Association of AKT1 and SELP Polymorphisms with Cachexia and Survival of Patients with Pancreatic Cancer

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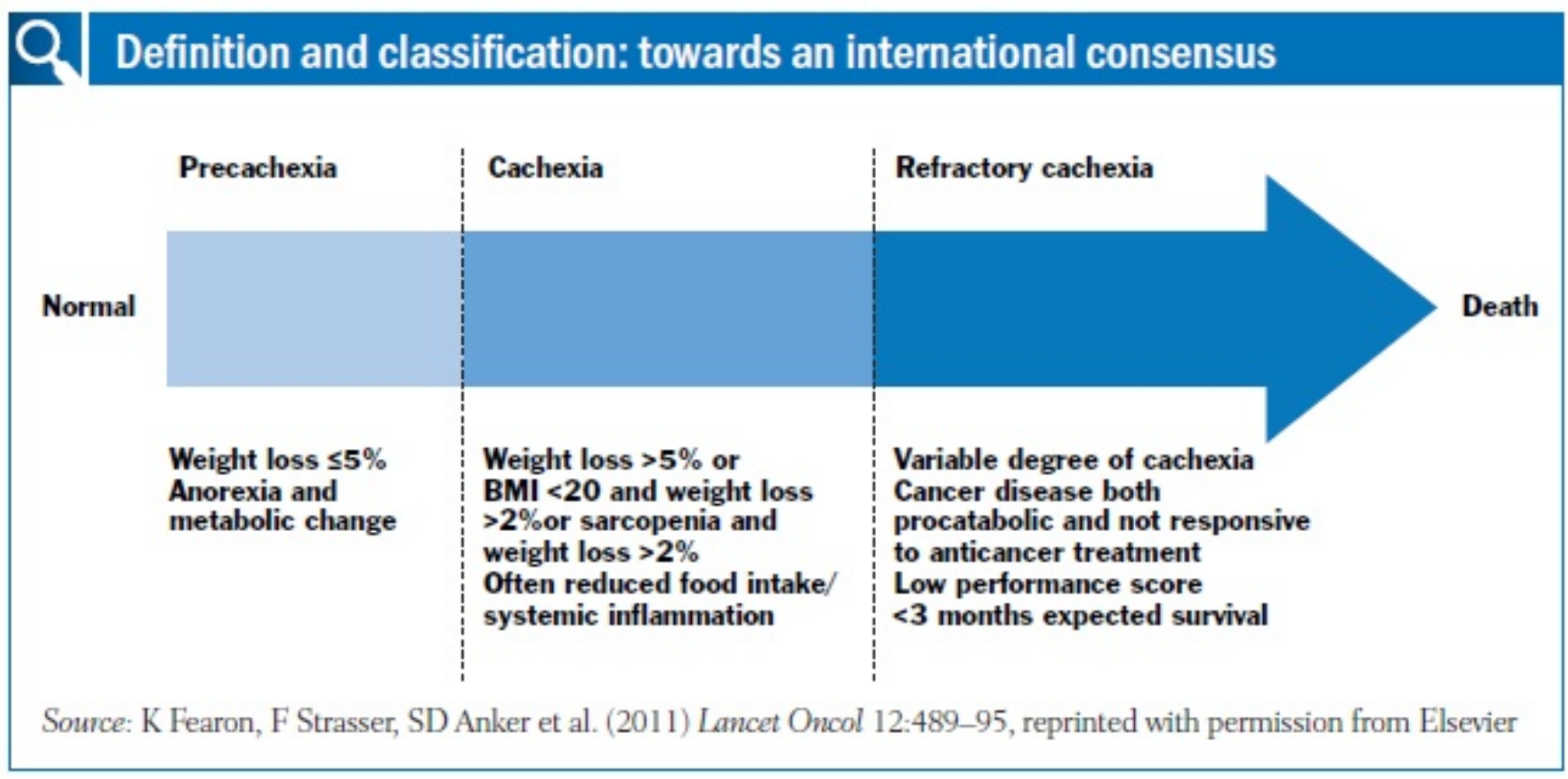
Let's introduce the "elephant in the room": **Cachexia**

