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Proteins are potent biomarkers to detect colon cancer progression

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Abstract Colon cancer is the most common type of cancer and major cause of death worldwide. The detection of colon cancer is difficult in early stages. However, the secretory proteins have been used as ideal biomarker for the detection of colon cancer progress in cancer patients. Serum/tissue protein expression could help general practitioners to identify colon cancer at earlier stages. By this way, we use the biomarkers to evaluate the anticancer drugs and their response to therapy in cancer models. Recently, the biomarker discovery is important in cancer biology and disease management. Also, many measurable specific molecular components have been studied in colon cancer therapeutics. The biomolecules are mainly DNA, RNA, metabolites, enzymes, mRNA, aptamers and proteins. Thus, in this review we demonstrate the important protein biomarker in colon cancer development and molecular identification of protein biomarker discovery.

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1. Introduction

Colon cancer is a complex process involving multiple changes in genomic and proteomic levels. Colon cancer is a gradual progression from benign polyps/cysts and development late stage and metastasis (Reimers et al., 2013). It is primary metastasis to the liver and other distinct organs such as GIT and pancreas, etc. The serum protein expression is often significantly raised in patient's samples which are affected by colon and other cancers (Kievit et al., 1990; Ward et al., 2006). Despite the numerous clinical studies which demonstrate that there is importance for monitoring cell adhesion proteins that can be used to control further invasion and metastasis (Polanski et al., 2006). Biomolecules such as protein, DNA, miRNA and enzymes are the most accessible biological materials and detection of biomarkers in patients with chronic diseases could be of great clinical relevance, because malignant tumor cells release several proteins and extracellular fluids (ECF) into the blood stream and tissues. Some literature state that tumors released numerous proteins into the blood stream

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when compared to healthy human beings (Bünger et al., 2011; Fijneman et al., 2012).

Still, proteome study is particularly challenging due to its complex structure and function. Clinical specimens such as blood, stool and tissues contain several hundreds of secretory proteins. The proteins are essential for normal cell mechanisms and signaling, they secreted from different cellular organs (Maurya et al., 2007). Some of the proteins are specially secreted under pathological conditions, it's mainly due to the alternative mRNA synthesis and other chromosomal genetic variations including cancer, diabetes and Alzheimer disease (Deschoolmeester et al., 2010). Therefore, the vast dynamic range of proteins has been increased to study under cancer's pathological conditions and it is one of the novel biomarkers to find out the disease status. These kinds of proteins are released by tumor cells and will be found in blood and tissues (Rangiah et al., 2009). The receptor proteins could be identified by advanced analytical techniques such as mass spectrometry (MS) and MALDI-TOF, etc. Those methods have reduced the high amount of sample usage, rather it uses threshold or nanogram levels (Tanaka et al., 2010). Proteins are widely important for cellular mechanisms such as cell growth, cell signaling, protein metabolic process and cell motility (Karley et al., 2011). Ultimately, the proteomic studies identified the number of protein biomarkers. These protein biomarkers are benchmarked for colon cancer identification and therapeutics. For instance, the over-expressed glycoprotein is responsible for tumor growth and spread into distinct parts. The secreted protein is multifunctional and is implicated in malignancies including the stomach, lung, prostate, liver and colon (Ward et al., 2006). Also, these up regulated proteins stimulated tumorigenesis, including cell adhesion and invasion and migration of tumor cells.

Biomarker discovery has been developed in different steps. Firstly, the discovered biomarkers are subjected to verification and followed by development and finally confirmed in clinical validation (Bodovitz et al., 2003; Wahs-Oquendo et al., 2012). These biomarkers can be classified into different forms such as genetic biomarker, epigenetic biomarker, protein biomarker, metabolites' biomarker and immunological biomarker (Zhai et al., 2012). Fig. 1 shows the pipeline for protein biomarker discovery. The biomarkers can be assessed by various tools such as radiological techniques, high-throughput microarrays, 2DE (two-dimensional gel electrophoresis), western blotting and mass spectrometry. Also, the potentially important cancer biomarkers have been used to accurately detect the expression level of cell adhesion proteins and metabolites through these techniques (Bhatt et al., 2010). In this investigation, we focused on different protein biomarker expression in colon cancer and various proteomic techniques. These secretory proteins are novel biomarkers to identify colon cancer conditions.

