LABEL FREE PROTEOMIC ANALYSIS OF LAUREN'S INTESTINAL TYPE GASTRIC CANCER PATIENTS PLASMA

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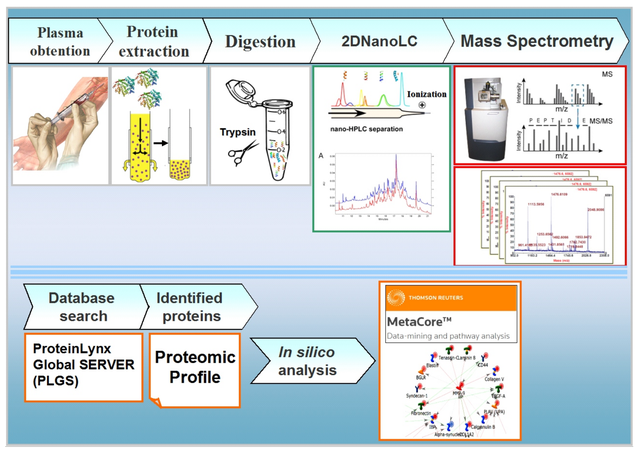
INTRODUCTION

Gastric cancer (GC) is the forth most common cancer in the world. This neoplasia is a multifactorial disease comprehending lifestyle, aging, genetic, socioeconomic and biological factors including *Helicobacter pylori* that have been attributed in 80% of the cases. According to Lauren classification the gastric adenocarcinoma presents two types: intestinal and diffuse. Innovative technologies have been used to identify alterations in gastric cancer cell biology. Genetic abnormalities, such as aberrant genes copy number variation and noncoding RNAs were identified as possible biomarkers. However, the molecular mechanisms leading to gastric cancer and its progression are not clearly understood. Currently, there are serum biomarkers for GC diagnosis, however, these markers are not sensible or specific enough. Thus, proteomic approaches are promising tool in investigation and identification of biomarkers, contributing to a better understanding and management of this pathology.

OBJECTIVE

The aim of this study was to investigate the proteomic profile of plasma samples from patients with IGC infected or not with *Helicobacter pylori* compared to plasma from healthy donors, in order to find new potential biomarkers.

METHODOLOGY



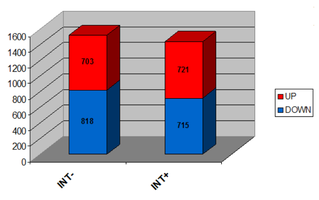
RESULTS

Proteomic profile evaluation of Laurent´s intestinal GC patient plasma infected and non infected with *H. Pylori*.

A

		Validation cohort
Number of patients	11	x
Mean age at diagnosis (range years)	69 (51-82)	x
Sex		
Men	8/11 (72,72%)	x
Women	3/11 (27,27%)	x
Histological type		
Intestinal +	n=5	x
Intestinal -	n=6	x
Tumor location		
Antrum	3/11 (27,27%)	x
Cardia	1/11 (9,09%)	x
Body	4/11 (36,36%)	x
Fundus	1/11 (9,09%)	x
Not identified	2/11 (18,18%)	x

B



C

Process Networks	INT+	INT-
Cell adhesion_Integrin-mediated cell-matrix adhesion	1	10
Immune response_Phagosome in antigen presentation	2	1
Cytoskeleton_Actin filaments	3	x
Cytoskeleton_Cytoplasmic microtubules	4	x
Cell adhesion_Cell junctions	5	x
Inflammation_Amphoterin signaling	6	x
Immune response_Antigen presentation	7	4
Cytoskeleton_Intermediate filaments	8	x
Development_Hedgehog signaling	9	x
Cell adhesion_Attractive and repulsive receptors	10	x
Apoptosis_Anti-Apoptosis mediated by external signals via PI3K/AKT	x	6
Cell adhesion_Platelet-endothelium-leucocyte interactions	x	2
Cytoskeleton_Macropinocytosis and its regulation	x	5
Immune response_Phagocytosis	x	3
Inflammation_IL-6 signaling	x	7
Proteolysis_ECM remodeling	x	9
Reproduction_Spermatogenesis, motility and copulation	x	8

D

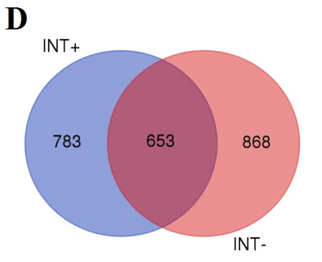


Figure 1. Label-free Nano LC/MSMS analysis of GC patient plasma. (A) Clinico-pathological data from patient. (B) Differential expression analysis using Expression[E] showing increased (UP) and decreased (DOWN) proteins from GC samples compared to normal controls. (C) Processes Networks related to INT- and INT+ plasma proteome. (D) Venn diagram of INT+ and INT- samples proteins showing overlapping and unique differentially expressed plasma proteins. INT+ = Intestinal Gastric Cancer *Helicobacter pylori* positive; INT- = Intestinal Gastric Cancer *Helicobacter pylori* negative.