Cachexia is a multi-factorial, systemic syndrome characterized by pathological wasting of skeletal muscle and adipose tissue mass that leads to pronounced weight loss

It can occur in the course of several chronic illnesses, but it is most frequently observed concomitantly with malignancies (30-50% cases) [*Argiles et al, Nat Rev Cancer 2014*]

Cachexia is **one of the most distressing conditions** for people with advanced cancer

Definition of Cachexia



Source: K Fearon, F Strasser, SD Anker et al. (2011) *Lancet Oncol* 12:489–95, reprinted with permission from Elsevier

Cachexia in pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) patients have the highest risk of developing cachexia, which is a direct cause of reduced quality of life and shorter survival:

- ✓ in advanced PDAC the presence of cachexia is associated with a worse prognosis [*Di Sebastiano et al, Br J Nutr 2013*]
- ✓ cachexia decreases the tolerance to systemic treatments and dramatically affects the quality of life [*Uomo et al, JOP 2006*]

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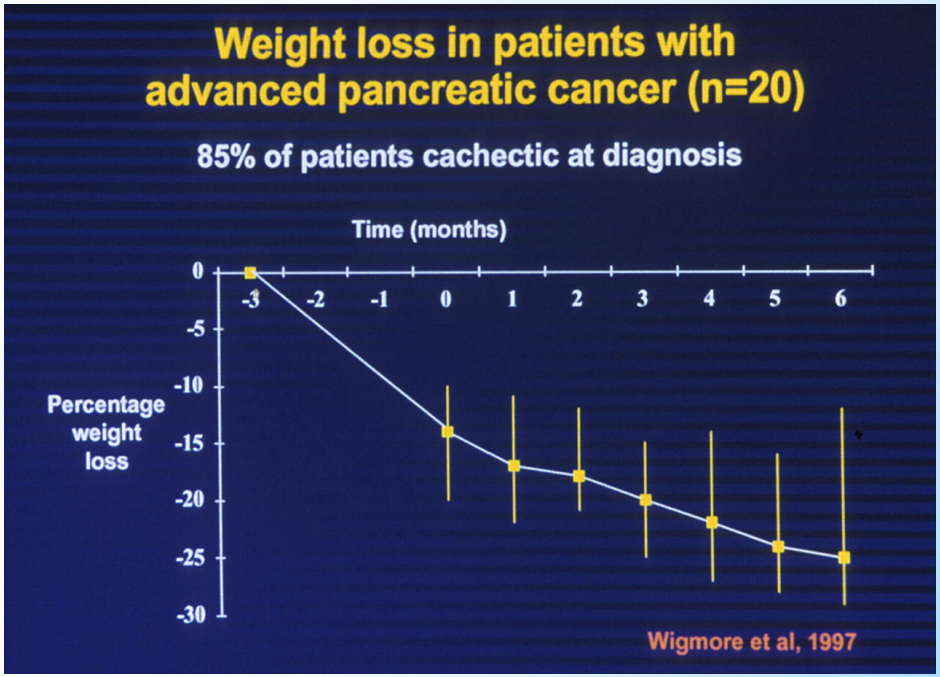
Tumor site	Incidence of weight loss (%)
Pancreas	83
Gastric	83
Esophagus	79
Head and neck	72
Colorectal	55–60
Lung	50–66
Prostate	56
Breast	10–35
General cancer population	63

