Study of Serum Proteomic Patterns in Patients with Primary Colorectal Cancer Based on Magnetic Bead Separation and Matrix Assisted Laser Desorption/Ionization- Time of Flight Mass Spectrometry (MALDI-TOF MS)

Thesis

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Contents

List of Abbreviations

ACF	: Aberrant crypt foci
APC	: Adenomatous polyposis coli
APCI	: Atmospheric pressure chemical ionization
APPI	: Atmospheric pressure photoionization
CEA	: Carcinoembryonic antigen
CI	: Chemical ionization
CRC	: Colorectal cancer
CTC	: Computed tomographic colonography
DHB	: Dihdroxybenzoic acid
EI	: Electron ionization (EI)
ESI	: Electrospray ionization
FAB	: Fast atom bombardment
FAP	: Familial adenomatous polyposis syndrome
FOBT	: Faecal occult blood test
GC	: Gas chromatography
HCCA	: α Cyano-4-hydroxycinnamic acid
HPLC	: High performance liquid chromatography
ICR	: Ion cyclotron resonance
JPS	: Juvenile polyposis syndrome
kDa	: Killo Dalton
LC	: Liquid chromatography
L-TOF	: Linear TOF
m/z	: Mass-to charge
MALDI	: Matrix-assisted laser desorption/-ionization
MB	: Magnetic beads
MB WAX	: Weak anion-exchange magnetic beads
MB WCX	: Weak cation-exchange magnetic beads
MS	: Mass Spectrometry
MS/MS	: Tandem MS
pI	: Isoelectric point
PJS	: Peutz-Jeghers syndrome
SIM	: Selected ion monitoring
TOF	: Time-of-flight
TOFR	: Time-to-flight reflection

List of Table

List of Figures

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide after lung and breast cancers with two-thirds of all CRCs occurring in the more developed regions of the world ***(Gado et al., 2013)***. Each year there are nearly one million new cases of CRC diagnosed worldwide and half a million deaths. In Egypt, it contributes for 5 % of all cancers ***(Elsabah and Adel, 2013).*** The nowadays applied tumor markers, carcinoembryonic antigen (CEA) and CA19.9 have shown poor sensitivity and specificity for diagnosis of CRC, as well as in judging the effectiveness of the surgical resection of the tumor ***(Bagaria B et al., 2013).*** Colonoscopy-guided biopsy is considered till now the gold standard for cancer colon diagnosis, in spite of its high cost and inconvenience and being an invasive procedure ***(Atkin et al., 2013).*** These facts have prompted the search for other non-invasive methods for the diagnosis.

The proteome is the protein complement of the genome. It is quite a bit more complicated than the genome because a single gene can give rise to a number of different proteins through alternative splicing of the pre-messenger RNAs (pre-mRNAs), RNA editing, proteolytic processing, and post-translational modifications. Proteomics is a branch of biotechnology concerned with applying the techniques of molecular biology, biochemistry, and genetics to analyze the structure, function, and interactions of the proteins

produced by the genes of a particular cell **(Dai et al., 2010).** This proteomic analyses can aid in the identification of all differentially expressed proteins and their post-translational modifications during cancer progression.

The development of mass spectrometry enabled the study of a large numbers of proteins in a single sample within a single run with the use of a small amount of sample making it possible to identify, simultaneously, up to thousands of proteins that can be used as biomarkers for cancer diagnosis, prognosis and monitoring of treatment regimens ***(Boja et al., 2012. Fan et al., 2012).*** Most proteomic analysis procedures mandates selective purification processes to remove impurities and reduce noise during signal analysis and protein identification. Magnetic beads (MB) have been developed and were considered as a promising material for convenient and efficient separation of peptides and proteins in biological samples ***(Yao et al., 2008).*** This method uses different chemical chromatographic surfaces on an outer layer of magnetic beads to selectively purify certain subsets of proteins, allowing unbound impurities to be removed by washing. Proteins bound to the magnetic beads are then eluted, diluted, and can be directly analyzed by MS ***(Guo et al 2014).***

Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a new MS technique ***(Deng et al., 2013)*** in which MALDI is attached

to a TOF analyzer which measures different times that are taken by the molecules to travel a fixed distance. This method requires relatively less intense, fast and easy sample preparation thus preserving the fragile proteins, peptides, and polymers that tend to fragment when ionized by other ionization techniques. The MALDI is less susceptible to interferences caused by salts and detergents and has high sensitivity for proteins in low molecular weight range or with extreme characteristics: highly hydrophobic, acidic, or basic. In this technique; after magnetic beads separation; the samples are applied with a suitable matrix material onto a metal chip where a pulsed laser triggers desorption and ionization of molecules which are subsequently detected in the TOF analyzer by their respective mass-to-charge ratios (m/z) ***(Bladergroen et al., 2014).*** Large numbers of detected protein molecules' signals are collected to form patterns identified as proteomic patterns that may characterize fingerprints of specific specimen's protein constituents in each biological subject, biological state, or disease category. The proteomic patterns have been shown to distinguish diseased and unaffected subjects to varying degrees in different cancers ***(Guo et al 2014)***. Proteomic studies generate an expanding list of candidate protein markers that are differentially expressed in blood of CRC patients and reproduce spectra proteomic profiles with different patterns ***(Kocevar et al., 2013).*** These peptide and protein biomarker patterns can be used to distinguish CRC patients from healthy controls with

high discriminative power ***(Guo et al., 2014)*** and may provide a novel non-invasive means of diagnosing CRC ***(Fan et al., 2012)***.