC-TOF, LC-MS	d), e) N, T
Kim 2010 (76)	Breast	50 / 0 / 50	Urine (not reported)	Untargeted	GC-qMS	a)
Lv 2012 (97)	Breast	40 / 40 / 34	Serum	Targeted (free fatty acids)	GC-qMS	a)
Chen 2009 (62)	Breast	20 / 0 / 18	Urine (creatinine)	Untargeted	LC-IT, LC-TOF	a)
Henneges 2009 (75)	Breast	85 / 0 / 85	Urine (creatinine)	Targeted (cis-diol metabolites)	LC-IT	a)
Frickenschmidt 2008 (63)	Breast	113 / 0 / 99	Urine (creatinine)	Targeted (cis-diol metabolites)	LC-IT	a) N
Nam 2009 (72)	Breast	50 / 0 / 50	Urine (total peak area)	Targeted (based on transcriptomics)	GC-qMS	a) V
			<i>Studies investigating colorectal cancer</i>			
Cheng 2012 (32)	CRC	101 / 0 / 103	Urine (total peak area)	Untargeted	GC-TOF, LC-TOF	a) N, V
Farshidfar 2012 (53)	CRC	103 / 0 / 0	Serum	Untargeted	GC-TOF, (NMR)	c), f) N, T+
Leichtle 2012 (91)	CRC	59 / 0 / 58	Serum	Targeted (amino acids)	LC-QqQ	a)

Study	Cancer type	Number of subjects (cancer patients/ disease control/ control)	Sample (normalization)	Targeted or untargeted study designs	Method	Aim
Ma 2012 (109)	CRC	30 / 0 / 30	Serum	Untargeted	GC-qMS	a), d)
Nishiumi 2012 (31)	CRC	119 / 0 / 123	Serum	Untargeted	GC-qMS	a)
Mal 2012 (6)	CRC	31 / 0 / 0	Tissue	Untargeted	GC-GC-TOF	e)
Kondo 2011 (98)	CRC	38 / 4 / 8	Serum	Targeted (fatty acids)	GC-qMS	a)
Qui 2010 (134)	CRC	60 / 0 / 63	Urine (total peak area)	Untargeted	GC-qMS	a), b)
Wang 2010 (65)	CRC	50 / 34 / 34	Urine (creatinine)	Untargeted + targeted (nucleosides)	LC-TOF	a)
Ritchie 2010 (30)	CRC	222 / 0 / 220	Serum	Untargeted + targeted (validation)	LC-IT, LC-QqQ (NMR)	a)
Mal 2009 (135)	Colon	6 / 0 / 0	Tissue	Untargeted	GC-qMS	d), e)
Chan 2009 (136)	CRC	31 / 0 / 0	Tissue	Untargeted	GC-qMS, (NMR)	e)
Ma 2009 (51)	CRC	24 / 0 / 80	Urine (creatinine)	Untargeted	LC-TOF	a), b)
Ma 2010 (119)	CRC	30 / 0 / 0	Serum	Untargeted	GC-qMS	b)
Qui 2009(80)	CRC	64 / 0 / 65	Serum	Untargeted	LC-TOF, GC-TOF	a)
Denkert 2008 (38)	CRC	27 / 0 / 0	Tissue	Untargeted	GC-TOF	e), f)
<i>Studies investigating gastric cancer</i>						
Aa 2012 (48)	Gastric	17 / 20 / 0	Plasma, Tissue	Untargeted	GC-TOF	a), b), e)
Song 2012 (137)	Gastric	30 / 0 / 30	Serum	Untargeted	GC-qMS	a)
Song 2011 (42)	Gastric	30 / 0 / 0	Tissue	Untargeted	GC-qMS	e)
Yu 2011 (125)	Gastric	22 / 57 / 0	Plasma	Untargeted	GC-TOF	a)
Wu 2010 (40)	Gastric	18 / 0 / 0	Tissue	Untargeted	Gc-qMS	e)
<i>Studies investigating hepatocellular cancer</i>						
Ressom 2012 (104)	HCC	78 / 184 / 0	Serum	Untargeted + targeted (lipids)	LC-TOF, LC-IT, LC-QqQ	a)
Tan 2012 (103)	HCC	262 / 150 / 0	Serum	Untargeted	LC-IT	a)
Wang 2012 (102)	HCC	82 / 48 / 90	Serum	Untargeted	LC-TOF	a)
Ye 2012 (47)	HCC	19 / 0 / 20	Urine	Untargeted	GC-TOF	c)
Zhou 2012 (96)	HCC	30 / 60 / 30	Serum	Untargeted	LC-TOF	f)
Soga 2011 (138)	HCC	32 / 159 / 57	Serum	Untargeted + targeted (peptides)	CE-TOF, LC-QqQ	a)
Cao 2011 (23)	HCC	23 / 22 / 23	Feces	Untargeted	LC-TOF	a)
Chen 2011 (113)	HCC	41 / 0 / 38	Serum	Untargeted	LC-QqQ	a)
Chen 2011 (79)	HCC	82 / 24 / 71	Serum, Urine (not reported)	Untargeted + targeted (bile acids)	GC-TOF, LC-TOF	a)