1.1. Proteomic signatures of colon cancer

The molecular study of cancer is mainly the study of protein expression profile and its prognostic information to support clinical decision making for cancer treatment. Signal mediating proteins are expressed in the normal biological processes, pathogenic conditions and pharmacologic responses of drugs. The up/down regulation of biological molecules such as proteins, DNA, abnormal methylation patterns, miRNAs, lipids, glucose and other biological molecules have shown serious pathogenic symptoms in the human system (Kocevar et al., 2013). The protein biomarkers are the mostly used parameters in clinical diagnosis for the detection of over-expressed proteins in clinical samples (Srivastava et al., 2001). The protein expression is characterized by different immunoassay techniques such as ELISA, immunohistochemistry and so on. These techniques have some limitation in clinical practice. For example, protein detection assays are of high specificity with antibody binding, less detection of low abundant proteins and low sensitivity (Kulasingam et al., 2008). However, the proteomic techniques encompass with nanotechnologies and enhance the application in identification, characterization and stability of the secretory proteins and their functions. The techniques are able to detect the complex proteins accurately and quickly.
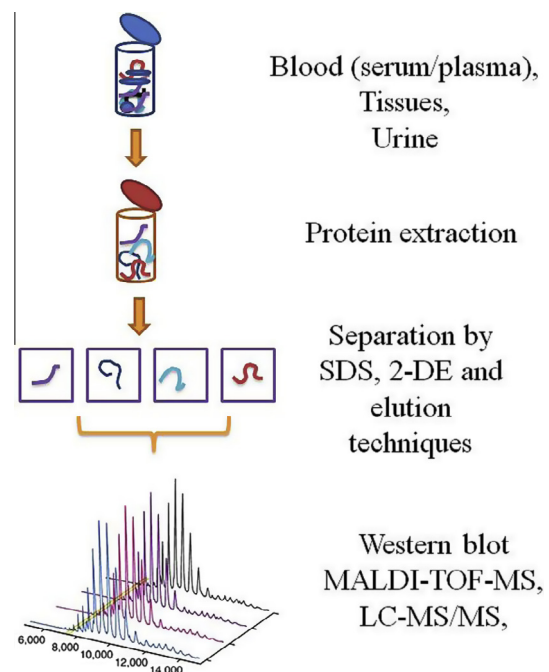

Figure 2 Flowchart for protein biomarker analysis using proteomic tools.
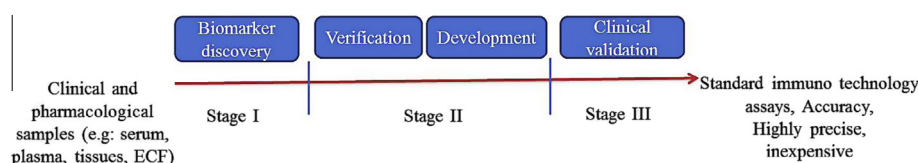

Figure 1 Pipeline for protein biomarker development.

Fig. 2 shows the mechanisms of protein biomarker evaluation from clinical samples.

1.2. Heat shock proteins

Heat shock proteins (HSP) are a group of proteins, which is present in all multicellular organisms. HSPs are classified into different groups based on their molecular weight such as hsp10, hsp40, hsp60, hsp70, hsp90 and hsp110 (Mikami et al., 2009; Vidyasagar et al., 2012). At present, the hsp over expression in cancer is not yet clear. However, the stress and temperature of the tumor environment may stimulate the HSP induction.

For instance, Hsp90 inhibitor chemically called as 17-(dimethyl amino ethyl amino)-17-demethoxygeldanamycin (17-DMAG) significantly inhibited the phosphorylation of epidermal growth factor receptor in HCT 116 and HT 29 cell lines. Similarly, it does activate the transcription factor-3, tumor suppressor and antimetastatic factor on mRNA and protein synthesis. Therefore, the overall studies show that 17-DMAG prominently induced apoptosis in colon cancer cells even though the Hsp90 has interesting phenomena in molecular target for colon cancer therapy in various clinical studies (Berney et al., 1999). Colon cancer cells secreted certain signaling components such as EGFR, FAK and c-Met. These signaling molecules are regulated by 17-DMAG treated colon cell lines, hence it controlled the cell migration *in vitro*. Ultimately, blocking Hsp90 leads to enhance the expression of a transcription factors and antimetastatic mechanism (Moser et al., 2007).

