MARKERS OF PROGRESSION RISK IN PATIENTS WITH SQUAMOUS CELL VULVAR CARCINOMA

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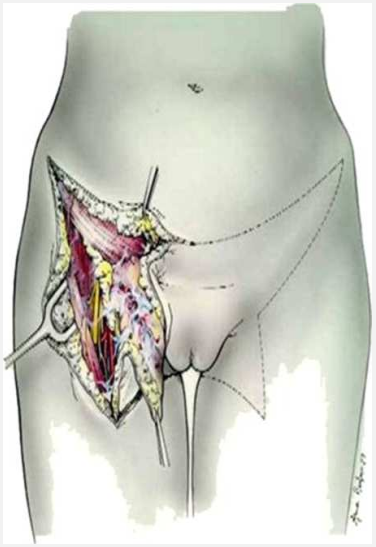
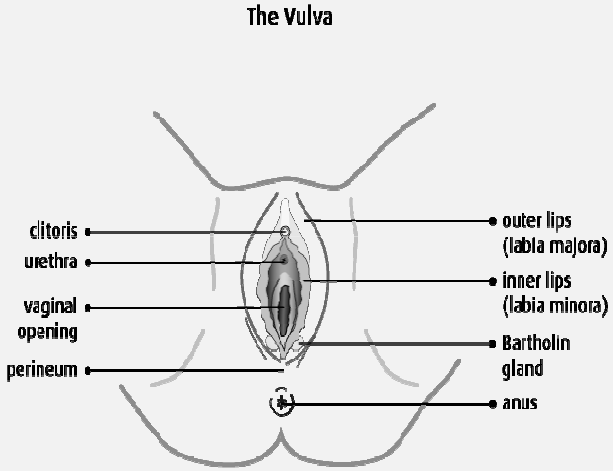
Vulvar Carcinoma

- Age-standardized incidence rate (ASR) - approx. 1 per 100,000
- 489 new cases and 315 VC-related deaths in Poland in 2015 (http://onkologia.org.pl/)
- The most common type - squamous cell carcinoma – 90% of patients
- VIN

  HSIL dVIN

  HPV+ VC HPV- VC





Canadian Cancer Society, http://www.cancer.ca/en/cancer-information/cancer-type/vulvar/vulvar-cancer/

- **Staging**
- **Margin size (AGO-CaRE-1, a large retrospective study)**
- **RTH**
- **Regional LN status – the only prognostic factor?**

Protein markers of VC progression

- to identify protein markers of VC that are indicative of a tumor that is more likely to progress
  - proteins that are differentially expressed in tumors of VC patients that progressed during ~10 years of follow-up period ("progVC")

vs those

who are disease-free at the time of last observation ("d-fVC").

Protein markers of VC progression

Materials & Methods

Patients

16 patients d-fVC

12 patients with progVC

14 control samples (normal vulvar tissue)

Method

iTRAQ (Isobaric tag for relative and absolute quantitation)

Results - iTRAQ

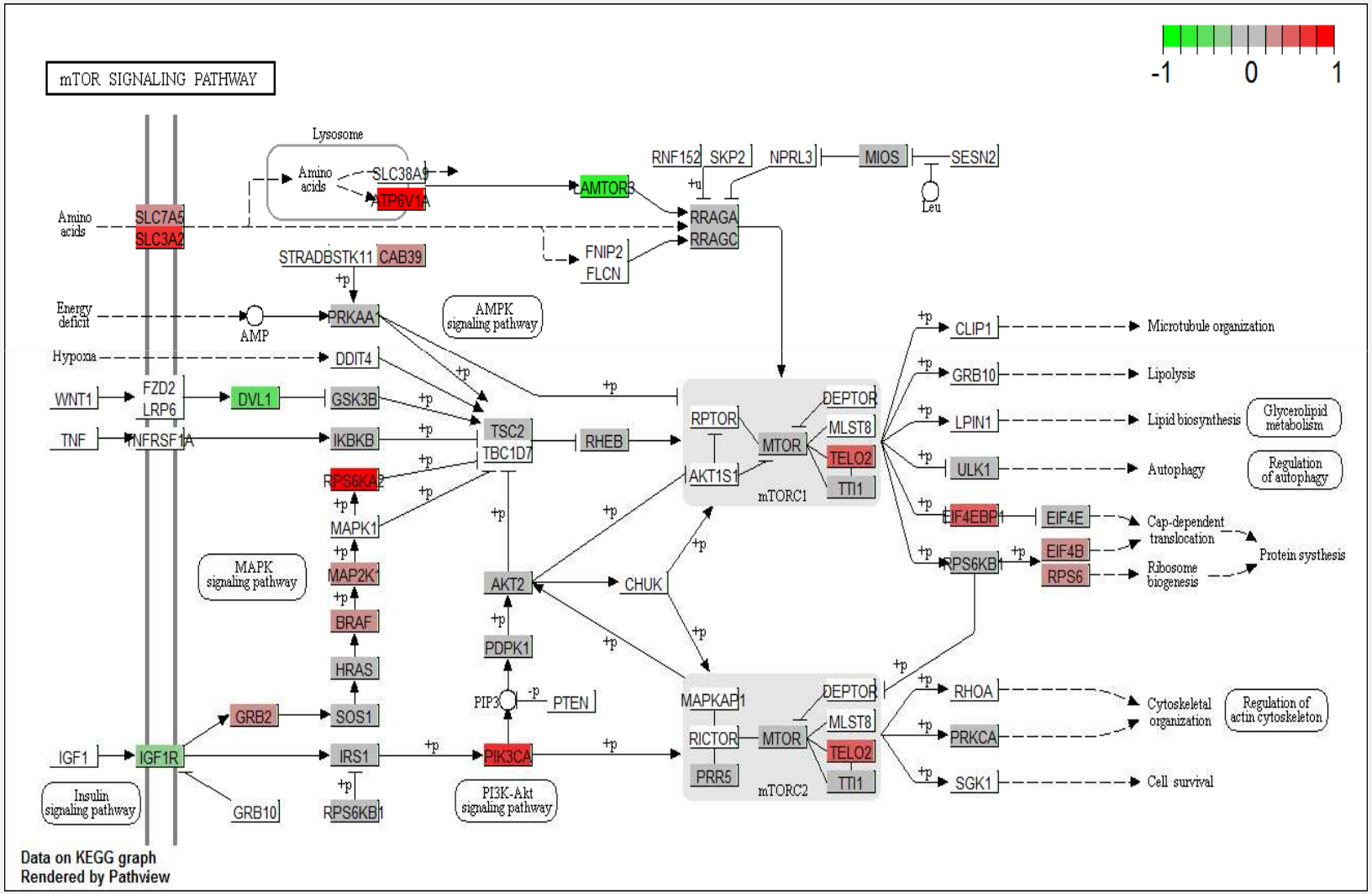
- 5510 proteins
- GO - VSCC *vs* control:

GO name	Terms	pvals	adjp	exp
GO:0051234	establishment of localization	0,000204	0,015108	199,2
GO:0048522	positive regulation of cellular process	0,000161	0,013502	157,3
GO:0007275	multicellular organism development	3,2E-06	0,000937	139,4
GO:0032502	developmental process	1,33E-05	0,002496	139,2
GO:0050896	response to stimulus	4,46E-07	0,000163	122,3
GO:1902578	single-organism localization	1,66E-08	1,75E-05	118,6
GO:0010646	regulation of cell communication	4,12E-05	0,005746	95,5
GO:0023051	regulation of signaling	8,8E-05	0,009377	94,3
GO:0016192	vesicle-mediated transport	0,000358	0,023101	91,8
GO:0050790	regulation of catalytic activity	0,000107	0,01025	84,9
GO:0032879	regulation of localization	0,000529	0,029662	83,7
GO:0065008	regulation of biological quality	9,32E-06	0,002025	77,5

Abb … itially expressed … value; Pa -

Results

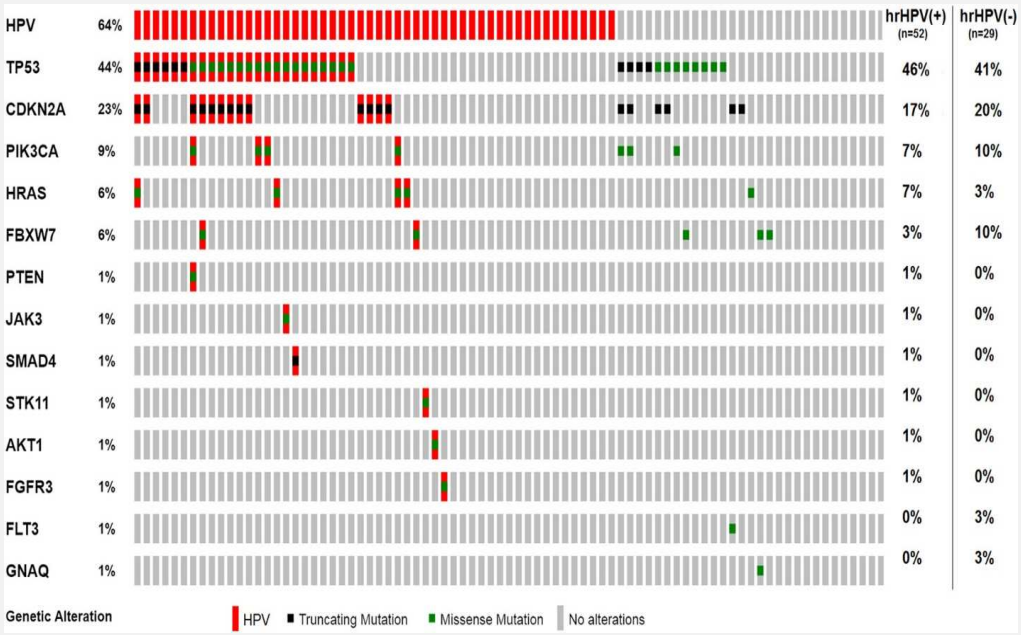
- 5510 proteins
- KEGG - VSCC *vs* control:

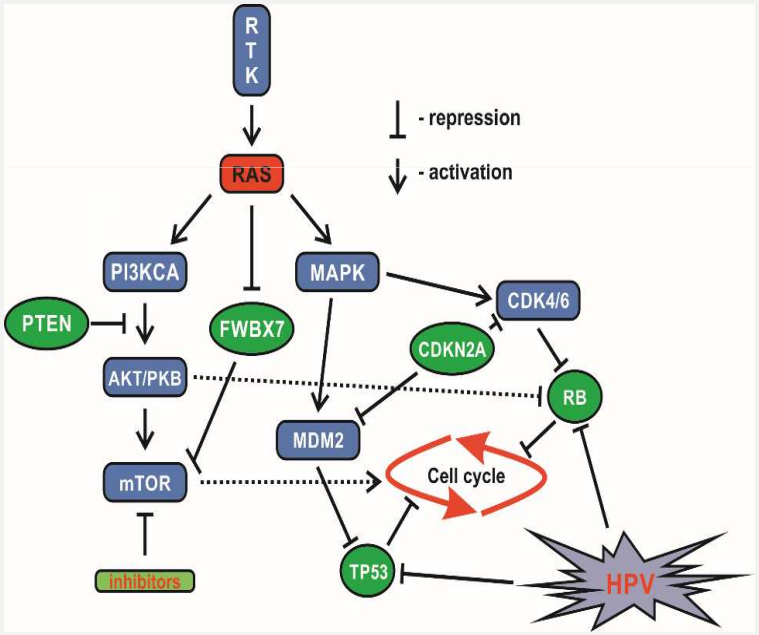


KEGG: Kyoto Encyclopedia of Genes and Genomes

Mutation profiling

- 83 VSCC
- 65% HPV(+) and 59% HPV(-): pathogenic mutations

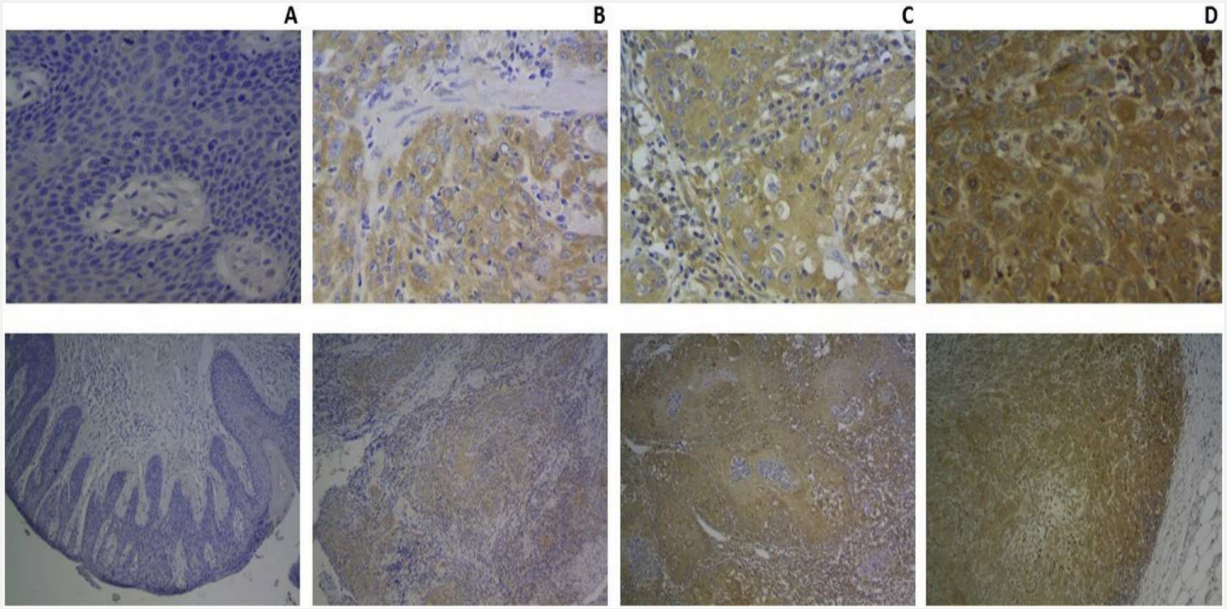




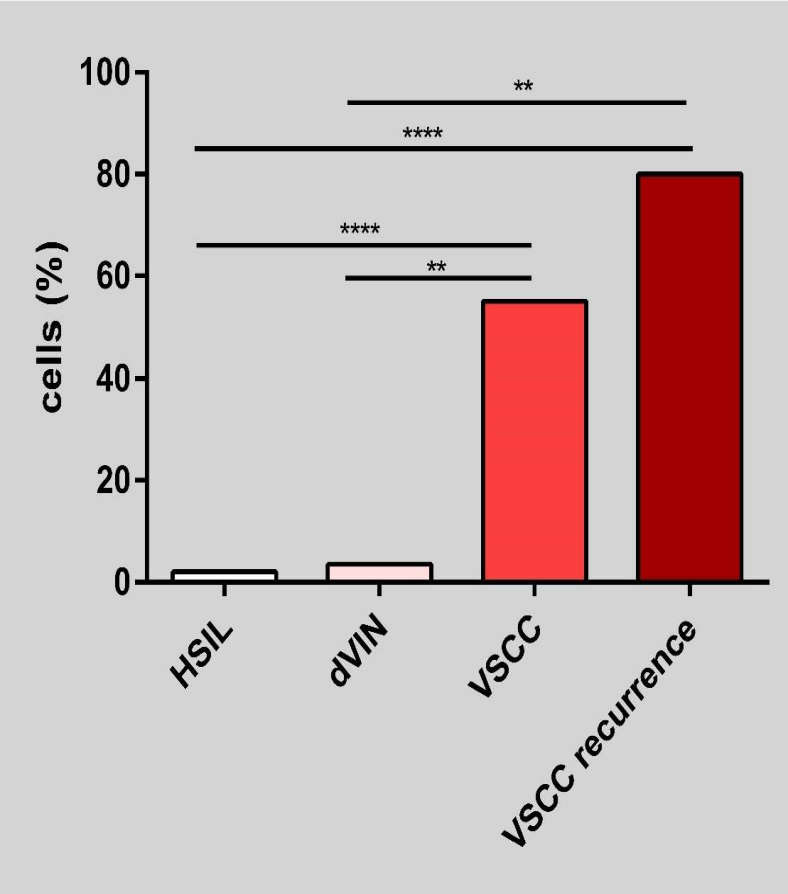
Zięba S et al., Gynecol Oncol. 2018;150(3):552-561.

mTOR expression

- IHC
- HSIL; dVIN; VSCC; recurrVC



HSIL d-FVC progVC recurrVC



anti-mTOR rabbit mAb
(7C10, Cell signaling)

Zięba S et al., Gynecol Oncol. 2018;150:552-561.

Results - iTRAQ

- 5510 proteins
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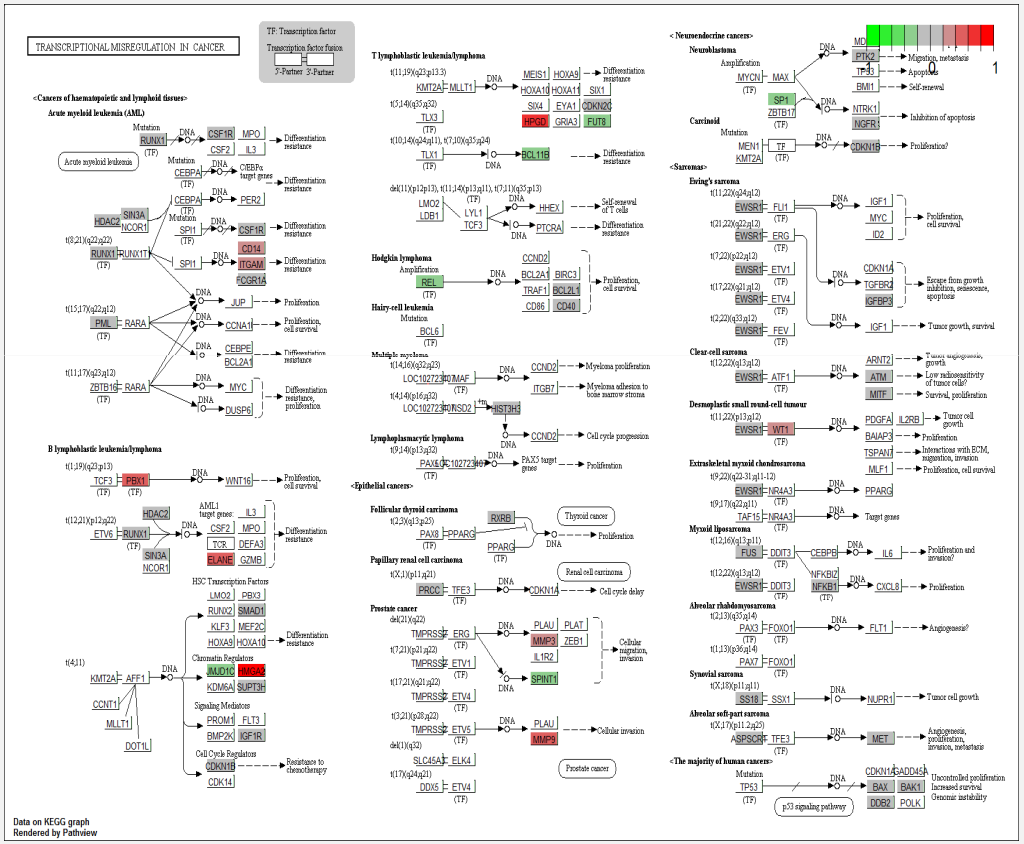
GO name	Term	P	Pa	exp
GO:1902578	single-organism localization	4,14E-06	0,001234	121,5
GO:0002252	immune effector process	0,000303	0,041061	54,7
GO:0009605	response to external stimulus	0,00022	0,032709	52,5
GO:0002684	positive regulation of immune system process	0,000326	0,041263	39,0
GO:0002253	activation of immune response	0,000119	0,021648	26,6
GO:0050878	regulation of body fluid levels	2,67E-10	5,03E-07	18,7
GO:0009611	response to wounding	0,000212	0,032436	16,8
GO:0002460	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	7,43E-07	0,000301	8,8
GO:0002449	lymphocyte mediated immunity	4,53E-07	0,000197	8,6
GO:0002576	platelet degranulation	8,45E-07	0,000302	8,3
GO:0016485	protein processing	9,66E-06	0,002486	8,2

