McNagny, K.M.; Hughes, M.R.; Graves, M.L.; DeBruin, E.J.; Snyder, K.J.; Cipollone, J.; Turvey, M.; Tan, P.C.; McColl, S.R.; Roskelley, C.D.
Podocalyxin in the diagnosis and treatment of cancer
Advances in cancer management, 2012 / Mohan, R. (ed./s), Ch.8, pp.155-194

8

Podocalyxin in the Diagnosis and Treatment of Cancer

Kelly M. McNagny[1], Michael R. Hughes[1], Marcia L. Graves[1], Erin J. DeBruin[1], Kimberly Snyder[1], Jane Cipollone[1], Michelle Turvey[2], Poh C. Tan[1], Shaun McColl[2] and Calvin D. Roskelley[1]

1. Introduction

Although their functions in tumour development and progression are not well understood, several secreted and membrane-associated mucins have proven to be valuable diagnostic and prognostic markers in human cancer and many of these are targets for cancer vaccines and therapies (Singh et al., 2008; Chauhan et al., 2009; Kufe, 2009). Podocalyxin-like protein 1 (**podocalyxin**, gene name ***PODXL***) is a member of the CD34 family of sialomucins. Podocalyxin (PC), also called PCLP1, gp135, MEP21, and thrombomucin, is a single-pass type I transmembrane protein primarily expressed by vascular endothelia, specialized kidney epithelial cells called podocytes, and a limited set of hematopoietic progenitor cells in adult mice and humans (Nielsen & McNagny, 2009b). Expression of PC has also been noted on the luminal face of kidney tubule cells, breast and ductal lumens, oviductal luminal cells, mesothelial cells and neurons in normal mammalian tissues. Polymorphisms and inappropriate or increased expression of PC is linked to several human cancers including germ-line cancers, several carcinomas, malignant astrocytoma and leukemia. In many of these cancers, detection of high levels of PC expression is associated with high-grade, aggressive tumours, increased risk of metastases and poor prognosis. In this review, we will examine the known and proposed functions of PC in normal (adult and embryonic) and cancerous cells, the molecular mechanisms regulating these functions, and potential applications of PC as a molecular marker in the diagnosis, prognosis and treatment of cancer.

2. Podocalyxin expression and normal function in mammals

2.1 Podocalyxin has an essential role in the development and function of the kidney glomerulus

PC is named for its expression on specialized kidney epithelial cells called podocytes. On these cells, PC is required for the development of the foot process that, together with fenestrated vascular endothelia, form the filtration apparatus of the glomerulus (reviewed in Nielsen & McNagny, 2009b). Unlike vascular and hematopoietic tissues where other members of the CD34 family are co-expressed, PC is the only CD34-family sialomucin

[1]*The University of British Columbia, Canada*
[2]*The University of Adelaide, Australia*

expressed on podocytes and therefore serves a non-redundant function in podocyte development. Germ-line deletion of *Podxl* in mice results in anuria and death within 24 hours of birth due to the failure of embryonic podocytes to undergo appropriate morphogenesis and form foot processes - one of the most potent mucin-dependent knockout phenotypes described (Doyonnas et al., 2001).

Although many tissue types and cell lineages express PC during mouse embryogenesis (and presumably human), PC's only essential function identified so far is the formation of the glomerular filtration apparatus of the kidney. However, we are beginning to discover more subtle roles for PC in the development, morphogenesis, polarization and motility of cells that form the lumen of tubules and ducts (or boundary elements in tissues) including kidney tubules, ovary ducts, mammary ducts and vascular lumens. For example, germ-line deletion of *Podxl* in mice causes a delay in the formation of lumens between adjacent endothelial cells of the developing aorta (Strilic et al., 2009) and reduces the adhesion of the mesothelia lining of the gut surface mesothelia to the umbilicus during embryogenesis (Doyonnas et al., 2001). From these and other embryonic analyses we have developed the concept that podocalyxin acts a general anti-adhesive that aids in the demarcation of tissue boundaries (McNagny et al., 1997; Doyonnas et al., 2001). PC expression is also required for efficient neurite outgrowth and branching in the developing brain (Vitureira et al., 2005, 2010). Despite these subtle developmental delays or abnormalities, in all tissues we have examined so far, save the kidney, *Podxl*$^{-/-}$ mouse neonates appear to be remarkably normal.