Study	Cancer type	Number of subjects (cancer patients/ disease control/ control)	Sample (normalization)	Targeted or untargeted study designs	Method	Aim
Chen 2009 (139)	HCC	21 / 0 / 24	Urine (not reported)	Untargeted	RP / HILIC LC-TOF	c), d)
Yin 2009 (101)	HCC	24 / 25 / 25	Serum	Untargeted	RP / HILIC LC-TOF	a)
Wu 2009 (140)	HCC	20 / 0 / 20	Urine (no normalization)	Untargeted	GC-qMS	a)
<i>Studies investigating lung cancer</i>						
Cai 2011 (46)	Lung	66 / 0 / 28	Plasma	Untargeted	LC-TOF	a), b) V, T ₊ ¹
Dong 2011 (121)	Lung	102 / 0 / 34	Plasma	Untargeted	LC-TOF	a), d) N
Hori 2011 (39)	Lung	33 / 0 / 29	Tissue, Serum	Untargeted	GC-qMS	a), e)
Wedge 2011 (25)	Lung	29 / 0 / 0	Plasma, Serum	Untargeted	GC-TOF, LC-IT	T
An 2010 (61)	Lung	19 / 0 / 22	Urine (creatinine)	Untargeted	LC-TOF	a)
Dong 2010 (141)	Lung	12 / 0 / 12	Plasma	Targeted (lyso-PCs)	LC-TOF	a), d)
Maeda 2010 (34)	Lung	303 / 0 / 4340	Plasma	Targeted (amino acids)	LC-QqQ	a) N, V
Yang 2010 (142)	Lung	35 / 0 / 32	Urine (creatinine)	Untargeted	RP / HILIC LC-IT	a)
Gaspar 2009 (21)	Lung	18 / 0 / 10	Exhaled air	Targeted (Hydrocarbons)	GC-TOF	a)
Fan 2009 (143)	Lung	1 case report	Tissue, plasma	Untargeted	GC-qMS, (NMR)	b), e)
Fan 2009 (144)	Lung	12 / 0 / 0	Plasma	Targeted (C13 Glucose tracer endproducts)	GC-IT, (NMR)	a) T ₊ ²
<i>Studies investigating head, neck and oral cancer</i>						
Xie 2012 (86)	Oral	37 / 32 / 34	Urine (total peak area)	Untargeted	GC-qMS	a)
Yi 2011 (145)	Nasopharyngeal	102 / 0 / 107	Serum	Untargeted	GC-qMS	a) N, V
Zhang 2010 (146)	Osteosarcoma	24 / 19 / 32	Serum, Urine (total peak area)	Untargeted	GC-TOF	a)
Yan 2008 (147)	Oral	20 / 27 / 11	Saliva	Untargeted	LC-MS	a)
<i>Studies investigating ovarian cancer</i>						
Fan 2012 (148)	Ovarian	80 / 0 / 93	Plasma	Untargeted	LC-TOF	a) V
Zhang 2012 (149)	Ovarian	80 / 90 / 0	Plasma	Untargeted	LC-TOF	a) V
Buckendahl 2011 (111)	Ovarian	33 ³ / 14 / 5	Tissue	Untargeted	GC-TOF	d), e) T
Chen 2011 (35)	Ovarian	235 / 135 / 218	Serum	Untargeted + targeted (validation)	LC-TOF	a) N, V
Chen 2011 (77)	Ovarian	82 / 0 / 24	Serum	Untargeted	LC-TOF	a)
Silva 2010 (52)	Ovarian	85 / 60 / 35	Serum	Untargeted	LC-IT	a) V
Guan 2009 (74)	Ovarian	37 / 35 / 0	Serum	Untargeted	LC-TOF	a)
Denkert 2006 (37)	Ovarian	66 / 9 / 0	Tissue	Untargeted	GC-TOF	e), f)