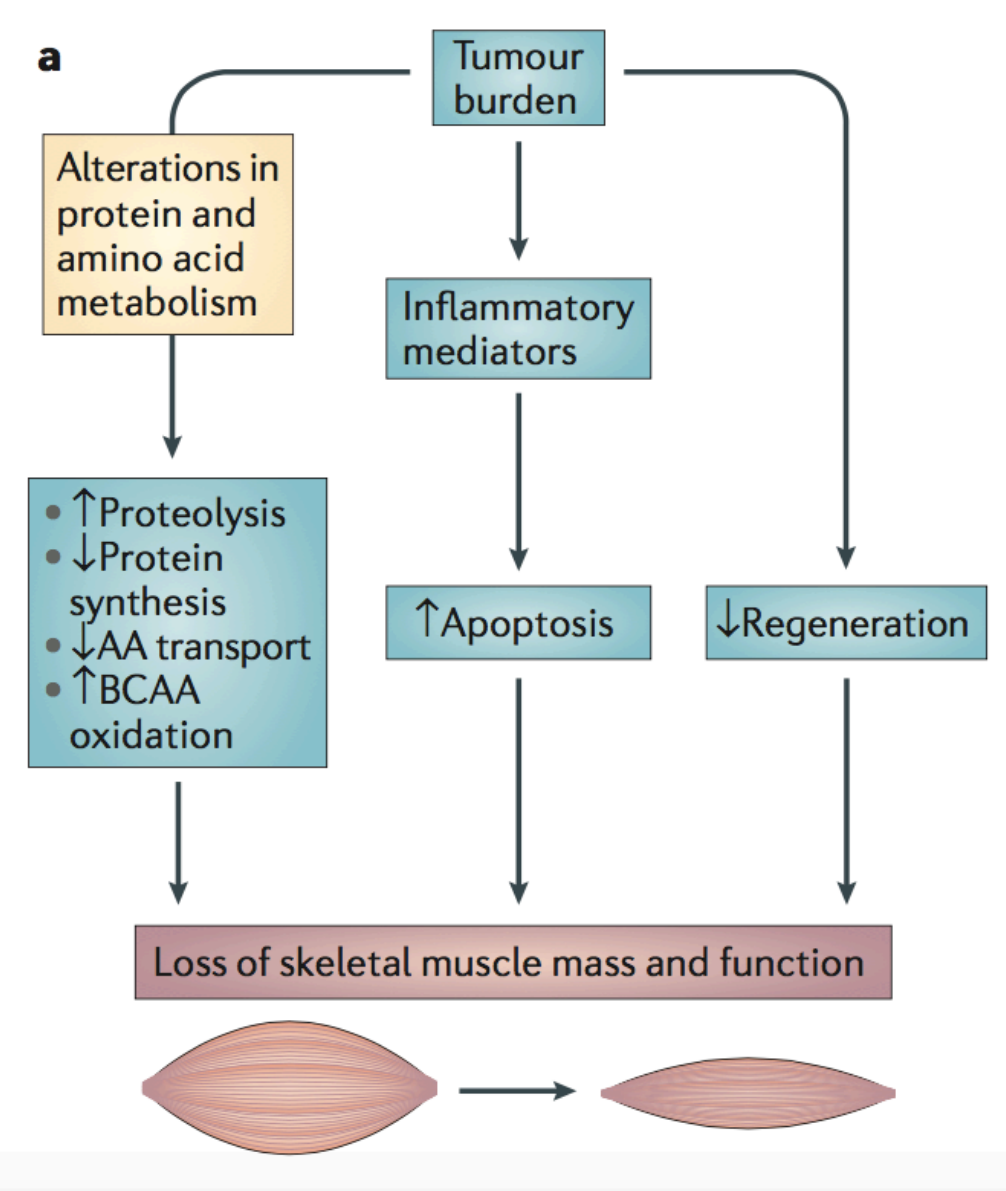
Source: Nat Clin Pract Oncol © 2005 Nature Publishing Group