Heat shock proteins (HSPs) are highly conserved and involved in various cellular signaling that mediate cell survival rate in cancers. It has different forms of molecular weight and presence in different types of cancer. For instance, the inhibition of HSP 70 expression has to stimulate the intracellular Ca^{2+} levels in colon cancer cell lines and led to the release of intracellular Ca^{2+} in cell culture environment. Ca^{2+} induces the caspase dependent cell death mechanism in colon cancer cell line. HSP70 inhibits programed cell death in colon cancer cells and decreased cytosolic calcium level in tissues and stabilization of lysosomes. Also HSP70 upregulates cell survival in other types of cancer cells such as pancreatic and prostate cancer (Dudeja et al., 2009). Morita et al. (2014) found that HSP 40 family member such as DNAJB8 is highly expressed in colorectal cancer. Overexpression of DNAJB8 enhanced the expression in tumorigenicity indicating that DNAJB8 has a major role in colorectal cancer prognosis.

1.3. Carcino embryonic antigen (CEA)

CEA is a glycoprotein, the molecular weight ⩽ 200 kDa. CEA was first identified in 1965 by Gold and Freedman, the antigen was detected in serum sample of colon cancer patients (Gold and Freedman, 1965). Elevated CEA levels were observed in colorectal, breast, lung, or pancreatic cancer patient. CEA is a member of the immunoglobulin superfamily. It contains two types of immunoglobulin domains namely an N-terminal domain such as IgV-like variable domain and IgC2-like constant domain (Duffy et al., 2001). CEA is a well known serum protein marker which belongs to the immunoglobulin (Ig) superfamily. It has been acting as a mediator for cell adhesion

on cancer cells (Hatakeyama et al., 2013). Parkhurst et al. (2011) reviewed the current use of 31 active clinical drugs that target CEA, among which 18 have target action and control the tumor development in colon cancer patients. But the other 11 drugs are in trials and have been found to less active in targeting CEA and lack tumor response and related toxicities. These drugs are needed to improve the immunologic activity against CEA target in colon cancer by using standard vaccine strategies.

Recent studies showed that the CEA overexpression occurs in > 90% of colorectal cancers and 60% of other types of cancer including gastric, lung and pancreatic(Michor et al., 2005). Moreover, identification of the receptor of CEA mediates its prometastatic activities to other organs and would have great impact on drug development for cancer treatment (Dwyer et al., 2011). This CEA-receptor (CEAR) has been identified as a homolog of the heterogeneous nuclear ribonucleoprotein M4 (hnRNP M4). The hnRNP M4 mainly promotes the pre-mRNA binding mechanisms. In cancer state, hnRNP M4 does not bind with RNA homopolymers but helps as a nuclear mRNA-transporter and involves in mRNA splicing. Due to these biological mechanisms that CEA interacts with a receptor-like molecule (receptor at the membrane of Kupffer cells and macrophages) to transmit signal transduction activity and release of various cytokines (Laguinge et al., 2005).

CEA increases the concentration in metastatic CRC to colonize in the liver and develop spontaneous pancreatic and lung metastasis (Li et al., 2010). CEA protein and epitopes have been identified from human T lymphocytes (T cells). CEA-expressing in cancer cells is weakly recognized by the immune system. Recently, the proteomic studies have introduced several new strategies to enhance immune reactions against CEA. This includes using antibodies directly against CEA, inserting the CEA gene into recombinant viruses and bacteria as viral and bacterial vaccines, peptides and DNA or RNA onto dendritic cells to increase vaccine effectiveness (Hörig et al., 2000).

