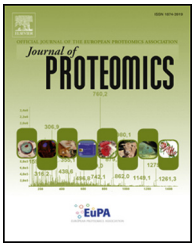
JOURNAL OF PROTEOMICS XX (2014) XXX–XXX

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot



In-depth proteomic delineation of the colorectal cancer exoproteome: Mechanistic insight and identification of potential biomarkers

George S. Karagiannis[a,b], Maria P. Pavlou[a,b], Punit Saraon[a,b], Natasha Musrap[a,b], Annie Xie[a], Ihor Batruch[b], Ioannis Prassas[b], Apostolos Dimitromanolakis[b], Constantina Petraki[c], Eleftherios P. Diamandis[a,b,d,*]

[a]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada
[b]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada
[c]Pathology Department, Metropolitan Hospital, Athens, Greece
[d]Department of Clinical Biochemistry, University Health Network, Toronto, Ontario, Canada

ARTICLE INFO

Article history:
Received 20 December 2013
Accepted 18 March 2014

Keywords:
Colorectal cancer
Exoproteome
Kallikrein-related peptidase
Olfactomedin-4
Proteomics
Enrichment map analysis

ABSTRACT

Systemic mining of cancer exoproteome/secretome has emerged as a pivotal strategy for delineation of molecular pathways with mechanistic importance in cancer development, as well as the discovery of diagnostic/prognostic biomarkers. Although major advances in diagnostic and therapeutic management of colorectal cancer have been underscored in the last decade, this cancer still remains the second leading cause of cancer-related deaths in the developed world. Despite previous studies on deciphering the colorectal cancer exoproteome, such studies lack adequate depth and robustness due to technological limitations. Here, using a well-established LC–MS/MS method on an LTQ-Orbitrap mass spectrometer, we extensively delineated the exoproteome of 12 colon cancer cell lines. In total, 2979 non-redundant proteins were identified with a minimum of two peptides, of which ~62% were extracellular or cell membrane-bound, based on prediction software. To further characterize this dataset and identify clinical opportunities, first, we investigated overrepresented molecular concepts of interest via enrichment map analysis and second, we demonstrated translational importance of certain proteins, such as olfactomedin-4 and kallikrein-related peptidases-6 and -10, by investigating their expression levels in patient

Abbreviations: ATCC, American Type Culture Collection; AUC, Area Under the Curve; AZGP1, Zinc-alpha 2-glycoprotein; CA9, Carbonic Anhydrase IX; CDCHO, Chemically-defined Chinese Hamster Ovary; CEA, Carcinoembryonic Antigen; CM, Conditioned Media; CRC, Colorectal Cancer; DTT, Dithiothreitol; ECM, Extracellular Matrix; EGFR, Epithelial Growth Factor Receptor; EMEM, Eagle's Minimum Essential Medium; EMT, Epithelial-to-Mesenchymal Transition; FAP, Familial Adenomatous Polyposis; FBS, Fetal Bovine Serum; FDR, False Discovery Rate; GO, Gene Ontology; GREM1, Gremlin-1; HL, Hosmer–Lemeshow; (HP)LC, (High Performance) Liquid Chromatography; IBD, Inflammatory Bowel Disease; KLK, Kallikrein-related Peptidase; LOXL2, Lysyl-Oxidase Homolog-2; MS/MS, Tandem Mass Spectrometry; NME, Nucleoside Diphosphate Kinase A; OLFM4, Olfactomedin-4; PBS, Phosphate-buffered Saline; RER, Replication Error; ROC, Receiver Operating Characteristic; RPMI, Roswell Park Memorial Institute; SRPX2, sushi repeat-containing protein-2; VCAN, Versican.

* *Corresponding author at:* Mount Sinai Hospital, Joseph & Wolf Lebovic Ctr., 60 Murray St., Box 32, Floor 6, Room L6-201, Toronto, ON M5T 3L9, Canada. Tel.: +1 416 586 8443; fax: +1 416 619 5521.

E-mail addresses: gkaragiannis@mtsinai.on.ca (G.S. Karagiannis), mpavlou@mtsinai.on.ca (M.P. Pavlou), punit.saraon@utoronto.ca (P. Saraon), Natasha.musrap@mtsinai.on.ca (N. Musrap), anniexie31@gmail.com (A. Xie), ibatruch.acdclabs@gmail.com (I. Batruch), yprassas@gmail.com (I. Prassas), apostol@cs.toronto.edu (A. Dimitromanolakis), constantinapetraki@gmail.com (C. Petraki), ediamandis@mtsinai.on.ca (E.P. Diamandis).

http://dx.doi.org/10.1016/j.jprot.2014.03.018
1874-3919/

tissues and/or fluids. Overall, the present study details a comprehensive colorectal cancer exoproteome dataset, and may be used as future platform for biomarker discovery, and hypothesis-generating studies.

Biological significance

This article represents one of the most extensive and comprehensive proteomic datasets regarding the secreted/extracellular proteome of colorectal cancer cell lines. The reported datasets may form a platform for a plethora of future, discovery-based or hypothesis-generating studies, attempting to either delineate putative cancer biomarkers for CRC, or elucidate questions of mechanistic importance (e.g. investigation of deregulated pathways for CRC progression).

