Podocalyxin-like protein as a prognostic marker in colorectal cancer: Functional studies and clinical implication

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# Podocalyxin-like protein (PODXL)



- A cell surface adhesion molecule; member of the CD34 subfamily of sialomucins
- Expressed in endothelial venules
- Expressed in developing hematopoetic stem cells
- First identified in the kidney; helps form a highly negatively charged cell surface coat that maintains the filtration slit in the glomerulus

# **PODXL** expression in cancer

Link to more aggressive forms of:

- Breast cancer (Somasiri, 2004)\*
- Blasts in acute leukemia (Kelley, 2005)
- Liver cancer (Heukamp et al., 2006)
- Astrocytoma (Hayatsu, 2008)
- Pancreatic cancer (Dallas, 2012)
- \* correlation with survival (n= 272)

High podocalyxin expression is associated with decreased disease-specific survival.





## PODXL expression in testicular cancer

"Human embryonal carcinoma tumor antigen, Gp200/GCTM-2, is podocalyxin". Schopperle et al., 2003 "This is the first report of podocalyxin expression on malignant cells."





Embryonal carcinoma

Seminoma





www.bjcancer.com

Overexpression of podocalyxin-like protein is an independent factor of poor prognosis in colorectal cancer

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## Negative staining



## Weak staining



## Moderate ("fluffy") staining











## Correlation with clinicopathological parameters

T-stage <sup>x</sup>				
1	32(12.5)	13(6.8)	1(1.4)	<0.001**
2	36(14.1)	21(11.0)	7(10.0)	
3	157(61.6)	130(68.1)	40(57.1)	
4	30(11.8)	27(14.1)	22(31.4)	
missing	13	5	2	
N-Stage <sup>x</sup>				
0	157(64.6)	103(56.9)	26(37.7)	<0.001**
1	53(21.8)	48(26.5)	21(30.4)	
2	33(13.6)	30(16.6)	22(31.9)	
missing	25	15	3	
M stage <sup>x</sup>				
0	228(86.4)	157(80.9)	52(73.2)	0.009**
1	36(13.6)	37(19.1)	19(26.8)	
missing	4	2	1	
Differentiation grade				
High	21(8.0)	12(6.3)	1(1.4)	<0.001**
Intermediate	202(76.8)	140(72.9)	31(43.7)	
Low	40(15.2)	40(20.8)	39(54.9)	
missing	5	4	1	
Vascular invasion				
No	85(55.2)	52(45.6)	15(33.3)	0.008**
Yes	69(44.8)	62(54.4)	30(66.7)	
missing	114	82	27	

# Cancer specific and overall survival according to PODXL expression



	Colorectal cancer specific survival			5-year overall survival		
	HR(95%CI)	p-value	n(events)	HR(95%CI)	p-value	n(events)
		Univariate			Univariate	
PODXL low PODXL high	1.00 1.98(1.38-2.84)	<0.001	464(147) 72(37)	1.00 1.85(1.29-2.64)	0,001	464(153) 72(37)
PODXL low PODXL high	1.00 1.57(0.99-2.18)	<i>Multivariate</i> 0.055	417(126) <i>68(34)</i>	1.00 1.52(1.03-2.25)	<i>Multivariate</i> 0.036	417(126) 68(34)

## Prognostic value in Stage II disease?

 In patients with stage II disease (n=205), high PODXL expression was associated with a significantly shorter OS (HR.3.03; 95% CI 1.45– 6.34) and 5-year OS (HR.2.83; 95% CI 1.14– 7.16)

# Survival in strata according to PODXL expression and adjuvant chemotherapy in curatively treated Stage III patients



# Conclusions

- PODXL expression is an independent predictor of poor prognosis in colorectal cancer.
- Colorectal cancer patients with PODXL high tumours benefit from conventional chemotherapy
- Useful marker for prognostic stratification of patients with Stage II-III disease?