1.4. Tissue inhibitor of matrix metalloproteinase 1

Generally, cancer cells are interacting with neighboring cells through cell receptors and extracellular matrix (ECM). The ECM and receptor connections with surrounding cells are largely modified by the matrix metalloproteinases (MMPs) and normal protein function. The degrading of ECM mechanism is mainly involved by MMPs. On the other hand MMP activities have been regulated by the tissue inhibitors of metallo proteinases (Bourboulia et al., 2010). TIMPs can inhibit the function of MMPs with varying conditions and different MMPs. Human colon cancer enhances the synthesis of TIMP-1 in cancer model compared to their normal counterparts, eventually increasing the growth of cancers. Moreover, TIMP-1 promotes colon cancer progression and accumulation of CAFs (Cancer Associated Fibroblasts) in colon cancer. Also, TIMP-1 has been stimulating the protumor effect in prostate, colon and other type of cancers (Gong et al., 2013).

Tissue inhibitor metaloprotenase-1 is a glycoprotein present in various cancerous and noncancerous tissues and bodily fluids. TIMPs have four important classes namely, TIMP-1, -2, -3, and -4. TIMPs, TIMP-1 and TIMP-2 are mainly involved in enzyme-inhibitory properties in cancer models (Offenbra et al.,

2008). TIMP-1 has growth-promoting properties and stimulates tumor growth by inhibiting the apoptosis in colon cancer. TIMP-1 is a novel and specific protein marker for the identification of early-stage colon cancer (CC). In CC patient, blood plasma sample has significantly elevated TIMP-1 levels compared with healthy individuals (Holten-Andersen et al., 2002). Table 1 explains various important protein biomarkers discovered from colon cancer clinical samples. Different molecular marker detection from CRC has been achieved extensively in clinical use. For instance, variety of enzymes and other biomolecules have been developed to assess the status of CRC, but this approach is also needed to be elaborated in clinical studies (Ross et al., 2010; Kaler et al., 2014).

Progression of colon cancer is mainly reciprocal interactions between stroma and cancer cells. Furthermore, the major stromal cell types have been associated with various types of cancer including colon, liver and breast. Colon cancer is mainly mediated by different signaling and secretary molecules such as cytokines, growth factors, chemokines, proteases, and components of the extracellular matrix. Some of the potent serum protein biomarkers identified are proteinglutamine gamma-glutamyltransferase 2 (TGM2), insulin-like growth factor-binding protein 7 (IGFBP7) and calcyclin binding protein (CacyBP) as involving in colorectal metastasis (Pan et al., 2009; Katayama et al., 2006; Zhao et al., 2007; Ghosh et al., 2011). Other tumorigenic factors such as cyclooxygenase 2 (COX2), selenoproteins and inducible nitric oxide synthase (iNOS/NOS2) have often been involved in colon tumorigenesis (Mcintosh et al., 2008). These protein biomarkers have an influence to detect the early changes in carcinogenesis (Xuezhi et al., 2006).

Evaluating the changes of protein profiles associated with the colorectal tumorigenesis will be potential therapeutic targets for early colorectal cancer treatment. The alteration in protein expression levels in cancer patients revealed that significant changes in glycolytic pathway, and decreased gluconeogenesis, glucuronic acid pathway, eventually reduced tricarboxylic acid cycle process. Moreover, the increasing pH range between 4 and 7 has been found and the over expression of some metabolic enzymes such as succinate dehydrogenase subunit A, succinyl-CoA 3-ketoacid coenzyme A transferase and aldehyde dehydrogenase was down-regulated even though several other proteins such as triosephosphate isomerase and keratins 8 were up-regulated in colon cancer (Sastre et al., 2011; Di Nicolantonio et al., 2008; Mao et al., 2011; Coppedè et al., 2014).

1.4.1. Enzymes

The biological markers such as enzymes are very strong tools for monitoring the progression of cell functions. It effectively shows a new therapeutic way to control chronic diseases including cancers. Telomerase is RNA-containing enzymes that involve transcriptional process to synthesize DNA and genome integrity (Roig et al., 2009). Mathioudaki1 et al., 2008 studied/found that some telomerase was involved in the colonic crypt activity and progressively increases the gastrointestinal tract function. Thus, increasing the telomerase activity may risk treated patients who are likely to have cancer recurrence and may give an indication for postoperative chemotherapy or future telomerase-targeting therapy. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GRx), arginine methyltransfer-

ase 1 and glutathione-S-transferase (GST) contribute as plasma biomarkers that could find prognostic of CRC risk (Maffei et al., 2011).