1. Introduction

Colorectal cancer (CRC) represents one of the most important causes of cancer-related death, and is the second most frequent type of cancer after lung cancer [1–3]. While CRC is mostly identified in the sporadic form, a significant portion can also occur in the context on inflammatory bowel disease (IBD) [4,5] or genetic syndromes, such as familial adenomatous polyposis (FAP) [6,7] and Lynch syndrome [8]. The development of sporadic CRC is caused by the accumulation of genetic and epigenetic changes, which can be generally categorized into two types: (I) Approximately 80% of CRC patients undergo a well-characterized series of molecular events, described as *adenoma–carcinoma sequence* [9–11], involving chromosomal aberrations and mutations in several genes, such as *APC*, *KRAS*, *P53* and *DCC* [12–17]. (II) The remaining 20% of CRC patients undergoes a secondary molecular pathway, which causes genetic instability in microsatellite loci attributable to alterations in the DNA mismatch repair genes, such as *MLH1*, *MSH2*, *MSH6* and *PMS2* [10,11,18]. These latter cancers are considered as replication error-deficient (RER+), while the former ones as replication error-proficient (RER–) [19].

Recently, high-throughput proteomic pipelines coupled to mass spectrometry have played a pivotal role in protein research, especially in the simultaneous identification, quantification and characterization of thousands of proteins in complex biological samples [20,21]. The emergence of these technologies has enabled the field of cancer research (i.e. oncoproteomics) with a plethora of opportunities, such as the diagnosis and therapeutic management of cancer [20,22]. An emerging subfield of oncoproteomics originates from the so-called 'secretome analysis', which attempts to delineate the extracellular proteome of cancer and/or other types of cells. The term 'secretome' was originally adapted by Tjalsma et al. [23] and Antelmann et al. [24] as a concept providing an integrated and global view of the protein secretion by considering both to the secretion systems and their protein substrates. It should be mentioned that proteins found in the extracellular milieu, i.e. the exoproteins, are not systematically secreted. Secreted proteins are defined as proteins actively transported across biological membrane by a secretion system (i.e. canonical or non-canonical) [25–28]. The term 'exoproteome' was later coined by Tjalsma et al. (2007) [29] to specifically refer to the subset of proteins present in the extracellular milieu, i.e. the extracellular proteome.

The indications, thus far, point to the fact that the exoproteome is a promising source of candidate biomarkers and therapeutic targets for various types of cancer, in the era of personalized medicine [30–34]. With the exception of a small number of studies, attempts to decipher the colorectal cancer exoproteome have been lacking. The aforementioned ones have yielded a set of candidate CRC biomarkers, of which a subset was selected for validation studies in human tissues and serum. Wu et al. [35] identified dataset of 325 unique proteins, of which collapsing response mediator-2 (CRMP-2) was validated by immunohistochemistry and its levels were significantly higher in CRC patients versus healthy controls. Xue et al. [36] performed differential proteomic analysis of the SW480/SW620 model, using label-free quantification. This study yielded a total of 910 proteins, of which 145 exhibited differential expressions. Trefoil factor 3 and growth/differentiation factor 15 were further validated in a large cohort of clinical tissues and serum, in which they could predict colorectal cancer metastasis. In an integrative approach, Wu et al. analyzed the secretomes of 23 human cancer cell lines derived from 11 cancer types including CRC, using one-dimensional SDS-PAGE and nano-LC–MS/MS, and proposed a list of candidate serological biomarkers [37]. Additional studies have quantitatively compared the extracellular proteomes between metastatic and primary cell lines [38], or coculture models to mimic the CRC microenvironment [39], as well as the CRC stem cell exoproteome [40,41] and identified key candidates of CRC development, progression and/or drug resistance.

To complement efforts for characterization of the CRC exoproteome, here, we performed in-depth proteomic analyses, integrating and comparing the proteomes of conditioned media (CM) from 12 different CRC cell lines (SW1116, SW480, LS174T, LS180, WiDR, SW620, RKO, LoVo, HCT116, DLD1, Colo320HSR and Colo205), which were chosen to recapitulate, as much as possible, the heterogeneity of the disease. As such, these cell lines represent individuals of varying ethnic backgrounds and age groups, mutational profiles and disease stage and/or differentiation status. All samples were analyzed in triplicate using strong cation exchange (SCX) chromatography followed by liquid chromatography (LC)–tandem mass spectrometry (MS/MS) on a linear trap quadrupole (LTQ)-Orbitrap mass spectrometer. The reported dataset may form a platform for a plethora of future, discovery-based or hypothesis-generating studies, attempting to either delineate putative cancer biomarkers for CRC, or elucidate questions of

Please cite this article as: Karagiannis GS., et al, In-depth proteomic delineation of the colorectal cancer exoproteome: Mechanistic insight and identification of potential biomarker..., J Prot (2014), http://dx.doi.org/10.1016/j.jprot.2014.03.018