In adult mice and humans, PC is primarily restricted to vascular endothelia, kidney podocytes and a subset of hematopoietic stem cells. In addition, PC expression has also been reported on some mature blood types including mouse "stress" erythroid progenitors (and anemia-induced erythroblasts) (Doyonnas et al., 2005; Sathyanarayana et al., 2007; Maltby et al., 2009) and activated rat platelets (Miettinen et al., 1999). Although others and we have proposed a role for PC (and the CD34 antigen) in facilitating the trafficking of hematopoietic progenitors cells to the bone marrow (BM) as well as more mature blood cells from the BM or periphery to sites of inflammation (Nielsen & McNagny, 2008, 2009a, 2009b), the function of PC expression on normal hematopoietic cells remains enigmatic.

In summary, although PC has a critical role in kidney development, PC appears to be dispensable for the normal development of other tissues we have examined including vascular endothelia, breast duct epithelia and hematopoietic tissues. However, after increasingly more detailed analyses, we have also found that PC has subtle roles in these tissues under conditions of tissue remodeling and development that may have consequences in disease pathogenesis (unpublished observations).

2.2 Transcriptional regulation of *PODXL* expression

The regulation of *PODXL* expression is modulated by several transcription factors, epigenetic methylation of CpG islands in the *PODXL* promoter and by expression of micro RNA (miR-199a2). Depending on the cell lineage and disease state, *PODXL* expression can be induced by Sp1 (specificity protein 1) and Wilms' tumour suppressor protein 1 (WT1). The *PODXL* promoter contains multiple binding sites for Sp1 and there is evidence of direct binding of WT1 to the *PODXL* promoter (Palmer et al., 2001; Butta et al., 2006). While Sp1 is expressed in many cell types and has many targets in the human genome, Sp1 is

upregulated in many cancers including epithelial carcinomas (reviewed in Li & Davie, 2010). WT1 is primarily expressed in developing kidney and in the podocytes of the glomerulus in normal kidney. However, WT1 is overexpressed in cancers of the kidney, lung and breast and in acute leukemia and myelodysplastic syndromes. Thus, both Sp1 and WT1 may influence the expression of *PODXL* in cancers.

Adult erythroid cells express PC in response to anemic stress via erythropoietin receptor (EpoR)-dependent activation of the signal transducer and activator of transcription 5 (STAT5) (Sathyanarayana et al., 2007). Although *PODXL* expression via STAT5 has not been demonstrated in other cell types, STAT5 activation can be induced in epithelial cells by a variety of mechanisms. For example, with respect to the pathogenesis of carcinomas (especially prostate and breast cancers) STAT5 activation downstream of the prolactin receptor activation is one possible route to enhanced PC expression (Jacobson et al., 2010).

Transcriptional repressors of *PODXL* include the ubiquitously expressed tumour suppressor transcription factor p53 (*TP53*) and the integrin-associated adaptor protein PINCH1 (*LIMS1*) (Stanhope-Baker et al., 2004; Wang et al., 2011). PINCH1 is normally associated with focal adhesion complexes in podocytes but becomes dissociated from these in response to transforming growth factor beta (TGFβ) stimulation (Wang et al., 2011). PINCH1 then enters the nucleus and antagonizes WT1 transcriptional activation of *PODXL* (Wang et al., 2011). Since TGFβ can induce *PODXL* expression in a human lung adenocarcinoma cell line (**see §3.5.1**) (Meng et al., 2011), the role of TGFβ and PINCH1 in the regulation of *PODXL* expression may be specific to podocytes (or normal, adherent epithelia). Finally, *PODXL* is a target for miR-199a2, a micro RNA that is normally expressed in epithelial cells of many tissues (Cheung et al., 2011). miR-199a2 targets (represses) a collection of transcripts and has a putative role as a tumour suppressor in several human cancers (**see §3.7.2**).