1.4.2. MiRNAs

MicroRNAs (miRNAs) are very small elements of less than 22 nucleotide sequences of genome, and it is smaller piece of RNA. miRNA is involved in different cellular functions like proliferation, differentiation, apoptosis and metabolism (Reid et al., 2012). Even though the functional studies on miRNA have not yet been fully understood, since the last two decades, a rapidly increasing number of miRNA studies have discovered different types of miRNA and their role in oncogenesis and gene expression level of miRNA as antioncogene. Moreover, it is predicted that approximately 30% of protein-encoding genes are controlled by miRNAs (Liu et al., 2011). miRNA is a widely used biomarker for CRC detection. Clinically identified different circulating miRNAs were altered in colon cancer patients. Circulating MiRNA-92 levels were significantly higher in CRC patients than in healthy controls and miRNA-141 was significantly associated with stage IV colon cancer. miRNA is used to evaluate the sensitivity and specificity of unknown clinical samples (Allegra et al., 2012).

The prominent miRNAs such as miRNA-21 and miRNA-31 have up-regulation in colon cancer patients and also increase the progression of other human cancers (Markou et al., 2008). Subsequently, Overexpression of miR-34a strongly inhibited colon cancer cell migration and invasion. Also, the miRNA-34a was down-regulated by more than 60% in colon cancer as compared to the adjacent normal colon samples (Wu et al., 2012; Yang et al., 2014). MiR-31 was one of the first miRNAs to be found deregulated in colorectal cancer (Bandres et al., 2006). Other miRNA serum levels such as miR-92a, miR-141, miR-601 and miR760 have also shown to predict colon cancer progression and help in diagnosis (Cortez et al., 2011; Mitchell et al., 2008). On the other hand, miRNA is validated as a powerful cancer biomarkers for the detection of tumor location and different subtypes of colorectal cancer such as MIN (microsatellite instability), CIN (chromosomal instability), and CIMP (CpG island methylator phenotype) (Wang et al., 2012; Locker et al., 2006).

1.4.3. Cytokines

Malignant tumor has the ability to remodel the structure and create a permissive microenvironment for their growth and development. Consecutively, tumor cells produce soluble factors such as cytokines, growth factors and proteases. These regulatory factors induce the growth, differentiation and survival of tumor cell progression and promotion (Klampfer et al., 2011). Colon cancer cells induce the macrophage functions to release IL-1β. The IL-1β induced NF-κB activation is coupled to the inactivation of GSK3β function and stimulation of Wnt signaling in colon cancer cells. The human colon cancers are infiltrated by inflammatory cells including mast cells and macrophages, which secrete TNFα. TNFα is increased in tumor bearing mice and significantly mast cells were depleted in the animal model. The reduced levels of TNFα, confirmed that mast cells are important sources of TNFα. Predominantly, the depletion of mast cells or anti-TNFα treatment significantly suppressed polys in colon cancer induced mice model (Kaler et al., 2009). Kemik et al. (2010)

Table 1 Summary of identified protein biomarkers from colon cancers.