Study	Cancer type	Number of subjects (cancer patients/ disease control/ control)	Sample (normalization)	Targeted or untargeted study designs	Method	Aim
<i>Studies investigating prostate cancer</i>						
Saylor 2012 (45)	Prostate	36 / 0 / 0	Plasma	Untargeted	LC-IT, GC-qMS	b)
Lokhov 2010 (28)	Prostate	40 / 0 / 30	Plasma	Untargeted	Direct injection-TOF	a)
Thysell 2010 (100)	Prostate	17 / 30 / 0	Tissue, Plasma	Untargeted	GC-TOF	e), f)
Sreekumar 2009 (73)	Prostate	59 / 0 / 51	Tissue, Urine (sediments), Plasma	Untargeted + targeted (validation)	GC-IT, LC-IT	a), c), e) f)
<i>Studies investigating renal cell cancer</i>						
Ganti 2012 (99)	RCC	29 / 0 / 33	Urine (creatinine)	Untargeted + targeted (acylcarnitines)	LC-IT, GC-qMS LC-QqQ	a)
Catchpole 2011 (150)	RCC	96 / 0 / 0	Tissue	Untargeted	GC-TOF	e), f)
Lin 2011 (41)	RCC	33 / 0 / 25	Serum	Untargeted	RP / HILIC LC-TOF	a)
Kim 2010 (76)	RCC	29 / 0 / 33	Urine (no normalization)	Untargeted	LC-IT, GC-qMS	a)
Lin 2010 (27)	RCC	31 / 0 / 20	Serum	Untargeted	Direct injection-TOF, LC-TOF	
Kim 2009 (50)	RCC	50 / 0 / 13	Urine (osmolality)	Untargeted	LC-IT	T+
Kind 2007 (151)	RCC	6 / 0 / 6	Urine (total peak area)	Untargeted	RP / HILIC LC-IT, GC- TOF	a), f)
Perroud 2006 (64)	RCC	5 / 0 / 5	Urine (total peak area)	Untargeted	GC-TOF	a)
<i>Studies investigating other types of cancer</i>						
Locasale 2012 (19)	Glioma	10 / 0 / 7	Cerebrospinal fluid	Untargeted	LC-IT	a), c)
Kotowska 2011 (107)	Adrenal glands	28 / 0 / 30	Urine (not reported)	Targeted (steroids)	GC-qMS	a)
Arit 2011 (106)	Adrenal glands	102 / 45 / 0	Urine (mean value of healthy control)	Targeted (steroids)	GC-qMS	N, T
Yoo 2010 (29)	Lymphoma	96 / 0 / 125	Urine (total peak area)	Untargeted	MALDI-TOF, LC-IT	V, T
Abafy 2010 (24)	Melanoma	23 / 25 / 20	Tissue	Untargeted	GC-qMS	e)
Nishiumi 2010 (152)	Pancreatic	20 / 0 / 9	Serum	Untargeted	GC-qMS	a)
Urayama 2010 (153)	Pancreatic	5 / 3 / 2	Plasma	Untargeted	GC-TOF, LC-IT	a)
Wiborn 2010 (20)	Glioblastoma	11 / 0 / 0	Cerebrospinal fluid	Untargeted	GC-TOF	T+
Jiye 2010 (44)	CML	59 / 0 / 18	Plasma	Untargeted	GC-TOF	T
Wu 2009 (154)	Esophageal	20 / 0 / 0	Tissue	Untargeted	GC-qMS	e)
Zhao 2006 (155)	unspecified	27 / 0 / 26	Urine (not reported)	Targeted (cis-diol metabolites)	LC-TOF, (LC-UV)	a)

¹ repeated sample collection only for a minority of patients;

² repeated sample collection within 12 hours;

³ Total number of patients was 70. Data on metabolic profiling was available for 33 patients