3. Podocalyxin is a diagnostic marker and prognostic indicator in cancer

Within the last 5-7 years, retrospective analysis of tumour tissue archives by histology, protein and transcript expression analyses have identified increased PC expression or *PODXL* gene polymorphisms in several human cancers. In this section, we will outline what is known of PC expression patterns in human cancers including epithelial carcinomas (kidney, breast, thyroid, lung, ovarian, prostate and gastrointestinal cancer), germ-cell tumours (testicular cancer), astrocytomas (brain cancer) and leukemia (**Table 1**). In general, in studies where PC expression has been correlated with tumour behaviour and patient outcome data, PC expression in primary tumour cells is associated with increased tumour aggressiveness, risk of distant metastases and poor prognosis. Mechanistic studies using human cancer cell lines suggest that PC expression in tumour cells is not just merely correlative with aggressiveness, but may have a direct contribution to tumour progression, survival and metastases. In addition to the potential this wealth of data offers for the development of PC-targeted adjuvant treatments for systemic cancer, these findings and accompanying mechanistic studies provide insights into the molecular mechanisms of tumour progression and metastases. While it is clear that there remain many technical challenges for the development of PC-targeted cancer treatments, implementation of clinically beneficial diagnostics is feasible and compatible with existing technology and methods, requiring only a concerted validation effort.

Review Section (§) Primary Tissue	Podocalyxin expression profile	Diagnostic & Prognostic Significance
§3.1.1 Kidney (Renal cell carcinoma)	• PC protein expression 4-fold higher in cancerous cells of rare subset of RCC tumours (~10%) • PC expression in primary RCC correlates with risk of metastasis and poor patient outcome. Hazard ratios (HR) (PC^+ *vs*. PC^- RCC primary tumors) HR = 3.6 (metastasis-free survival) HR = 7.5 (disease-specific survival) (Hsu et al., 2010)	• PC expression in tumor cells correlates with increased risk of aggressive tumor phenotype, and is an independent predictor of distant metastases and poor prognosis
§3.1.2 Kidney (Nephroblastoma)	• Higher PC transcript expression (↑ 1.5 fold) in more aggressive anaplastic (advanced grade) compared to low grade nephroblastoma (Stanhope-Baker et al., 2004)	• Diagnostic marker (transcript) of anaplastic grade nephroblastoma
§3.2 Breast carcinoma	• High PC protein expression in rare "node-negative" subset of breast carcinoma (~6%) • PC overexpression in primary breast tumours associated with increased relative risk of poor outcome (RR = 8.45) (Somasiri et al., 2004)	• PC expression in tumor cells correlates with increased risk of aggressive tumor phenotype, and is an independent predictor of distant metastases and poor prognosis
§3.3 Ovarian carcinoma	• High expression of PC on tumour cells in 67% of all ovarian epithelial tumours and 87% of high-grade serous epithelial ovarian carcinoma • PC cytoplasmic expression not associated with disease-outcome in high-grade serous ovarian tumours, but cell surface expression of PC is an independent predictor of poor patient outcome with HR ~ 1.6 (compared to PC-negative high-grade serous ovarian tumor) **(**Cipollone et al, submitted)	• Prognostic marker of poor outcome in high-grade serous ovarian cancer when PC overexpression is cell surface localized
§3.4 Thyroid carcinoma	• High expression of PC protein common (~52% cases) in undifferentiated thyroid carcinoma (UTC) subtype but not detected in normal thyroid epithelia or other thyroid cancer cells (Yasuoka et al., 2008)	• Diagnostic and staging marker of undifferentiated thyroid carcinoma
§3.5 Lung (small-cell lung carcinoma)	• PC protein expression detected in tumour cells in majority (~87.5%) in small-cell lung carcinoma (SCLC) (Koch et al., 2008)	• Diagnostic marker (tissue biopsy)
§3.6 Prostate carcinoma	• *PODXL* germ-line polymorphism and SNP are associated with increased risk of developing prostate cancer with an aggressive tumor. In-frame single or double deletion polymorphism in exon 1 associated with increased risk of prostate cancer Odds ratio (OR) = 2.14-2.58 (all prostate cancer) OR = 3.04-4.42 (aggressive disease) SNP (G340A) resulting in missense mutation associated with increased risk of prostate cancer OR = 1.48 (all prostate cancer) (Neville et al., 2002) • Possible increased PC expression in metastatic tumor but not primary prostate tumours (**Fig. 5**) (Yu et al., 2004; Chandran et al., 2007)	• Genetic marker of prostate cancer risk • High expression of PC a potential prognostic marker of tumour aggressiveness and metastases