Molecular basis of cachexia

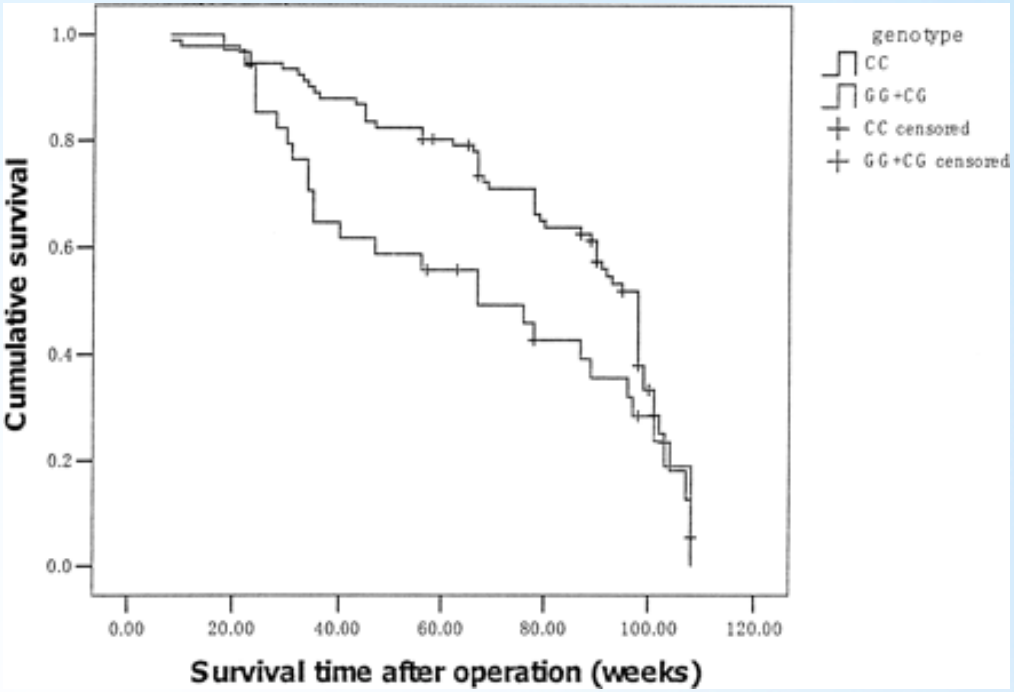
PDAC patients have significantly reduced hemoglobin and albumin, associated to the systemic reaction to both the tumor and the inflammation

Specific pro-inflammatory cytokines such as interleukin-6 (IL-6) have been shown to be associated with progressive weight loss

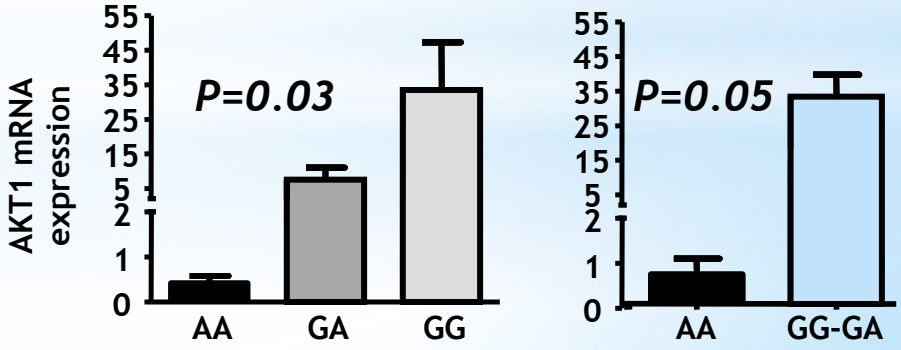


Genetic basis of cachexia

Previous analysis supported the role of the IL6-rs1800796 and SELP-rs6136 SNPs as susceptibility biomarkers for PDAC cachexia [*Zhang et al, 2008; Tan et al, 2011]*

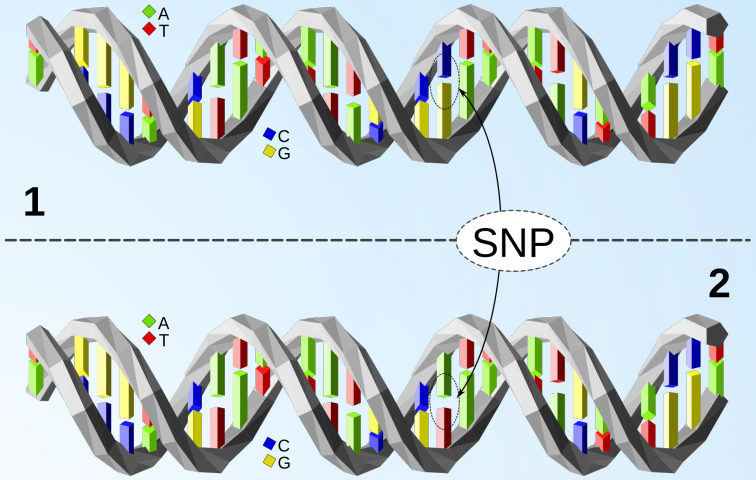


Our recent studies revealed a key functional role of the AKT1-rs1130233 SNP *[Giovannetti et al; MCT 2010; Avan et al.; CCR 2014*]



