PRC

P-selectin genotype is associated with the development of cancer cachexia.

Benjamin H. L. Tan, Torill Fladvad, Theodore P. Braun, Antonio Vigano, Florian Strasser, D. A. Christopher Deans, Richard J. E. Skipworth, Tora S. Solheim, Sambasivarao Damaraju, James A. Ross, Stein Kaasa, Daniel L. Marks, Vickie E. Baracos, Frank Skorpen, Kenneth C. H. Fearon, European Palliative Care Research Collaborative. *EMBO Mol. Med*. 2012 Apr 4. doi: 10. 1002/emmm.201200231. [Epub ahead of print]

Presenting author: Frank Skorpen, NTNU

NTNU – Trondheim Norwegian University of Science and Technology · ST. OLAVS HOSPITAL TRONDHEIM UNIVERSITY HOSPITAL · Norwegian Cancer Society · Kreft foreningen · EAPC · Fondazione IRCCS Istituto Nazionale dei Tumori · Regione Lombardia

Problem

- More than half of cancer patients suffer from cachexia, and it is responsible for death in up to 20% of cases.
- Cachexia is also a significant cause of morbidity in cancer patients.
- Based on our current knowledge of demographic and clinical factors, we are unable to predict, for any given cohort of patients, who will develop cancer cachexia and who will not.

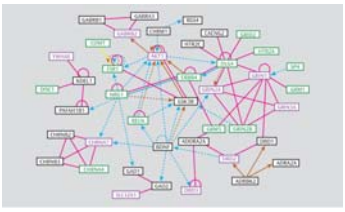
Cachexia and genetics

- Genetic variations in regulation of the inflammatory response, appetite regulation, and in muscle and fat metabolic pathways are likely to affect the risk of developing cachexia
- Knowledge of genotypic variation associated with cachexia would contribute to early identification of patients at risk and allow institution of prophylactic measures

3

Approach

Candidate genes and gene variants from systematic review and gene expression experiments



Main cohort
(*n*=775)

Significant associations ($p<0.05$)

Validation cohort
(*n*=101)

Most significant gene tested in relevant animal model

4

Phenotype definitions

- There is no consensus diagnostic criteria for cancer cachexia, but the condition is defined by the presence of involuntary weight loss
- The current study defined cachexia as:
  - mild or greater (WT loss >5%)
  - moderate or greater (WT loss >10%)
  - severe (WT loss >15%)
- CRP >10mg/l was used as an indicator of systemic inflammation

5

Description of the cachexia cohorts

Main cohort

Table 1. Patient demographics (main cohort). Patients were recruited from (2004 to 2008) from the NHS Lothian, UK, Cross Cancer Institute, Edmonton, Canada, and McGill University Health Centre, Montreal, Canada

	No. of patients ($n = 775$)
Age (years) †	65.5 ± 11.8
Range	27–97
Sex	
M	476 (61.4)
F	299 (38.6)
Tumour type	
Oesophageal or gastric	389 (50.2)
Pancreatic	114 (14.7)
Non-small cell lung cancer	232 (29.9)
Other	40 (5.2)
Stage	
I	38 (4.9)
II	95 (12.3)
III	216 (27.9)
IV	392 (50.5)
Unknown	34 (4.4)
Body mass index (kg/m^2) †	24.9 ± 4.9
Range	12.9–46.7
Percentage weight loss †	7.95 ± 8.16
Range	0–43.8
C-reactive protein (mg/l)† ($n = 569$)	23.0 ± 35.9
CRP > 10 mg/l	235 (41.3)
CRP ≤ 10 mg/l	334 (58.7)

Values are number of patients with percentages in parentheses unless indicated otherwise.
†values are mean ± SD. Characteristics were measured at first presentation to a surgical or oncology clinic.

Validation cohort

Table 4. Patient demographics (validation cohort). Patients recruited from (2007 to 2008) from the Oncology & Palliative Medicine, Cantonal Hospital, St. Gallen, Switzerland

	No. of patients ($n = 101$)
Age (years) †	62.0 ± 11.5
Range	35–88
Sex	
M	60 (59.4)
F	41 (40.6)
Tumour type	
Oesophageal or gastric	18 (17.8)
Pancreatic	6 (5.9)
Non-small cell lung cancer	19 (18.8)
Other	58 (57.4)
Stage	
I	0
II	3 (3.0)
III	2 (2.0)
IV	96 (95.0)
Body mass index (kg/m^2) †	23.7 ± 4.3
Range	15.4–37.8
Percentage weight loss †	5.54 ± 7.91
Range	0–43.1
C-reactive protein (mg/l)† ($n = 95$)	75.5 ± 76.4
CRP > 10 mg/l	78 (82.1)
CRP ≤ 10 mg/l	17 (17.9)

Values are number of patients with percentages in parentheses unless indicated otherwise.
†Values are mean ± SD. Characteristics were measured at first presentation to an oncology clinic.