No	Type of cancer	Clinical samples	Techniques used	Identified Proteins	References
1	Colon cancer	Serum	SELDI-TOF-MS, LC–MS /MS, ELISA	Transferrin, α-1 antitrypsin, apolipo protein 1, complement c3a	Ward et al. (2006)
2	Colorectal cancer	Serum	SELDI-TOF-MS	Two unknown proteins	Zhen et al. (2006)
3	Colon cancer	Serum	PCR, blotting techniques	Caveolin-1, Kallikrein 6	Henkhaus et al. (2008)
4	Colon cancer	Serum	2DE, RT-PCR, ESI-T-MS, Immunoblotting	Glucose regulated protein- 8	Xing et al., 2006
5	Colon cancer	Plasma and tissue extract	SELDI-TOF-MS, size exclusion chromatography	α defensing -1,-2,-3	Albrethsen et al. (2005)
6	Colorectal cancer	Serum	SELDI-TOF-MS, MALDI-TOF-MS, LC–MS /MS, HP	3.9 KDa protein	Zhai et al. (2012)
7	Colon tumor	Extra cellular fluid	ELISA, TENDEM-MS	-	Fijneman et al., 2012
8	Colorectal cancer	Blood serum	Immunometric assay	Serum C-peptide	Kaaks et al., 2000
9	Colorectal cancer	Serum	SELDI-TOF	Three different unknown proteins	Liu et al. (2010)
10	Colon cancer	Serum	ClinProt profiling technology, LTQ orbitrap XL	Alpha -2- HS glycol protein	Fan et al. (2014)
11	Colon cancer	Serum	LC–MS and MS/MS	Tryptic KRT 8 peptide	Zhou et al. (2009)
12	Colorectal cancer	Blood plasma	Lectin glycoarray/lectin blot, Nano LC–MS /MS	Plasma glycol protein	Qiu et al. (2008)
13	Colorectal cancer	Serum	MALDI-TOF-MS, Magnetic bead separation	Low mass peptides	Deng et al. (2013)
14	Colon cancer (CT-29)	Serum	LC-MRM/MS, western blot	Catenin	Rangiah et al. (2009)
15	Colon cancer	Serum	2D- PAGE, Western blotting, RT-PCR	Defensin α 6	Nam et al. (2005)
16	Colorectal cancer	Serum	2D- PAGE, Western blotting, RT-PCR	MMPs	Zucker and Vacirca (2004)
17	Colorectal cancer	Serum	2D- PAGE, Western blotting, RT-PCR	Cyclin D	Arber et al. (1996)
18	Colon cancer (DLD-1)	Serum	RT-PCR, Western blot	S100 P	Jiang et al., 2011
19	Colon cancer (HT-29)	Serum	RT-PCR, FACS, 1D-SDS PAGE	Lamin A/C filament protein	Willis et al. (2008)
20	Colorectal cancer	Tissues	2-DE, LC-tandem MS	Heat shock protein, aldehyde dehydrogenase	Dwyer et al. (2011)
21	Colon cancer	Serum, tissue	Western blotting, RT-PCR, Immunohistochemical assay	Txl-2, (thioredoxin like protein-2)	Lu et al. (2013)
22	Colorectal cancer	Colon tissues	Immunohistochemistry	P53, nm23, u-PA, VEGF	Berney et al. (1999)
23	Colon cancer	Colon tissues	Western blot, RT-PCR	Eph B4	Stephenson et al. (2001)

studied that the serum IL-6 increased levels was noticed in the metastatic-stage of colon cancer patients than without metastasis patients. Also, IL-17 stimulates the tumor cell functions to secrete a variety of angiogenic factors, including VEGF, PGE1, PGE2, keratinocyte-derived chemokine (KC), and macrophage inflammatory protein-2 (MIP-2), which promote angiogenesis in cancer (Wu et al., 2013).

1.4.4. Chemokines

Chemokines are a large subfamily which is classified into 4 different groups mainly C, CC, CXC and CX3. The CC chemokines are effectively functioning on various cell types, including monocytes and lymphocytes whereas, the CXC chemokines merely act on neutrophils and T-lymphocytes. Chemokines have been first discovered from man and it effects by binding to 7-transmembrane domain G protein-coupled receptors (Kulbe et al., 2004). Chemokine 25 (CCL25) and its associated receptor chemokine receptor 9 (CCR9) inhibit colorectal cancer (CRC) invasion and metastasis. Also, CCR9 protein expression levels were the highest in colon adenomas and gradually decreased in invasive and metastatic CRCs (Chen et al., 2012). CCL4 has a direct effect on the tumors and CCL5 has been the only CK found to be more abundant in normal mucosa than in colon carcinoma tissue. It seems that these CKs contribute more to tumor growth rather than antitumor immunity (Baier et al., 2005).