Aim of the study

To evaluate the association of candidate single nucleotide polymorphisms (SNPs) in the *AKT1*, *IL6*, and *SELP* genes with cachexia



Patients and methods

- 151 patients, enrolled between 31/03/2004 and 10/01/2009 [first cohort]
- 152 patients, enrolled between 15/01/2009 and 15/01/2013 [validation cohort]

All the eligible patients were chemo-naive patients with diagnosis of histologically confirmed locally advanced or metastatic PDAC, treated at the Carrara Civic Hospital

Genomic DNA extracted from blood was analysed for the *rs1800796*, *rs6136*, and *rs1130233* SNPs using Taqman®-based PCRs in the ABIPRISM-7500 instrument

Results: Clinical characteristics and outcome

	First cohort			Second/validation cohort		
Characteristics	**Patients, n (%)**	**OS median mo. (95% CI)**	***p*-value***	**Patients, n (%)**	**OS median mo. (95% CI)**	***p*-value***
No. patients	151	12.5 (10.9–14.1)		152	12.0 (9.9–14.1)	
Age, years						
≤65	92 (60.9%)	13.5 (11.7–15.3)	0.031	100 (65.8%)	11.6 (9.8–13.4)	0.864
>65	59 (39.1%)	10.9 (8.4–13.4)		52 (34.2%)	13.3 (11.4–15.4)	
Sex						
Male	98 (64.9%)	12.3 (10.2–14.3)	0.697	92 (60.5%)	11 (8.2–13.8)	0.044
Female	53 (35.1%)	13.3 (9.5–17.1)		60 (39.5%)	13.7 (10.9–16.5)	
Cachexia						
yes	53 (35.1%)	9.9 (8.4–11.4)	0.0006	59 (38.8%)	9.1 (6.9–11.2)	0.005
no	98 (64.9%)	14.3 (12.4–16.2)		93 (61.2%)	14.2 (12.7–15.7)	

**p-values were calculated with Log-rank test.*
OS: Overall survival; mo, months.

Importantly, cachexia was significantly associated with shorter OS in both cohorts ($p<0.001$ and $p=0.005$, respectively)

Results: SNPs

The allelic frequencies were comparable with those reported in Caucasian populations, in NCBI and NCI-SNP500 databases

No significant correlations were detected between genotype and baseline demographic characteristics

Genotyping of PDAC patients for the candidate *SELP*, *AKT1* and *IL-6* SNPs

	First cohort			Second/validation cohort	
SNP		**Patients n (%)**	**HWE *p*-value**	**Patients n (%)**	**HWE *p*-value**
SELP-rs6136					
	AA	81 (53.6)		76 (50.0)	
	AC	60 (39.7)		66 (43.4)	
	CC	10 (6.6)	0.911	10 (6.6)	0.859
AKT1-rs1130233					
	GG	70 (46.4)		74 (49.3)	
	GA	65 (43.1)		64 (42.7)	
	AA	16 (10.6)	0.980	12 (8.0)	0.972
IL6-rs1800796					
	GG	91 (60.3)		100 (65.8)	
	GC	51 (33.8)		44 (29.0)	
	CC	9 (6.0)	0.923	8 (5.3)	0.527