Results

- 5510 proteins
- KEGG - d-fVC *vs* progVC:



KEGG: Kyoto Encyclopedia of Genes and Genomes

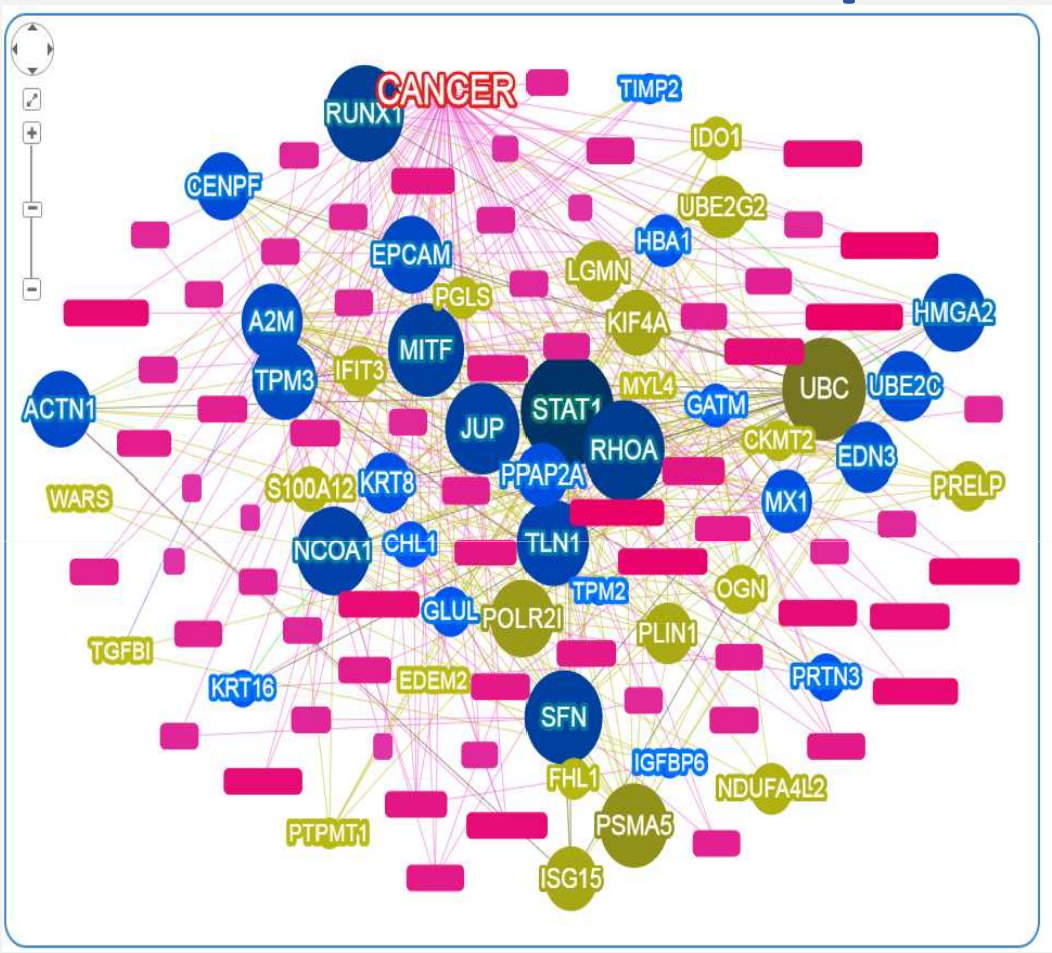
High Mobility Group AT-Hook 2

Tissue	Cancer staining	Protein expression of normal tissue	Tissue	Cancer staining	Protein expression of normal tissue
Breast cancer			Melanoma		
Carcinoid			Ovarian cancer		
Cervical cancer			Pancreatic cancer		
Colorectal cancer			Prostate cancer		
Endometrial cancer			Renal cancer		
Glioma			Skin cancer		
Head and neck cancer			Stomach cancer		
Liver cancer			Testis cancer		
Lung cancer			Thyroid cancer		
Lymphoma			Urothelial cancer		

http://www.proteinatlas.org/

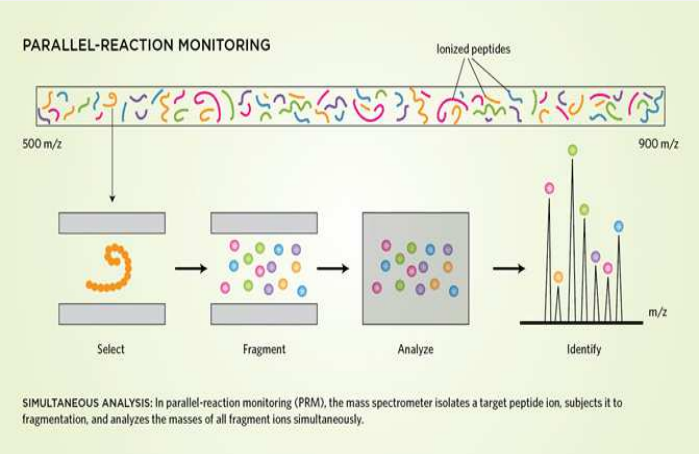
HMGA2 expression pattern and TERT mutations in tumors of the vulva. Oncol Rep. 2015; 33:2675-80.

Validation – 47 proteins



Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. Nat Methods. 2015;12:841-3.