Review Section (§) Primary Tissue	Podocalyxin expression profile	Diagnostic & Prognostic Significance
§3.7 Testicular (germ cell tumour)	• PC antigens (GCTM-2, TRA-1-60/81) detected in serum of testicular cancer patients (35-75% NSGCT cases) (Schopperle et al., 2003) • *PODXL*-targeting miR-199a downregulated in testicular cancer (Cheung et al., 2011)	• Potential serum marker for testicular cancer • PC potentially equally or more robust marker of testicular cancer compared to α-fetoprotein or chorionic gonadotrophin
§3.8.1 Liver (hepatocellular carcinoma (HCC))	• High expression of PC protein detected in sinusoidal endothelial associated with HCC but not normal adjacent tissue or cirrhotic lesions (Chen et al., 2004; Heukamp et al., 2006)	• Differential diagnosis of HCC *vs.* cirrhotic lesions (tissue biopsy)
§3.8.2 Pancreatic ductal adenocarcinoma	• PC protein expressed in primary tumours of 42% pancreatic ductal adenocarcinoma (PDAC) and 30% ampullary ductal carcinoma but rarely in other primary tumours of GI origin (e.g., liver, colon, esophagus) • High PC expression more often detected in high-grade PDAC (53% Grade 3, 48% Grade 2 and 18% Grade 1) (Ney et al., 2007)	• Possible identification of primary tissue of metastatic tumours from a suspected GI source
§3.8.3 Colon carcinoma	• PC protein and peptides detected in media and lysate of colon carcinoma cell lines. Expression not yet determined in colon cancer primary tumours. (Thomas et al., 2009)	• Potential serum marker for colon cancer
§3.9 Hematopoietic	• PC protein expression commonly detected (77-87% cases) in diffuse pattern (immunohistology) in formalin-fixed bone marrow biopsy in majority cases of AML, ALL and myeloid sarcoma (Kelley et al., 2005). • Cell surface expression in 20% AML from fresh blood or bone marrow samples (flow cytometry) • (Riccioni et al., 2006).	• Potential diagnostic marker (tissue biopsy), however significance to disease phenotype and outcome not known • Potential surface marker of AML blasts in peripheral blood
§3.10 Brain (astrocytoma)	• High PC protein and transcript expression in ~50% cases of high-grade malignant astrocytomas (anaplastic astrocytoma and glioblastoma) but not low-grade astrocytic tumours (Hayatsu et al., 2008).	• Diagnostic marker of high grade/anaplastic astrocytoma

Table 1. Summary of podocalyxin expression profiles in human cancers.

3.1 Kidney cancer

3.1.1 Renal cell carcinoma

Renal cell carcinoma (RCC), which accounts for 85% of renal cancers, originates from epithelial cells of the renal tubules (Cohen & McGovern, 2005). In a retrospective study, PC protein expression was examined in a collection of 303 formalin-fixed, paraffin-embedded RCC tumours (Hsu et al., 2010). Of these, 29 tumour specimens (9.6%) were found to express PC protein in cancerous cells (4-fold over non-expressors) (Hsu et al., 2010). In contrast, in normal kidney and the majority of RCC patient tumours, PC is only detected in the glomerulus and vascular endothelia (Hsu et al., 2010). Patient outcome data showed that PC-positive RCC tumours were much more likely to be high-grade, advanced-stage tumours (Hsu et al., 2010). Importantly, patients with PC-positive RCC also had decreased