HWE: Hardy–Weinberg equilibrium

Results: Polymorphisms and cachexia

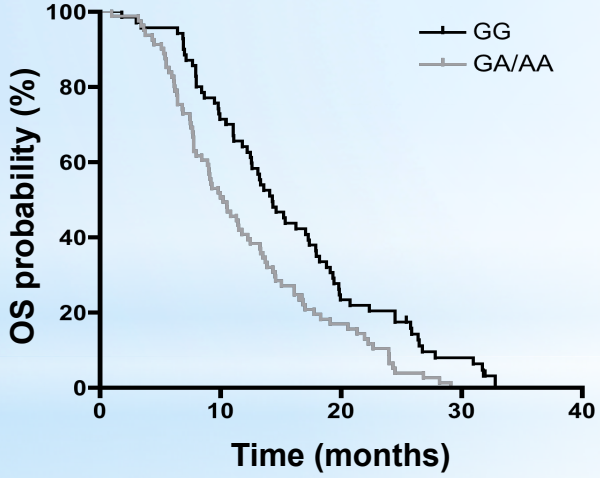
			First cohort		Second cohort	
SNP	**Cachexia**	**Genotype**	**Patients *n* (%)**	***p* value***	**Patients *n* (%)**	***p* value***
SELP rs6136	**Yes**	**AA**	36 (23.8%)	0.011	36 (23.8%)	0.045
		AC/CC	17 (11.2%)		23 (15.1%)	
	No	**AA**	45 (29.8%)		40 (26.3%)	
		AC/CC	53 (35.2%)		53 (34.8%)	
AKT1 rs1130233	**Yes**	**GG**	16 (10.1%)	0.004	21 (13.8%)	0.019
		GA/AA	37 (24.5%)		36 (23.7%)	
	No	**GG**	54 (35.7%)		53 (34.9%)	
		GA/AA	44 (29.7%)		40 (27.6%)	
IL-6 rs1800796	**Yes**	**GG**	17 (11.2%)	0.169	18 (11.8%)	0.486
		GC/CC	36 (23.4%)		41 (27%)	
	No	**GG**	43 (28.5%)		34 (22.4%)	
		GC/CC	55 (36.9%)		59 (38.8%)	

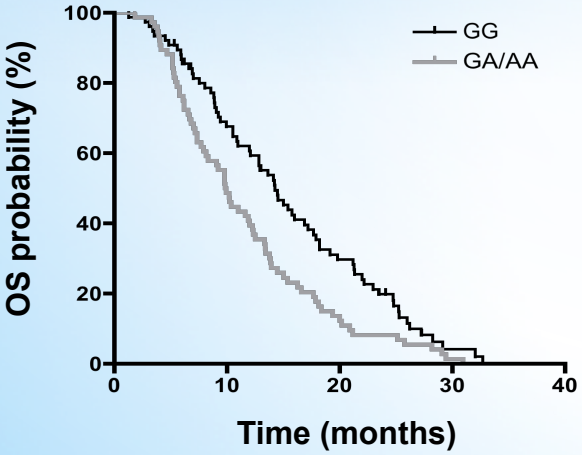
**p-values were calculated with Fisher's exact test.*
SNPs, single nucleotide polymorphisms.
Note: *SELP* and *IL6* SNPs was detectable in all the samples, while the *AKT1* genotype could not be determined in 2 of the patients of the second cohort.

A significantly higher proportion of patients harboring at least one A-AKT1-rs1130233 SNP has cachexia
Similarly, 44% of the patients with the SELP-rs6136-AA variant experienced cachexia, compared to 24% of the patients harboring the SELP-rs6136-AC/CC

Results: Polymorphisms and outcome

- AKT1-rs1130233 was associated with significantly differential OS in both cohorts
- The GA/AA genotype emerged as a significant predictor for shorter survival in the multivariate analysis





Factors associated with overall survival in the multivariate analysis

		First cohort			Second/validation cohort		
Covariates for OS		**HR (95%CI)**	**df**	***p*-value**	**HR (95%CI)**	**df**	***p*-value**
Age, years	**≤65**	1 (ref)	1	0.032	1 (ref)	1	0.864
	>65	1.4 (1.0-2.0)			1.0 (0.7-1.4)		
Sex	**Male**	1.1 (0.8-1.5)	1	0.698	1.4 (1.0-1.9)	1	0.046
	Female	1 (ref)			1 (ref)		
Cachexia	**Yes**	2.2 (1.6-3.2)	1	<0.001	1.6 (1.1-2.3)	1	0.006
	No	1 (ref)			1 (ref)		
AKT1 rs1130233	***GG***	1 (ref)	1	0.002	1 (ref)	1	0.004
	GA/AA	1.7 (1.2-2.4)			1.6 (1.2-2.3)		