In silico analysis

Common proteins between INT- and INT + plasma samples

Table 1: Processes related to the 653 common proteins

Functional Enrichment Analysis a	Protein b UP	Protein b DOWN
Development_Regulation of cytoskeleton, telomere length and cellular immortalization		KLHL1, Cofilin, PDGF-R-alpha, Tubulin alpha, MAP-1B, HSP90, Tankyrase 2, Ku80,Kinesin heavy chain,Desmuslin
WNT signaling pathway	TLE	TCF7L2 (TCF4), Tcf(Lef)
TGF-beta 1-induced transactivation of membrane receptors signaling in HCC		Cofilin, PDGF-R-alpha, Lef-1
Cell adhesion and ECM remodeling	Vitronectin	TIMP2, PLAT (TPA), Collagen IV, Cofilin, Tcf(Lef), Tubulin alpha, Myosin X, HSP90, DNAM1, PDGF-R-alpha, Protein C

a Enrichment analysis was performed using Metacore TM.
b Proteins which were identified to be significantly up- or down-regulated by proteomic experiment were shown.

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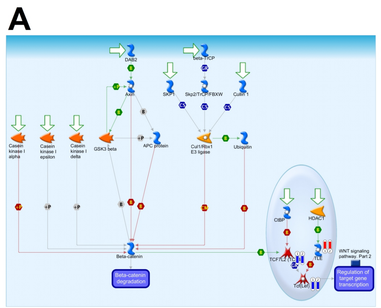

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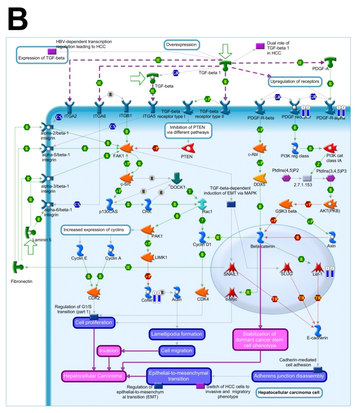

Figura 2: Biologic Pathways associated with common differentially expressed proteins between INT+ and INT- samples. (A) WNT signaling pathway. Part 1. Degradation of beta-catenin in the absence WNT signaling . (B) TGF-beta 1-induced transactivation of membrane receptors signaling in HCC. *In silico using* MetaCoreTM software (GeneGO Inc., Encinitas, CA). Thermometers indicates pathway proteins that were found differentially expressed in our work. Thermometers also indicates the expression levels: red thermometers- up regulation; blue thermometers- down regulation.

Exclusive proteins found in INT-

Table 2: Functional enrichment analyses of 868 proteins differentially expressed found exclusively in INT-.

Functional Enrichment Analysis [a]	Protein [b] UP	Protein [b] DOWN
Protein folding and maturation_Bradykinin / Kallidin maturation		Bradykinin
wtCFTR and deltaF508-CFTR traffic / Clathrin coated vesicles formation (normal and CF)	c-Cbl, AP180, Dynamin-2	DAB2
Regulation of angiogenesis	SREBP1, EGFR, TWEAK(TNFSF12), Clusterin, Shc, N-cadherin	CARD11, NF-kB
Transcription_CoREST complex-mediated epigenetic gene silencing	RREB1, Synapsin I, HDAC2, BAF170	
Regulation of lipid metabolism	SREBP1, AMPK gamma subunit	
Development_ERBB-family signaling	Shc, c-Cbl, EGFR	NF-kB
Androgen receptor activation	Clusterin, Shc, c-Cbl, EGFR, VIL2 (ezrin), N-cadherin, Shc, RAD9	

a Enrichment analysis was performed using Metacore TM.
b Proteins which were identified to be significantly up- or down-regulated by proteomic experiment were shown.