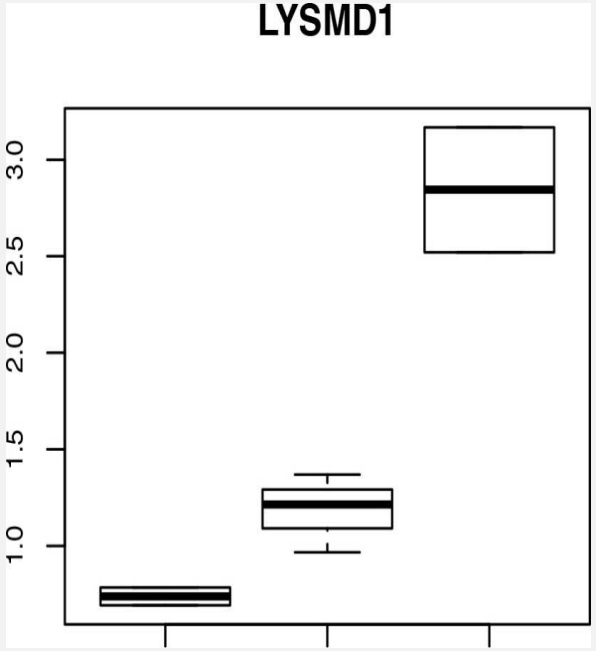
PRM

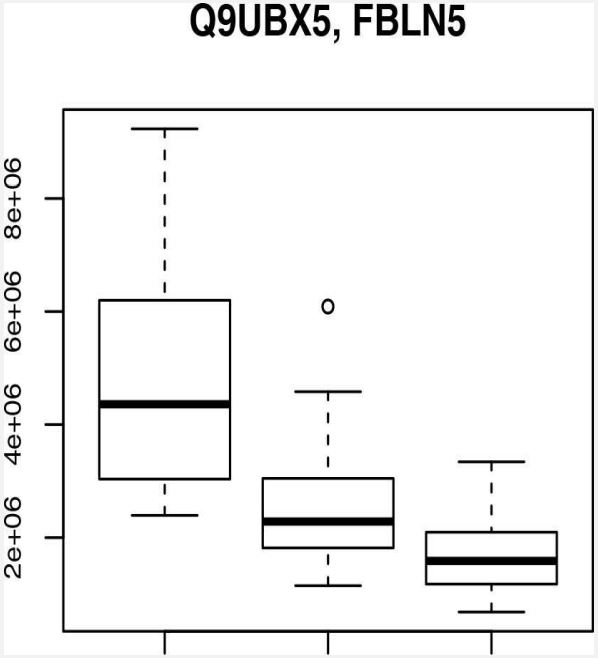


http://www.the-scientist.com/?articles.view/articleNo/40051/title/Moving-Target/

PRM - validation

VIN → early VSCC → late VSCC → recurrence





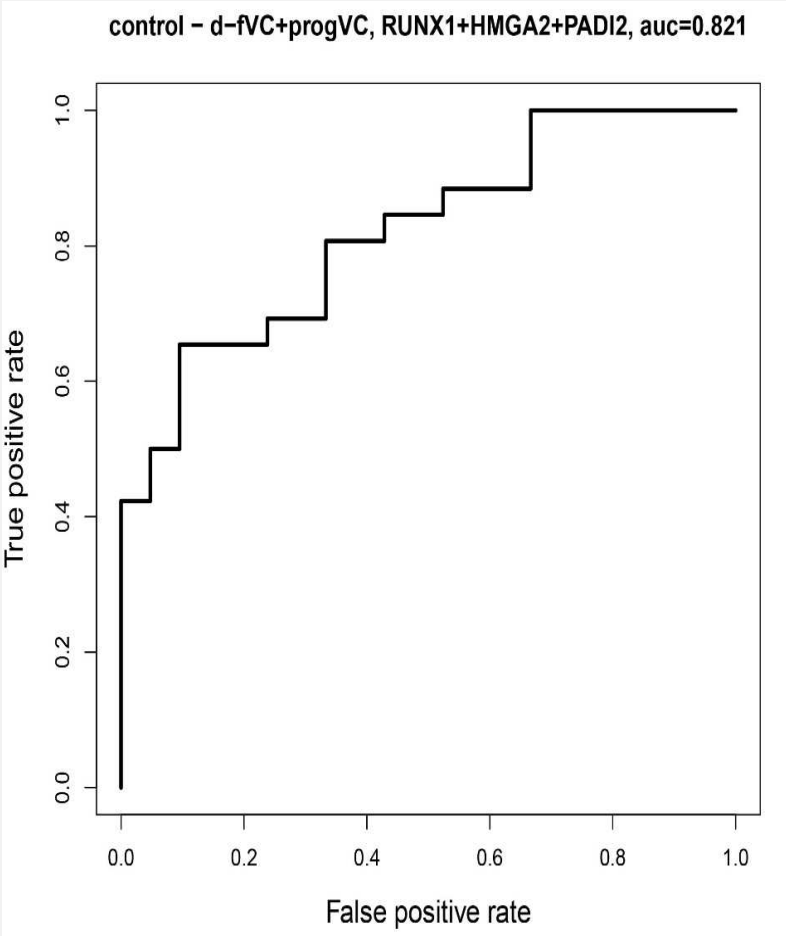
PRM - validation

- 96 samples
- d-fVC; progVC; normal tissue; prosp; VIN

protein	pVal.wilcox	dfVSCC-progVSCC	AUC	pVal.wilcox	control-VSCC	AUC
HMGA2	**0,006**	**14,4**	**0,73**	0,006	45,5	0,76
ANO1	**0,007**	**3,4**	**0,73**	ns	1,8	0,55
PRTN3	**0,012**	**2,7**	**0,71**	0,000	13,3	0,92
UBE2C	**0,007**	**2,7**	**0,73**	0,000	8,9	0,87
KRT18	**0,022**	**2,5**	**0,70**	0,000	8,2	0,91
S100A12	**0,016**	**2,4**	**0,71**	0,000	12,0	0,92
PTX3	**0,036**	**2,3**	**0,68**	ns	2,7	0,68
RUNX1	**0,019**	**2,1**	**0,70**	0,000	6,7	0,90
PADI2	**0,019**	**1,9**	**0,70**	0,000	3,7	0,88
ABI3BP	**0,023**	**0,7**	**0,69**	0,000	0,2	0,92
PRELP	**0,016**	**0,7**	**0,71**	0,000	0,3	0,93

