#4854 -A Translational, Multiplexed, Hypothesis-Generating Study on Circulating Cytokines in Patients with Cancer-Cachexia.

Mª Teresa Agulló-Ortuño, Virginia Pardo-Marqués, Elena Prieto-García, C. Vanesa Díaz-García, Irene Otero, José A. López-Martín.

Medical Oncology - Hospital Universitario 12 de Octubre & Clinical & Translational Cancer Research Group - Instituto de Investigacion Hospital Universitario 12 de Octubre. RedLab #324. Madrid. Spain

Introduction

Cancer cachexia is a multifactorial syndrome defined by consensus as an ongoing loss of skeletal muscle tissue - in the presence or absence of a decrease of adipose tissue - that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairments. (1) Cancer cachexia impacts approximately 60% of the 1.4 million patients diagnosed with cancer in the United States. Among late stage patients, cachexia affects 50 -80% patients and contributes in 20% of cancer related deaths. (2) Its underlying mechanisms are not fully understood, and remains an unmet medical need.

Objectives

To measure multiple cytokines, chemokines, and other secreted proteins (**CCSPs**) in plasma from cancer patients with cachexia, in order to develop new hypotheses on pathways and/or eventual therapeutic targets.

Methods

This is part of a pilot, observational, cross-sectional, case-control, IRB-reviewed translational research in cancer patients with/without cachexia. (3)

- Anthropometric, clinical and biochemical data from consenting eligible cancer patients were collected, as well as blood samples, from which plasma was separated and immediately stored at -80°C until analysis.
- Measurements of **CCSPs** were performed at the Human Immune Monitoring Center at Stanford University (CA, USA), where they used 63-plex immunoassays according to published protocols.(4) Plates were read using a Luminex 200 equipment.
- Data were analyzed with R-based packages and scripts, by univariate and multivariate methods, using error adjustments for multiple testings (False Discovery Rate, FDR).
- We defined **CCSPs** differentially associated with cachexia (**CDAC**) as those with either statistically significant adjusted p-values (FDR < 0.05), relevant log2FC changes (> 1.3 or < - 1.3), or top-ranked by a random forest algorithm.
- **CDAC** were mapped onto a protein-protein interaction network (STRING database). (5) Only empirically- and database-based interactions that resulted in molecular actions were selected to extract the subnetwork and to analyze functional enrichments.
- Significant results from a 2D-DIGE proteomics (**2DP**) study (results not shown) on plasma aliquots from the same patients, were also mapped in the networks.
- A new subnetwork was subsequently generated with up to 40 additional proteins that were 1st order interactors of the differentially present proteins (**CDAC** + **2DP**), defined as: associated to a molecular action, experimental- or database-based, and with a high confidence score (> 0.9)

References

1.-Fearon K, Strasser F, Anker SD,et al. .The Lancet Oncology. 2011;12(5):489-95

2.- Advani SM, Advani PG, VonVille HM, Jafri SH. BMC Cancer. 2018;18(1):1174..

3.- Cala MP, Agulló-Ortuño MT, Prieto-García E, ... Lopez-Martin JA. J Cachexia Sarcopenia Muscle. 2018;9(2):348-357.

4.- https://iti.stanford.edu/himc/immunoassays.html

5.- https://string-db.org/

jalopezmartin@gmail.com

Results: Patient / tumor characteristics

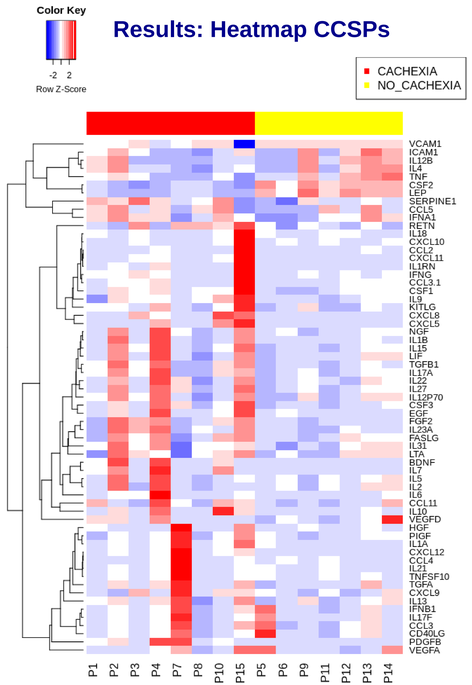
	Cachexia	No-cachexia	*p-value*
Overall, N (%)	8 (53.3)	7 (46.7)	
Female: Male	1 : 7	1 : 6	0.92
Median age (years) (range)	61.5 (36-81)	63.9 (48-80)	0.74
Relative weight loss in the last 3 months, %	17.6 ± 14.2	1.0 ± 2.7	0.01
BMI, kg/m^2	20.8 ± 3.4	24.5 ± 2.1	0.03
BSA (DuBois), m^2	1.7 ± 1.5	1.8 ± 1.2	0.07
CRP, mg/L	5.7 ± 5.6	0.7 ± 0.5	0.04
Tumour type, N (%)			
• Pancreas	3 (37.5)	1 (14.3)	
• Ampulloma	1 (12.5)		
• Melanoma	3 (37.5)	1 (14.3)	
• Cholangiocarcinoma	1 (12.5)		
• Stomach		1 (14.3)	
• Oesophageal		2 (28.6)	
• Colon		1 (14.3)	
• Sarcoma		1 (14.3)	

BSA: Body surface area. **BMI**: Body mass index. **CRP**: C-reactive protein.
Data are mean ± standard deviation.
Modified from:ref. 3