1.5. Stem-cell associated markers

Recently, cancer stem cell (CSC) has emerged as a tool to evaluate the status of several solid tumors including CRC. Cancer stem cell theory was originally proposed by Cohnheim in 1875. This theory mainly consists of different criteria: (1) A number of external or internal factors such as physical and chemical agents causing genetic damages in the stem cells, (2). The damaged stem cell gives a morphologically distinct type of tumor, (3) Different tumors from different stem cells have different biochemical and genomic profiles. However, CRCSC markers might be used as a novel and effective biomarker to predict cancer progression, and identify patients at risk for relapse (O'Brien et al., 2007; Ricci-Vitiani et al., 2007; Papailiou et al., 2011; Vaiopoulos et al., 2012; Rossell et al., 2013). Even though the CSC therapy raises many questions about diagnostic and therapeutic approaches for cancer and retro viral diseases, CSC will be used for more efficacious screening and early detection for cancer treatment in near future.
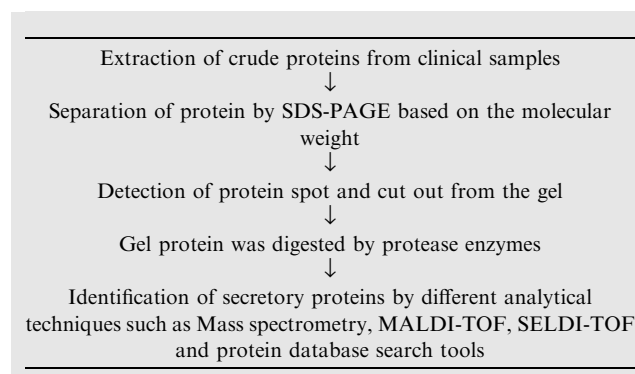
2. Techniques to predict the protein expression

2.1. Gel electrophoresis

2.1.1. 2D PAGE (2-dimentional electrophoresis)

2-D electrophoresis was first introduced by O'Farrell and Klose in 1975. It is commonly used to analyze proteins within the mass range of 20–250 kDa and pH of 3–11. Gel electrophoresis techniques have been widely used to identify the protein expression in clinical samples. SDS, 1D and 2D PAGE techniques are specifically used for the separation of complex proteins from the mixture, based on the molecular weight (Magdeldin et al., 2014). Nevertheless, it can identify molecular weight of less than 20,000 kDa of proteins. The advantage of 2DE is that it can detect peptide fragments with post translational modification and amino acid mutation. Even though 2D-electrophoresis techniques have some drawbacks, it can mainly detect only protein expression and cannot detect protein–protein interactions or protein function (Lilly et al., 2002). For the discovery of those protein specific functions additionally, we should use affinity electrophoresis and other functional techniques. Also, membrane proteins are difficult to separate in 2DE due to poor solubility. But this problem can be solved by using higher protein concentrations and by applying fractionation methods. Additionally, sometimes 2D-electrophoresis has insufficient resolution to detect the proteins. The following flow chart can clearly show the protein analysis (Friedman et al., 2004).

Extraction of crude proteins from clinical samples
↓
Separation of protein by SDS-PAGE based on the molecular weight
↓
Detection of protein spot and cut out from the gel
↓
Gel protein was digested by protease enzymes
↓
Identification of secretory proteins by different analytical techniques such as Mass spectrometry, MALDI-TOF, SELDI-TOF and protein database search tools

2.2. Analytical proteomic tools

2.2.1. LC–MS (liquid chromatography–mass spectrometry)

Liquid chromatography (LC) is a separation technique widely used in the field of proteomic studies and life sciences. LC–MS is commonly used for drug development at different stages including peptide mapping, glycoprotein mapping, natural products and metabolites identification and quality control (Bronsema et al., 2013). LC has emerged as a novel technique to quantifying peptides and proteins in biological samples and MS/MS fragmentation for each peptide sequence. Peptides consist of amino acid residues with molecular weight 6000 Da or smaller. Peptides comprise of approximately fifty or fewer amino acid molecules (Zhou et al., 2009). Peptides and cell adhesion proteins are significant functions in therapeutics and disease development. LC–MS analytical method is used to predict the proteins in clinical and non-clinical samples. LC–MS required a minimal quantity of sample, capable of measuring structurally or chemically similar proteins and peptides. The method has no requirement for antibodies to detect the proteins. LC–MS has good accuracy and high throughput. Additionally, some problems might occur when using LC–MS methods for protein and peptide characterization including poor solubility of protein in corresponding solvents, chromatographic behavior, nonspecific adsorption behavior and m/z of ionized molecules may exceed the instrument's proficiencies (Ewles et al., 2010).