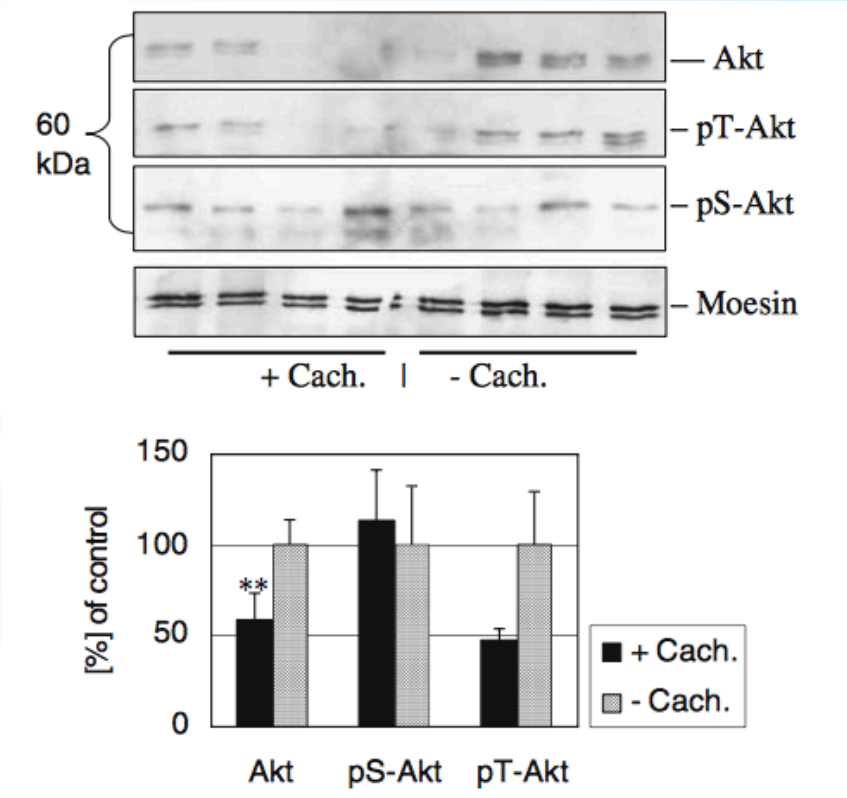
df: degrees of freedom; HR: hazard ratio; OS: overall survival

Our hypothesis on the role of Akt1 in cachexia

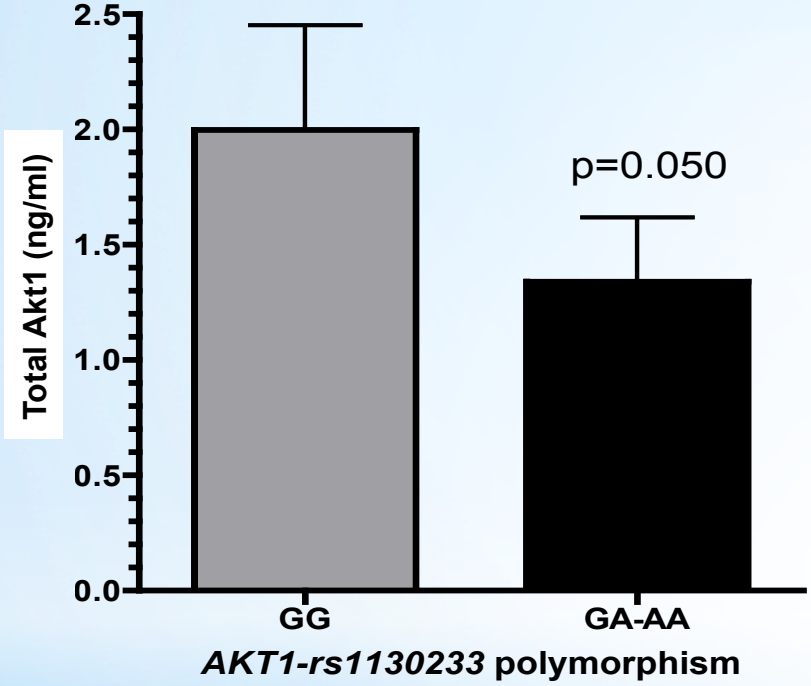
Akt1 is a serine/threonine kinase acting as a critical mediator of growth factor-induced survival