Exclusive proteins found in INT

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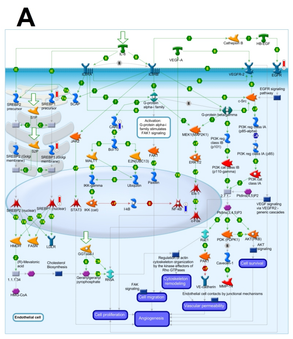

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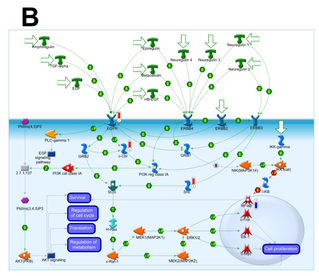

Figure 3: Signaling pathways associated to differentially expressed proteins exclusively found in INT. (A) *Role of IL-8 in angiogenesis*. (B) ERBB-family signaling . *In silico* analysis using MetaCoreTM software (GeneGO Inc., Encinitas, CA). Thermometers indicates pathway proteins that were found differentially expressed in our work. Thermometers also indicates the expression levels: red thermometers- up regulation; blue thermometers- down regulation.

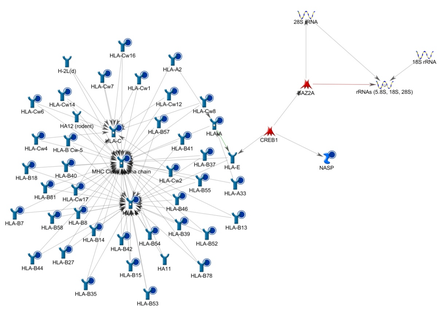

Figure 4: Network obtained by MetaCore analysis. Network: NASP, HLA-A, BAZ2A, CREB1, HLA-E. Colored circles indicates Interaction network proteins that were found differentially expressed in our work. Colored circles also indicates the expression levels: red circles- up regulation; blue circles- down regulation.

Exclusive proteins found in INT+

Table 3: Functional enrichment analyses of 783 proteins differentially expressed found exclusively in INT+

Functional Enrichment Analysis a	Protein b UP	Protein b DOWN
Stem cells	ITGA6, Oct-3/4, Keratin 18, MDM4, ETV2, PI3K cat class IA	SHH, Ihh, ERK1/2
LRRK2 in neurons in Parkinson's disease	MARK2, AP-2 alpha subunits	HSP90, ERK1/2
Nicotine / nAChR alpha-7 signaling in NSCLC	p90Rsk, Beta-arrestin1, CACNA1C, PI3K cat class IA	ERK1/2
Development	Beta-arrestin1, p90Rsk, Dynein 1 cytoplasmic heavy chain, DCTN1(p150Glued), PAFAH alpha (LIS1), PI3K cat class IA	ERK1/2
Immune response_Role of DAP12 receptors in NK cells	HLA-B, HLA-C, PI3K cat class IA, HLA-E, NIK(MAP3K14), p90RSK1	ERK1/2

a Enrichment analysis was performed using Metacore TM.
b Proteins which were identified to be significantly up- or down-regulated by proteomic experiment were shown.

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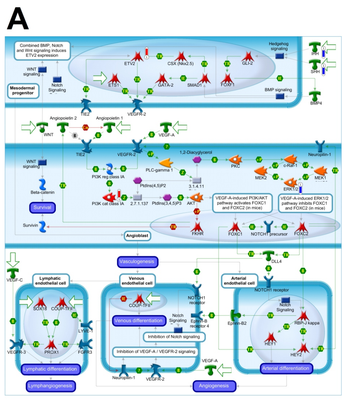

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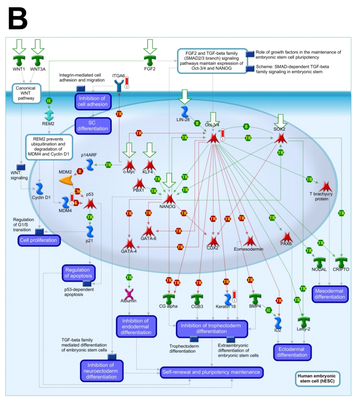

Figure 5: Signaling pathways associated with differentially expressed proteins exclusively found in INT+. (A) *Endothelial differentiation during embryonic development*. (B) *Self-renewal and pluripotency maintenance of human embryonic stem cells*. *In silico* analysis using MetaCoreTM software (GeneGO Inc., Encinitas, CA). Thermometers indicates pathway proteins that were found differentially expressed in our work. Thermometers also indicates the expression levels: red thermometers- up regulation; blue thermometers- down regulation.

Figure 6: Network obtained by MetaCore analysis. network HSP90, HLA-Cw3, CREB1, UHRF2, p21. Colored circles indicates Interaction network proteins that were found differentially expressed in our work. Colored circles also indicates the expression levels: red circles- up regulation; blue circles- down regulation.

CONCLUDING REMARKS

Altogether, our findings improved the comprehension of molecular biology of GC which highlights new potential targets for further investigation. Support: CAPES, CNPq, FAPERJ, Ministério da Saúde.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

MINISTÉRIO DA SAÚDE