2.2.2. SELDI-TOF-MS (surface-enhanced laser desorption/ionization-mass spectrometry)

SELDI-TOF-MS technology was first introduced by Hutchens and Yip in 1993. The instrumentation consists of different analytical parts mainly selective protein extraction and retention on chromatographic chip surfaces and their subsequent analysis by a simple laser desorption ionization mass spectrometer. SELDI-TOF-MS analytical techniques can provide a rapid protein expression profile from a range of biological and clinical samples (Zou et al., 2011). SELDI-TOF-MS is an established proteomic platform which is a characterization of signal mediating proteins from various clinical and pharmacological samples. The mass spectra data are used to identify the molecular weight of the proteins and peptides (Emanuele et al., 2012). Protein mass spectra from cancerous models and healthy controls are compared and analyzed for protein expression, which revealed novel expression of proteins in the samples (Zhai et al., 2012).

2.2.3. MALDI TOF-MS (matrix assisted laser desorption ionization-mass spectrometry)

Kamp, Karas and their colleagues have discovered a novel technique matrix-assisted laser desorption ionization (MALDI) in 1985. In 1987, Koichi Tanaka has modified the technique which added laser desorption ionization when he was working in Shimadzu Corp. MALDI/TOF spectra are mainly used for the identification of peptide fingerprinting. It may also identify the bacteria and fungi from different clinical samples. Mass spectrometry (MS) is a technique that identifies the metabolites by separating ions by their unique mass (mass-to-charge ratios) using a mass spectrometer (Carbonnelle et al., 2011; Wilson et al., 2012). MALDI-TOF is a technique to analyze proteins via gas ion production and detection based on their mass-to-charge ratio (m/z) (Deng et al., 2013). Identification of secretory proteins using a mass spectrometer is connected with ion source (e.g. matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI)), a mass analyzer and an ion detector. In the past ten years, the proteomic techniques are very fast growing that developed accuracy, higher sensitivity and resolution of instruments. The cell adhesion protein and peptide fragments have been identified by MALDI-TOF although, it is time consuming operating process, lacks automation and requires expertise to work in the instrumentation (Person et al., 2006; Karpova et al., 2010). It has some disadvantages though, for instance, it doesn't work well if two proteins are in very close proximity or the overlap of their relative bands and it cannot find the post translational modified proteins.

3. Factors upset the outcome of biomarkers

Different analytical variables such as sampling methods, storage, time of sample collection, sample processing, patient conditions especially fasting vs non-fasting states, medications and hormones can potentially affect biomarker concentrations. The stability of markers is mainly focused by immediate analysis following overnight storage at carefully 4 °C or −80 °C. In addition, the number of patient selection criteria for controls and experimental should balance the cohorts (e.g. age, gender) (Polanski and Anderson, 2006; Luo et al., 2011; Fung et al., 2014). These measures are important for exploring novel protein biomarker from cancer patients. Thus, this review manifests different secretory protein biomarkers used in colon cancer prediction and we addressed a few proteomic methods to characterize protein expression. However, this information has been extensively applied for further protein biomarker development and cancer drug discovery.

4. Future prospective of protein biomarkers in colon cancer

Identification of molecular marker particularly receptor/secretory proteins could improve the direction of treatment strategies in colon cancer. The biomarker development may lead to target therapies for cancers and improve the selection of adjuvants for drug development. The use of protein biomarkers might also decrease the economic burden in cancer treatment. Moreover, an automated and inexpensive standardized protein marker is necessary for the detection of colon cancer. Unfortunately, most of colon cancer cases are diagnosed at final stages. Therefore, by frequent evaluation of serum biomarker, changes can be found easily in the colon cancer development at early stages. Cell adhesion proteins/receptors are one of the novel biomarkers for finding colon cancer metastases in cancer biology. Also, the improvement is needed in the current colon cancer biomarker screening assays with high accuracy. More specific serum and tissue proteins are required to be explored in the colorectal cancer patients and it may enhance the new drug development. In this study, we conclude that serum and tissue proteins should be followed-up with colon cancer diagnosis and therefore can significantly control colon cancer progression. Also, the current biomarker research is needed to search for some unique molecular identities which could distinguish malignant tumor from normal cells.

Conflict of interest

We declared that there is no conflict of interest in the review studies.

Acknowledgements

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