disease-specific and metastasis-free survival and PC-positive RCC tumours (stage I-III) were more likely to result in distant metastases (i.e., non-lymph node sites) (Hsu et al., 2010). These findings suggest that PC expression in primary RCC tumours is a strong and independent predictor of distant metastasis and poor prognosis (Hsu et al., 2010). Thus, by identifying a rare, high-risk RCC sub-type, evaluation of PC expression has clear potential diagnostic and prognostic value.

3.1.2 Wilms' tumor (Nephroblastoma)

Wilms' tumour, or nephroblastoma, the most common pediatric kidney cancer, has a unique histological presentation indicative of aberrant or incomplete kidney development during embryogenesis (Huff, 2011). Unlike most of the cancers discussed in this review, PC expression (transcript) is significantly *reduced* in 64 patient nephroblastoma samples (relative mean 0.29) compared to pooled normal fetal kidney (Stanhope-Baker et al., 2004). Although WT1 is a positive regulator of *PODXL* expression (Palmer et al., 2001), and loss of WT1 expression is associated with some Wilms' tumours, there was no evidence of a correlation between *PODXL* and *WT1* expression in this study (Stanhope-Baker et al., 2004). However, perhaps of diagnostic value, this same study showed that *PODXL* expression was increased in more aggressive, anaplastic tumours compared to 40 non-anaplastic tumors (Stanhope-Baker et al., 2004). Notably, functional loss of p53 is associated with the most aggressive, anaplastic nephroblastomas with poor prognosis. As p53 negatively regulates *PODXL* expression (Stanhope-Baker et al., 2004), enhanced *PODXL* expression in anaplastic nephroblastomas may be explained directly by this genetic pathway. Although more study is required to know if enhanced *PODXL* expression in anaplastic nephroblastoma has a direct role in promoting tumour aggressiveness, expression of PODXL in nephroblastoma may be useful as a marker of poorly differentiated, anaplastic tumours.

3.2 Breast carcinoma

3.2.1 Expression of podocalyxin is an independent predictor of poor outcome in invasive breast carcinoma

In normal human breast tissue, expression of PC protein is relatively low and restricted to the apical face of the luminal duct epithelia and, as in most tissues, PC also marks the lumen of vascular endothelia in the breast (Somasiri et al., 2004). However, in a survey of tissue microarray constructed from 272 primary tumour samples from patients with locally invasive breast cancer, high expression of PC protein was detected in a small subset (6%) of invasive breast carcinomas (Somasiri et al., 2004). Notably, PC-positive primary tumours were associated with significantly higher risk of poor outcome (decreased disease-specific survival) compared to patients with tumours expressing little or no PC (Somasiri et al., 2004). PC-positive tumours were not significantly different in histological classification, size or risk of lymph node metastasis. However, PC expressing tumours were more likely to present at an advanced tumour grade, express p53, and be negative for estrogen receptor (ER) (*ESR1*) and HER-2 (*ERBB2*) (Somasiri et al., 2004). These data reveal that high expression of PC in primary, locally invasive breast carcinoma is a strong and independent predictor of poor outcome (Somasiri et al., 2004). As with its detection of rare RCC subsets, evaluation of PC expression in primary breast carcinomas may have immediate diagnostic and prognostic value in guiding treatment or surveillance strategies in breast cancer. In addition, detailed profiling of PC-tumours may provide a deeper understanding of tumour progression mechanisms. Related to these efforts, in the next section we discuss some of the

molecular mechanisms that underlay PC's role in promoting progression of invasive breast cancer to metastatic breast carcinoma.