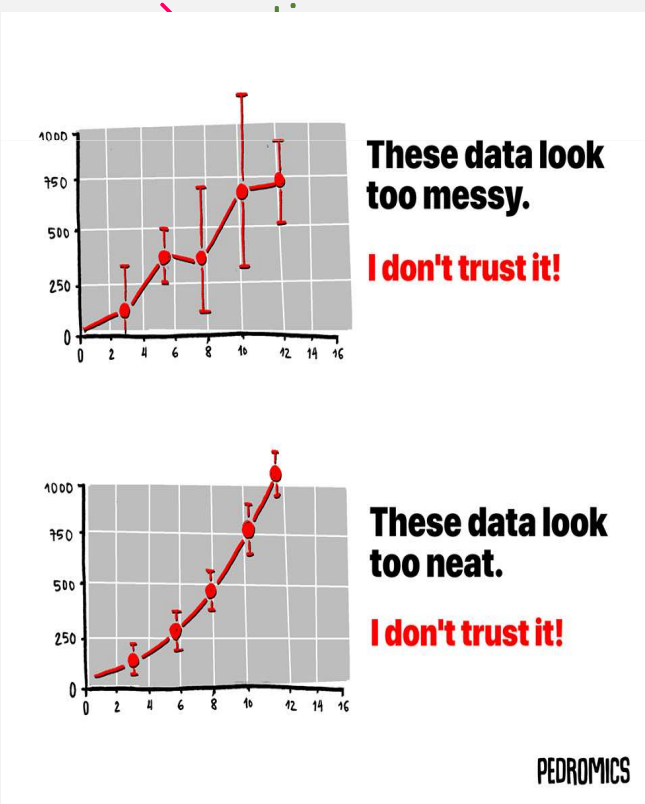
PRM - IHC

- 96 samples
- normal tissue; VIN; d-fVC; progVC; prosp

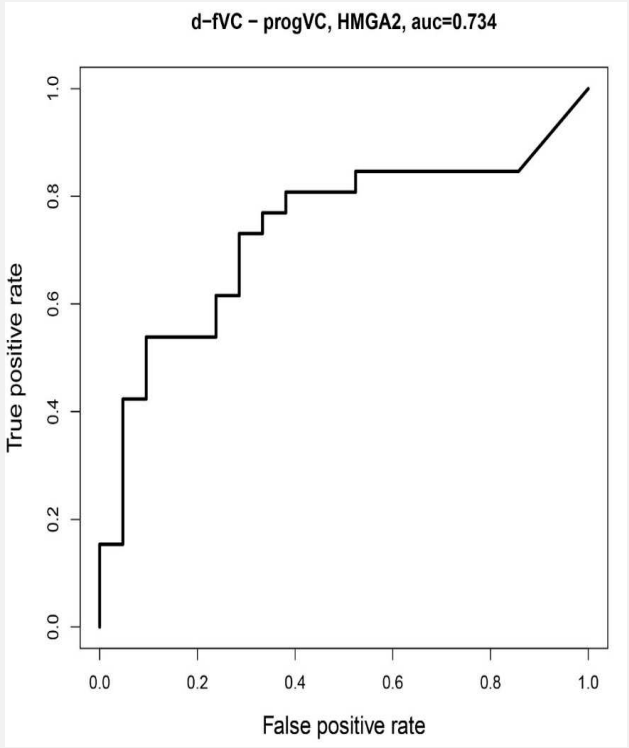


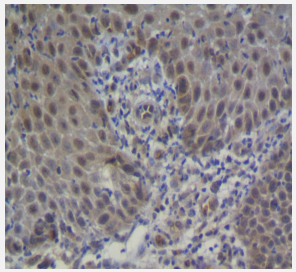
90 VSCC, 43 LN, 50 VIN

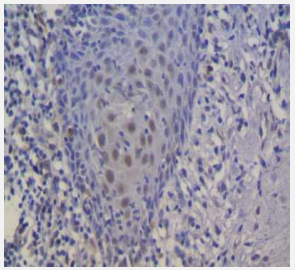
→ visual assesment



PRM – HMGA2 validation by IHC

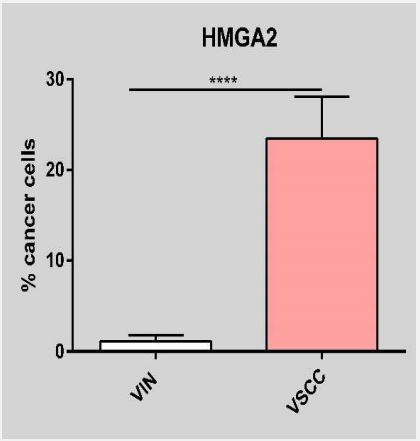


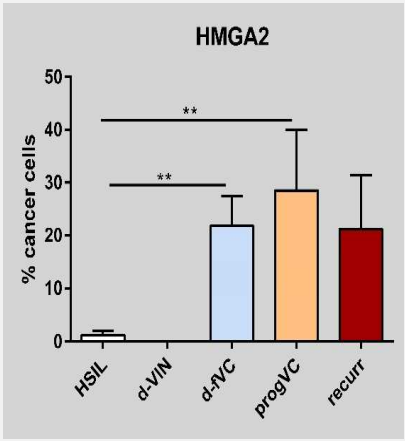


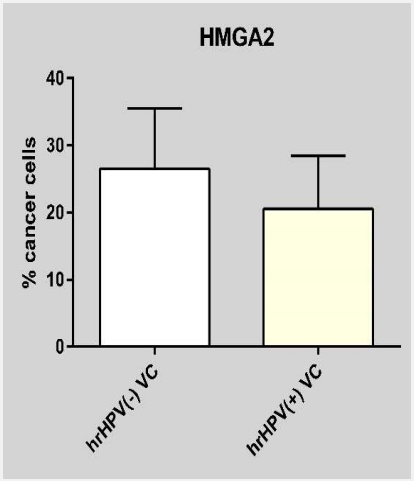


anti-HMGA2 (GenWay Biotech, Inc)

- 96 IHC
- VIN; d-fVC; progVC;







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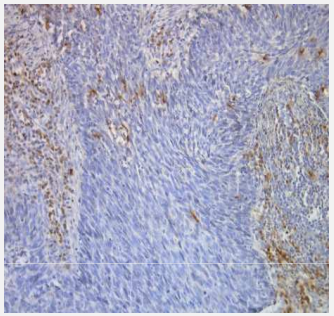

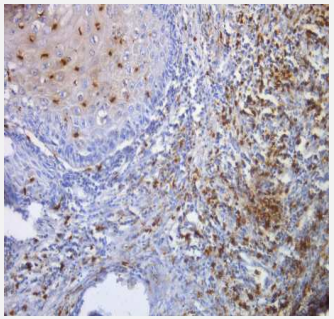

monoclonal anti-PR3 (PR3-G2, ThermoFisher Scientific)

Results - iTRAQ

- 5510 proteins
- GO - progVC *vs* d-fVC:

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GO:0016485	protein processing	9,66E-06	0,002486	8,2

Results - iTRAQ

names	path	pvals	adjp	count	exp
4610	Complement and coagulation cascades	4,97E-19	8,9E-17	31	5,227635
5150	Staphylococcus aureus infection	5,67	0,00038		
5020	Prion diseases	6,5			
5322	Systemic lupus erythematosus	5,33			
3320	PPAR signaling pathway	0,0			
4973	Carbohydrate digestion and absorption	0,02			
5142	Chagas disease (American trypanosomiasis)	0,04			
750	Vitamin B6 metabolism	0,05			
512	Mucin type O-Glycan biosynthesis	0,05			
5146	Amoebiasis	0,05			