6

Results

- Overall 191 SNPs in 99 genes were considered for the association study
- Following the relevant quality control checks, 129 SNPs in 80 genes remained for analysis in 775 patients
- Overall completion rate of genotyping was 95.6%

7

Genes with significant association with cancer cachexia classified according to weight loss alone

Table 2a. Genes with variants significantly associated with cancer cachexia in patients classified according to weight loss alone

Weight loss >15%. Number affected: 145/775 (18.7%)

Gene	SNP	Risk allele	OR (95% CI)	*p*-Value	Permutated *p*
SELP	rs6136	C	0.31 (0.14–0.72)	0.006615	0.008062
ICAM1	rs281432	G	1.53 (1.06–2.20)	0.02163	0.01652
DIO1	rs11206244	T	1.54 (1.06–2.24)	0.0226	0.02164
ADIPOR2	rs16928751	A	0.53 (0.29–0.96)	0.03521	0.03053
APEH	rs2960548	G	1.48 (1.03–2.11)	0.03384	0.03768

Weight loss >10%. Number affected: 266/775 (34.3%)

Gene	SNP	Risk allele	OR (95% CI)	*p*-Value	Permutated *p*
LEPR	rs1137100	G	0.66 (0.47–0.92)	0.01494	0.013
DIO1	rs11206244	T	1.52 (1.09–2.11)	0.0129	0.01512
SELP	rs6136	C	0.52 (0.29–0.93)	0.02746	0.02581
HYLS1	rs3088241	C	0.72 (0.53–0.97)	0.02829	0.02709
CAMK2B	rs10441113	A	0.73 (0.54–0.99)	0.04096	0.03419

Weight loss >5%. Number affected: 415/775 (53.5%)

Gene	SNP	Risk allele	OR (95% CI)	*p*-Value	Permutated *p*
TNFRSF1A	rs4149570	T	1.42 (1.08–1.87)	0.01134	0.01759
TNFRSF1A	rs767455	C	0.71 (0.53–0.95)	0.02034	0.02275
TNFRSF1B	rs976881	A	0.76 (0.57–1.00)	0.04804	0.04324
IL18	rs1946519	A	1.35 (1.02–1.79)	0.03895	0.04969

8

Genes with significant association with cancer cachexia classified according to weight loss and CRP > 10mg/l

Table 2b. Genes with variants significantly associated with cancer cachexia in patients classified according to weight loss with systemic inflammation (CRP >10 mg/l)

Weight loss >15% & CRP >10mg/l. Number affected: 76/569 (13.4%)

Gene	SNP	Risk allele	OR (95% CI)	*p*-Value	Permutated *p*
APEH	rs2960548	G	2.17 (1.36–3.47)	0.001125	0.000997
GHRL	rs42451	T	2.04 (1.25–3.31)	0.004031	0.004058
TNFRSF1A	rs4149570	T	1.84 (1.16–2.92)	0.009322	0.01031
SELP	rs6136	C	0.26 (0.08–0.79)	0.01765	0.01103
CNR1	rs1049353	A	1.82 (1.08–3.06)	0.02366	0.02254
IRS1	rs1025333	A	2.24 (1.07–4.69)	0.03257	0.03183
APEH	rs4855881	C	1.64 (1.04–2.59)	0.03431	0.03191
FOXO1	rs17446593	G	0.49 (0.26–0.92)	0.02704	0.03239
ICAM1	rs281432	G	1.63 (1.04–2.54)	0.03276	0.03941

Weight loss >10% & CRP >10mg/l. Number affected: 123/569 (21.6%)

Gene	SNP	Risk allele	OR (95% CI)	*p*-Value	Permutated *p*
APEH	rs2960548	G	1.80 (1.21–2.68)	0.003528	0.003499
GHRL	rs42451	T	1.79 (1.18–2.72)	0.006219	0.00467
TNFRSF1A	rs4149570	T	1.51 (1.04–2.18)	0.02958	0.01998
HYLS1	rs3088241	C	0.66 (0.46–0.95)	0.02374	0.02074
APEH	rs4855881	C	1.57 (1.06–2.32)	0.02334	0.02847
TSC2	rs7187438	C	0.64 (0.43–0.95)	0.0265	0.03438
TNFRSF1B	rs3397	C	0.67 (0.46–0.97)	0.03527	0.04286

Weight loss >5% & CRP >10 mg/l. Number affected: 166/569 (29.2%)

Gene	SNP	Risk allele	OR (95% CI)	*p*-Value	Permutated *p*
APEH	rs2960548	G	1.67 (1.17–2.38)	0.004924	0.004533
APEH	rs4855881	C	1.56 (1.10–2.21)	0.01321	0.01212
TNFRSF1A	rs4149570	T	1.51 (1.08–2.10)	0.01559	0.02074
ADIPOR2	rs16928751	A	0.56 (0.33–0.95)	0.03308	0.02096
ADIPOR2	rs35854772	T	0.57 (0.33–0.97)	0.03733	0.02667
TNFRSF1B	rs3397	C	0.70 (0.50–0.98)	0.03944	0.02923
LTBP1	rs817529	G	0.70 (0.49–0.98)	0.03719	0.03791
TNFRSF1A	rs767455	C	0.68 (0.48–0.96)	0.02682	0.03846