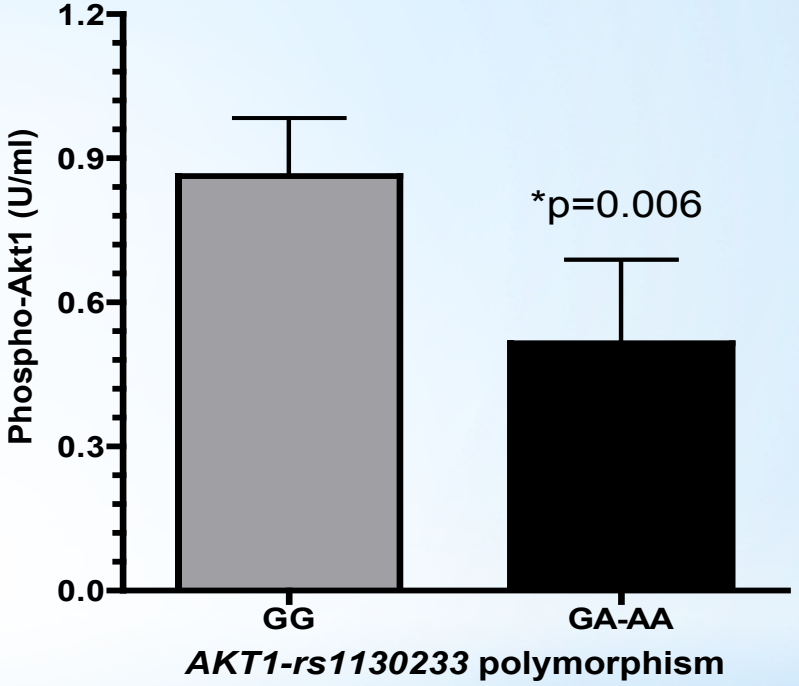
In skeletal muscle, Akt1 plays a very central role in the control of both muscle protein synthesis, via mTOR, and protein degradation, via the transcription factors of the FoxO family

Schmitt and colleagues demonstrated a cachexia-associated loss of Akt-dependent signaling in human skeletal muscle of cachectic compared to non-cachectic patients, using biopsies from 16 PDAC patients [J Mol Med 2007]



AKT1-rs1130233 and expression of Akt1 and phospho-Akt1 in skeletal muscle





AKT1-rs1130233-GA/AA correlated with reduced phospho-Akt1

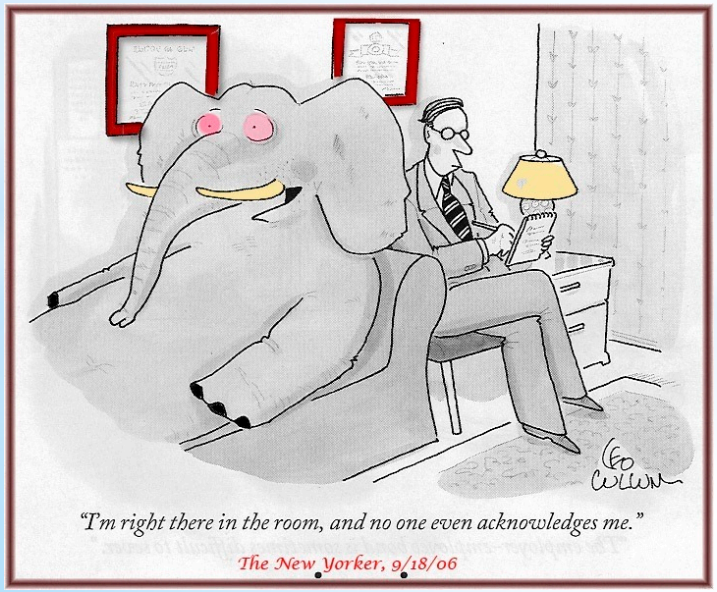
These genotypes might confer a reduced activity to Akt1, and thus reduce the antiapoptotic activity of this pivotal regulator of apoptotic signaling

Conclusions

AKT1-rs1130233 and *SELP-rs6136* polymorphisms emerged as a predictive risk factor of developing cachexia in locally-advanced and metastatic PDAC

AKT1 polymorphisms may also play a prognostic role

Validation of the value of the emerging candidate polymorphisms in future prospective trials might offer new tools to improve the clinical management of advanced PDAC patients



Acknowledgments

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