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#### Validation of podocalyxin-like protein as a biomarker of poor prognosis in colorectal cancer



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# Two independent cohorts

## Skåne University Hospital Malmö

- N=270
- 1990-91; consecutive
- Overall survival
- PODXL evaluated in 260 cases

## **Prospective cohort from Västerås**

- N= 320
- 2000-2003
- Recurrence free, diseasefree survival
- PODXL evaluated in 316 cases
- Fresh frozen tumour tissue available from ≈ 220 tumours

Table 1 Association between PODXL protein expression and clinicopathological parameters							
	Protein expression cohort 1			Protein expression cohort 2			
n (%)	low 235(90.4)	high 25(9.6)	p-value	low 291(92.1)	high 25(7.9)	p-value	
Age							
<=75	130(55.3)	16(64.0)	0.406	166(57.0)	137(69.9)	0.625	
>75	105(44.7)	9(36.0)		125(43.0)	59(30.1)		
Gender							
Female	116(52.2)	14(65.2)	0.528	143(49.1)	13(52.0)	0.784	
Male	119(47.8)	11(34.8)		148(50.9)	12(48.0)		
T stage							
1, 2	78(33.2)	8(32.0)	0.979	52(17.9)	1(4.0)	0.017	
3	124(52.8)	14(56.0)		193(66.3)	15(60.0)		
4	28(11.9)	3(12.0)		46(15.8)	9(36.0)		
Missing	5(2.1)						
N stage							
0	154(65.5)	9(36.0)	0.005	179(61.7)	5(20.0)	< 0.001	
1	53(22.6)	8(32.0)		57(19.7)	8(32.0)		
2	23(9.8)	7(28.0)		54(18.6)	12(48.0)		
Missing	5 (2.1)	1 (4.00)					
M stage							
0	201(85.5)	19(87.0)	0.150	262(90.0)	14(56.0)	< 0.001	
1	31(13.2)	6(13.0)		29(10.0)	11(44.0)		
Missing	3(1.3)						
Location							
Colon	189(78.3)	22(78.3)	0.376	194(66.7)	16(64.0)	0.057	
Rectum	45(21.7)	3(21.7)		97 (33.3)	9(36.0)		
Missing	1						
Differentiation grad	e						
High-intermediate	181(80.4)	13(78.3)	0.006	233(80.1)	15(60.0)	0.019	
Low	54(19.6)	12(21.7)		58(19.9)	10(40.0)		
Vascular invasion							
Absent	126(53.6)	6(95.7)	0.003	258(88.7)	18(72.0)	0.016	









#### Cohort II

Survival in strata according to PODXL expression and adjuvant chemotherapy in curatively treated Stage III patients



# Unpublished data

Prognostic value of PODXL expression in sigmoid colon cancer (n=305)

Five-year survival for American Joint Committee on Cancer fifth edition by tumor location.



JNCI

O'Connell J B et al. JNCI J Natl Cancer Inst 2004;96:1420-1425

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## Comparison of two antibodies



# Correlations of PODXL expression with other molecular characteristics

- KRAS mutation status: none
- MSI screening status : none
- Beta-catenin overexpression: none
- P53 mutation status :none
- Weak negative association with p27 (R=-0.16)
- Positive correlation with COX2 expression in sigmoid colon cohort (R=0.33 and R=0.26)

# Summary of clinical data

- PODXL expression is an independent predictor of poor prognosis in colorectal cancer
- PODXL may be a useful marker for prognostic stratification of patients with Stage II-III disease

## T4N2M1

## Dead 3 months from diagnosis

# Sialofucosylated podocalyxin is a functional E- and L-selectin ligand expressed by metastatic pancreatic cancer cells

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Membranous PODX expression 72/105 (69%) cases

Podocalyxin (PODXL) is overexpressed by a subset of pancreatic cancer cells.



Dallas M R et al. Am J Physiol Cell Physiol 2012;303:C616-C624 AMERICAN JOURNAL OF PHYSIOLOGY



## PODXL is a functional selectin ligand expressed by SW1990 metastatic pancreatic tumor cells.



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# Does PODXL expression contribute to transperitoneal metastasis?



"Infiltrative" morphology

## Serosal invasion



Clin Exp Metastasis (2012) 29:239–252 DOI 10.1007/s10585-011-9446-0

**RESEARCH PAPER** 

## The anti-adhesive mucin podocalyxin may help initiate the transperitoneal metastasis of high grade serous ovarian carcinoma

Jane A. Cipollone · Marcia L. Graves · Martin Köbel · Steve E. Kalloger · Tak Poon · C. Blake Gilks · Kelly M. McNagny · Calvin D. Roskelley



Force-expressed podocalyxin is targeted to the free surface of serous ovarian carcinoma cells



Cipollone et al., 2012

#### Podocalyxin decreases integrindependent cell adhesion



# **Future studies**

- Prospective testing of PODXL expression in tumours registered in Sydsvenska
  Tarmbiobanken (STABB) from Jan 1st 2012
- Plasma levels of PODXL as a prognostic marker?
- Circulating tumour cells
- Other cancer forms?

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