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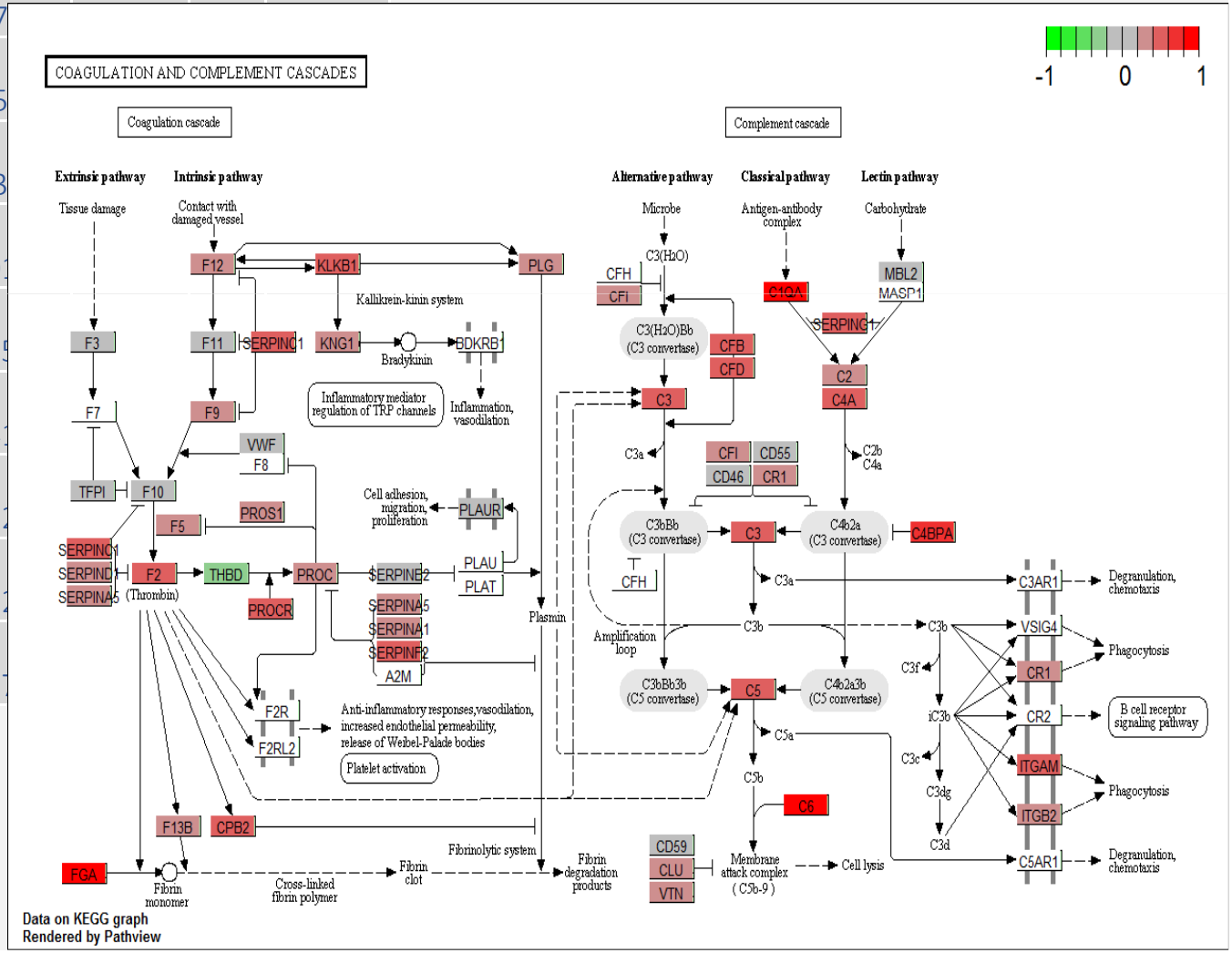
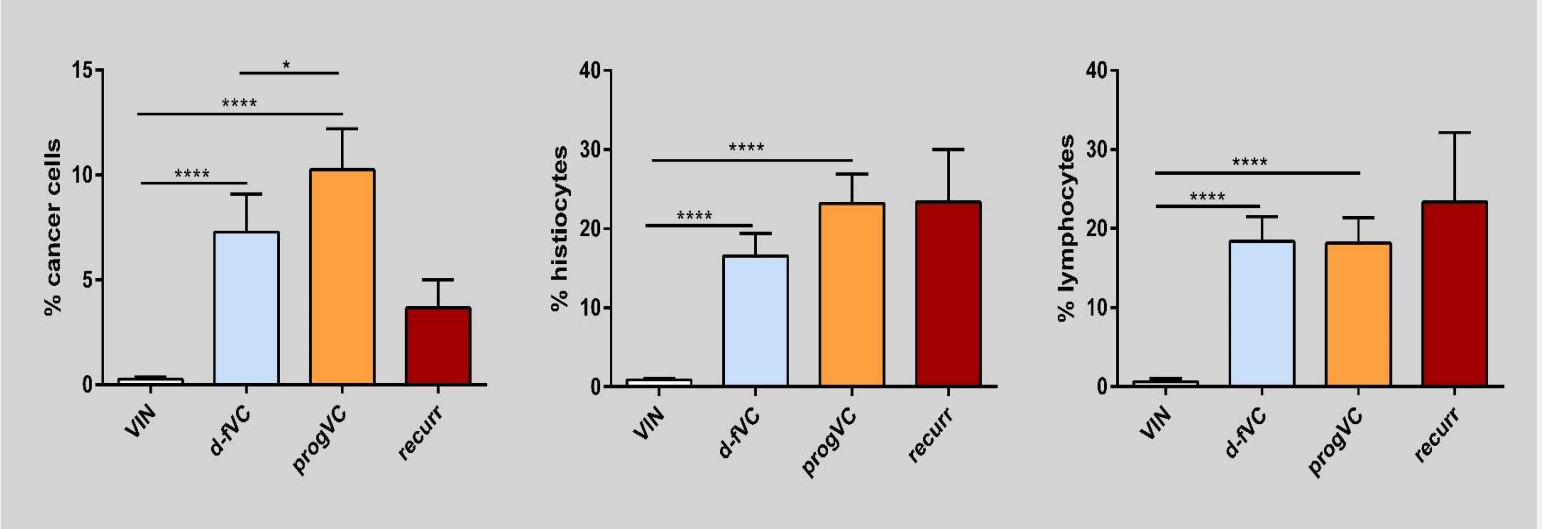
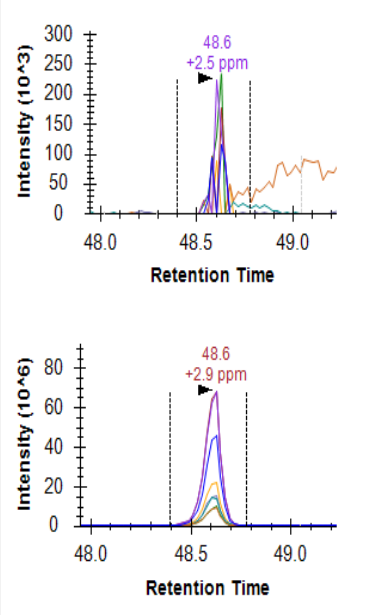
- KEGG - progVC *vs* d-fVC:



PRM – PRTN3 validation by IHC & PRM



45 VIN
35 d-fVC
31 progVC
3 recurrVC

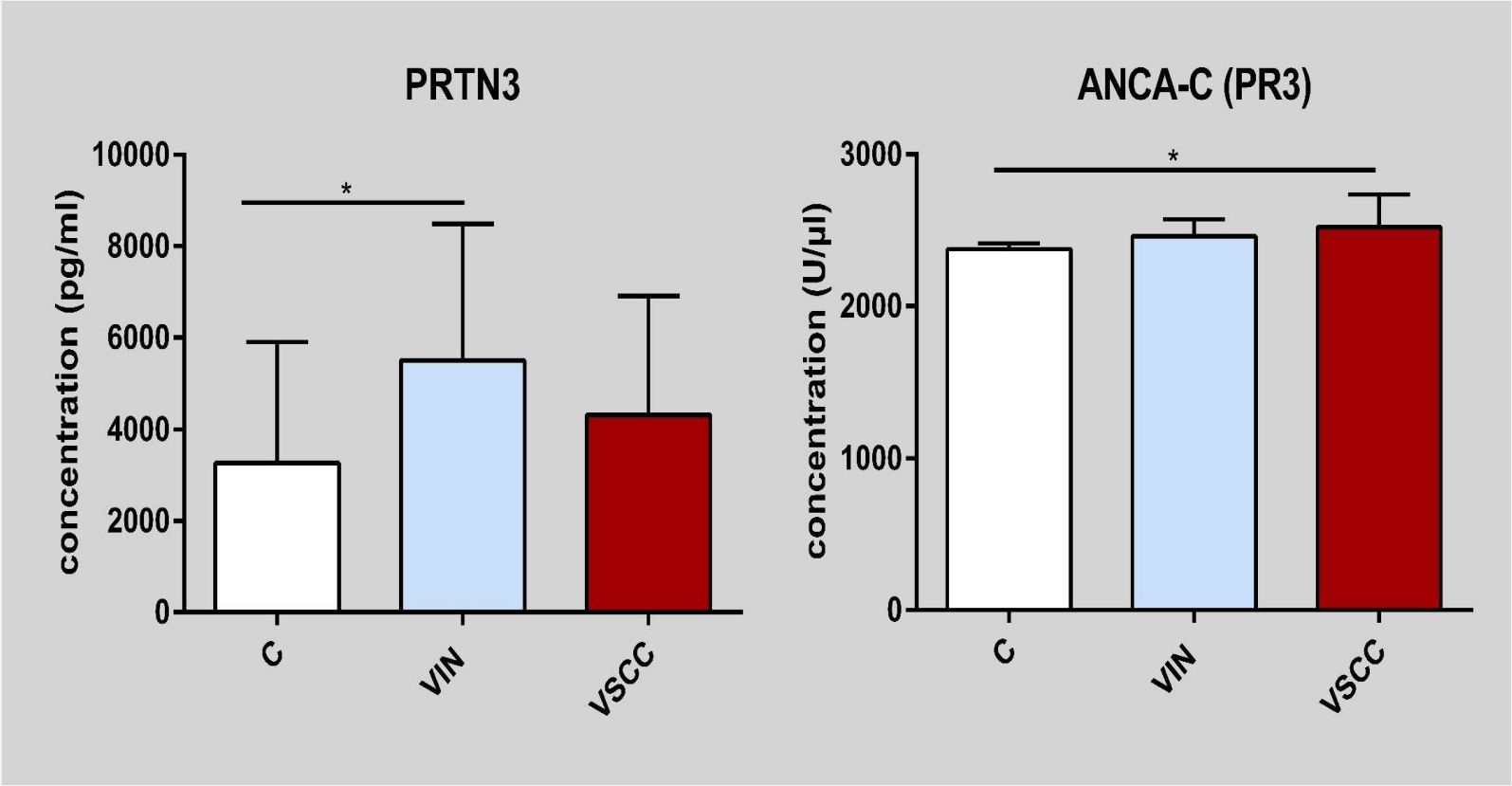


- 16 plasma samples
- 3 peptides

↓

- Possible signal but in noise
- Can't quantitate

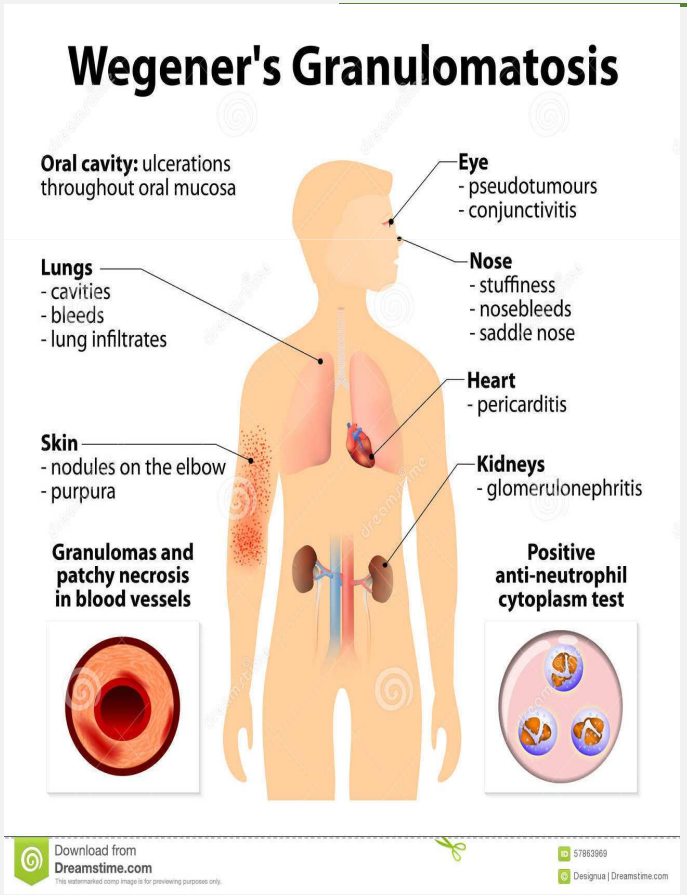
serum PRTN3 levels



14 C
22 VIN
51 VC

PRTN3

- serine protease
- enhances endothelial cell barrier function and thus vascular integrity
- during neutrophil transendothelial migration
- Granulomatosis With Polyangiitis and Vasculitis



Cancer Epidemiol Biomarkers Prev. 2009 Feb;18(2):472-8. doi: 10.1158/1055-9965.EPI-08-0905. Epub 2009 Jan 20.

Staphylococcus aureus and squamous cell carcinoma of the skin.

Kullander J[1], Forslund O, Dillner J.

Author information

Eur J Obstet Gynecol Reprod Biol. 2013 Nov;171(1):138-42. doi: 10.1016/j.ejogrb.2013.08.023. Epub 2013 Aug 19.

Can preoperative neutrophil:lymphocyte and platelet:lymphocyte ratios be used as predictive markers for lymph node metastasis in squamous cell carcinoma of the vulva?

Ertas IE[1], Gungorduk K, Akman L, Ozdemir A, Terek MC, Ozsaran A, Sanci M, Dikmen Y.

Conclusions

- inflammation in VC progression

- clinical relevance:
  - personalising VC treatment

Acknowledgements

D. Domański, A. Fatalska, J. Olędzki Mass Spectrometry Laboratory, IBB PAS, Warsaw

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