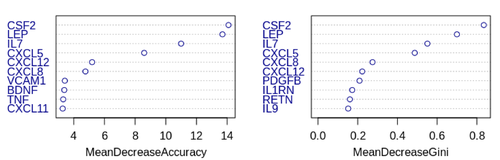
Results: Differentially abundant CCSPs

Protein	log2FC	p Wilcoxon	p adj Wilcoxon	p t test	p adj t test
LEP	-2.57	0.0003	0.0095	0.0001	0.0032
GM-CSF/CSF2	-1.85	0.0003	0.0095	0.0000	0.0000
TNF	-0.24	0.0721	0.3944	0.0458	0.3028
FGF2	0.38	0.1206	0.4221	0.0305	0.3028
IL23A	0.54	0.1520	0.4560	0.0412	0.3028
CSF3/GCSF	0.58	0.0289	0.2601	0.0357	0.3028
IL10	1.30	0.1307	0.4334	0.0792	0.3119
IL21	1.54	0.1893	0.4587	0.2736	0.4076
CCL4	1.54	0.6126	0.7422	0.2868	0.4076
EGF	1.55	0.2026	0.4727	0.1058	0.3124
HGF	1.55	0.2319	0.4918	0.1907	0.4005
IL6	1.57	0.2467	0.4918	0.2621	0.4076
CXCL10	1.60	0.8665	0.9252	0.3109	0.4167
CXCL8	2.16	0.0059	0.0743	0.0517	0.3028
BDNF	2.25	0.0721	0.3944	0.0721	0.3028
PDGFB	2.27	0.0289	0.2601	0.0575	0.3028
IL7	2.30	0.0037	0.0583	0.0401	0.3028
CXCL5	2.79	0.0022	0.0462	0.0692	0.3028
CXCL11	2.86	0.1893	0.4587	0.2976	0.4076
IL1RN	3.32	0.0401	0.3158	0.2594	0.4076
CCL2	3.47	0.0939	0.3944	0.3349	0.4393

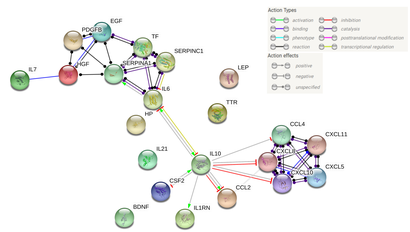
Results: Heatmap CCSPs



Results: Variable Importance (random forest)



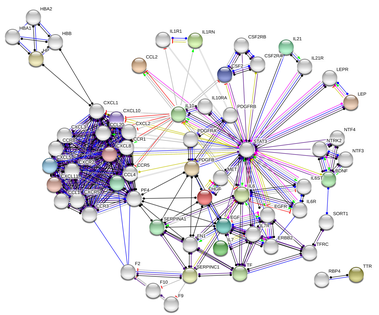
Results: Protein-protein interactions and functional enrichment (5)



KEGG pathway (*)	observed protein count	FDR
Cytokine-cytokine receptor interaction	15	1.94E-21
Jak-STAT signaling pathway	8	3.4E-10
IL-17 signaling pathway	6	2.97E-08
Malaria	5	6.54E-08
Chemokine signaling pathway	6	8.14E-07
Rheumatoid arthritis	5	8.14E-07
Toll-like receptor signaling pathway	5	1.47E-06
TNF signaling pathway	5	1.69E-06
Pertussis	4	1.7E-05
EGFR tyrosine kinase inhibitor resistance	4	1.85E-05
PI3K-Akt signaling pathway	6	1.85E-05
Salmonella infection	4	2.07E-05
Amoebiasis	4	2.94E-05
Chagas disease (American trypanosomiasis)	4	3.59E-05
Pathways in cancer	6	0.00011
Influenza A	4	0.00022
Cytosolic DNA-sensing pathway	3	0.00029
Kaposi's sarcoma-associated herpesvirus infection	4	0.00029
Inflammatory bowel disease (IBD)	3	0.00029
Melanoma	3	0.00037
Ras signaling pathway	4	0.00054
Hematopoietic cell lineage	3	0.00073
HIF-1 signaling pathway	3	0.00079
AGE-RAGE signaling pathway in diabetic complications	3	0.00079
MAPK signaling pathway	4	0.0012
FoxO signaling pathway	3	0.0016
Phospholipase D signaling pathway	3	0.0021
Non-alcoholic fatty liver disease (NAFLD)	3	0.0021
NOD-like receptor signaling pathway	3	0.0027
Transcriptional misregulation in cancer	3	0.0028
Focal adhesion	3	0.0039
Rap1 signaling pathway	3	0.0041
HTLV-I infection	3	0.007

(*) Only pathways represented with at least 3 observed proteins and a FDR < 0.05 are listed

Results: Subnetwork completion with 1st order interactors



Conclusions

- Despite sample size constraints and heterogeneity, this pilot study has been able to capture differences between the abundance of proteins in plasma from cancer patients with cachexia vs non-cachexia, which might be tumor-agnostic
- Most of the proteins point to STAT3 as a master regulator of the inflammatory responses that take place in cancer-cachexia
- Additional studies in pre-cachexia patients, are being designed

Acknowlegments

The study has been funded by ISCIII (PI 10/02072), Fundación Mutua Madrileña (FMM2010-018), and Comunidad de Madrid (Immunothercan S2011/BMD-2326 and S2017/BMD-3733). Dr Inés García-Consuegra Galiana (Proteomics Core Facility at our Institution) was instrumental for 2D-DIGE assays.