9

SNPs with association of $p<0.02$ with various cachexia phenotypes

Gene	SNP	Risk allele	OR (95% CI)	*p*-value
SELP	rs6136	C	0.31 (0.14-0.72)	0.006615
LEPR	rs1137100	G	0.66 (0.47-0.92)	0.01494
DIO1	rs11206244	T	1.52 (1.09-2.11)	0.0129
TNFRSF1A	rs4149670	T	1.42 (1.08-1.87)	0.0113
APEH	rs2960548	G	2.17 (1.36-3.47)	0.001125
APEH	rs4855881	C	1.56 (1.10-2.21)	0.01212
GHRL	rs42451	T	2.04 (1.25-3.31)	0.004031

10

Gene groups associated with at least one cachexia phenotype at the $p<0.05$ level

Table 3. Candidate gene groups associated with cancer cachexia phenotypes

Phenotype	Candidate gene group function	Number of genes†	Number of SNPs	*p*-Values
Weight loss >10% & CRP >10 mg/l	Appetite regulation	2	3	0.0155
	Glucocorticoid signalling	4	9	0.0351
	MAPK activity regulation	7	14	0.0481
Weight loss >15% & CRP >10 mg/l	Appetite regulation	2	3	0.008499
	Glucocorticoid signalling	4	9	0.0181
	MAPK activity regulation	7	14	0.0264

†The genes in each candidate gene group are listed in Supporting Information Table S2.

Appetite regulation : GHRL, LEP
Glucocorticoid signaling: IL-10, IL-6, TNF, GHRL
Reg. of MAPK activity : GHRL, IL-1B, IL-6, TGFB2, TNF, ADRB2, IGF2

11

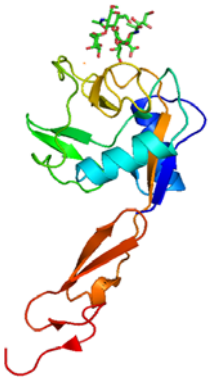
Main finding

- The C allele of *SELP* rs6136 (P-selectin) was found to be inversely associated with weight loss >10% both in the main study and the validation study!

Main: OR 0.52 (CI 0.29 – 0.93)
Valid.: OR 0.09 (CI 0.01 – 0.98)

12

P-selectin



140 kDa protein encoded by the *SELP* gene located on chromosome 1q21-q24. The gene spans > 50 kb and contains 17 exons in human

- Essential role in initial recruitment of leukocytes to the site of injury during inflammation
- Upon activation, P-selectin moves from an internal cell location to the surface of endothelial cells or megakaryocytes/platelets
- Functions as a cell adhesion molecule
- Two forms – membrane-bound and soluble (sP-selectin)
- sP-selectin may have a modulating effect on leukocyte recruitment

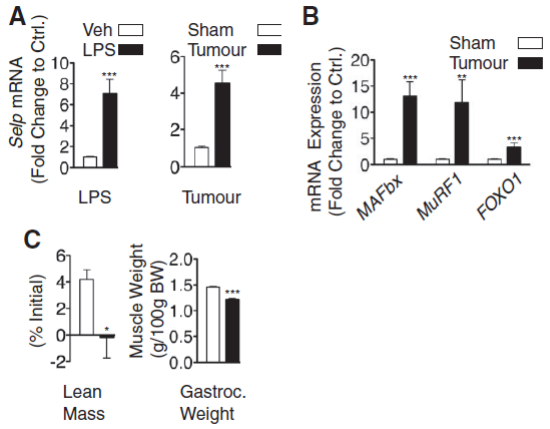
13

SELP rs6136

- Located in exon 13
- Missense variation changing a Threonine to a Proline
- Functional effect of changing the conformation of P-selectin near the cleavage site for the generation of sP-selectin
- Associated with lower levels of circulating sP-selectin

14

P-selectin is significantly upregulated in muscle following both tumor-induced cachexia in rats and intra-peritoneal injection of LPS in mice



15

Conclusion

- The study suggests that multiple pathways are likely to be involved in cachexia development
  - P-selectin, appetite regulation, glucocorticoid signaling, MAPK activity
- Upregulation of P-selectin in skeletal muscle accompanies muscle atrophy
- P-selectin genotype may prove useful in the risk stratification of pre-cachectic cancer patients
- P-selectin as a therapeutic target?

16

Limitations of the study

- Patients with diverse cachexia phenotypes, recruited at various stages of the disease process
  - Dilutes the phenotype and reduces power to detect true associations
- Possible dysphagia in patients with upper GI malignancy may contribute to malnutrition and WT loss
- Recall bias
  - pre-morbid weight was recalled by the patient (but verified where possible)
- Low power to detect weak association at the severe end of the cachexia spectrum
- Small size of the validation cohort
  - Increases the risk of false negatives

17

PRC

Thank you for your attention!

NTNU – Trondheim Norwegian University of Science and Technology
ST. OLAVS HOSPITAL TRONDHEIM UNIVERSITY HOSPITAL
Norwegian Cancer Society Kreft foreningen
EAPC
Fondazione IRCCS Istituto Nazionale dei Tumori
Sistema Sanitario Regione Lombardia

18

www.ntnu.no/prc European Palliative Care Research Centre (PRC)