3.2.2 Podocalyxin regulates breast cancer tumour cell invasiveness and migration *in vitro*

In order to gain insight into the biological role of PC in breast cancer, we, as well as other investigators, have surveyed the expression of *PODXL* and its intracellular ligands, NHERF-1 and -2, in human breast cancer cell lines and examined the effects of altering its expression on the behavior of these cells *in vitro* and *in vivo*. Recent microarray analysis of a collection of 50 human breast cancer cell lines revealed a wide range of expression of *PODXL*, NHERF-1 (*SLC9A3R1*) and NHERF-2 (*SLC9A3R2*) transcripts (Kao et al., 2009) **(Fig. 1)**. *PODXL* tends to be highly expressed in estrogen receptor- and progesterone receptor (PR)-negative (ER^-/PR^-) basal-like breast cancer lines. Conversely, high NHERF-1 and NHERF-2 expression correlates with luminal-type, ER^+ breast cancers. MCF7 cells are a line of luminal-like breast epithelial cells (Neve et al., 2006; Kao et al., 2009) obtained by pleural effusion (i.e., lung metastases) of a patient with metastatic invasive ductal adenocarcinoma (ER^+/PR^+)(Soule et al., 1973). Relative to other breast cancer cell lines, MCF7 expresses low-moderate levels of endogenous *PODXL* transcripts and high levels of NHERF-1 (and low to moderate expression of NHERF-2) (Kao et al., 2009) (**Fig. 1**). MDA.MB.231 cells, which express high levels of endogenous PODXL, are a basal B-subtype breast cancer cell line derived from pleural effusion of human metastatic breast adenocarcinoma (Kao et al., 2009) **(Fig. 1)**. In contrast to MCF7 cells, MDA.MB.231 are ER^-/PR^- and have a highly invasive phenotype in *in vitro* assays and *in vivo* xenograft models (Lacroix and Leclercq, 2004; Neve et al., 2006).

By overexpressing exogenous PC and structural mutants in MCF7 cells or by suppressing endogenous PC in MDA.MB.231 cells using RNA-interference (RNAi) methods, we (and others) have found that PC promotes a more aggressive, invasive phenotype *in vitro* and *in vivo* (Somasiri et al., 2004; Sizemore et al., 2007). In addition, our preliminary results corroborate a role for PC in breast cancer invasion since suppression of endogenous *PODXL* in MDA.MB.231 impairs serum-dependent migration *in vitro* (Turvey & McColl, unpublished observations). We are currently working to determine if silencing *PODXL* expression down regulates invasion-associated adhesion molecules and matrix proteinases and how loss of *PODXL* alters the morphology and cytoskeletal dynamics in MDA.MB.231.

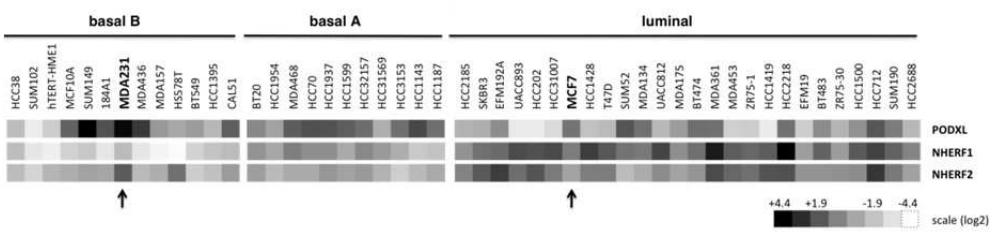


Fig. 1. Transcript expression levels of PODXL, NHERF1 and NHERF2 in a collection of human breast cancer cell lines. Arrows indicate the luminal-like MCF7 and basal (B)-like MDA.MB.231 breast cancer cell lines commonly used in the study of PC function. The transcript expression heat map was generated from meta-analysis of publically available microarray gene expression profiles of human breast cancer cell lines (Kao et al., 2009) using Gene Cluster 3.0 and Java TreeView.

3.2.3 Podocalyxin modulates EMT-independent breast cancer progression and metastatic potential

Epithelial-to-mesenchymal transition (EMT) is an important paradigm in the understanding of tumor progression to systemic, metastatic cancer (discussed in more detail in **§3.5.1**). However, EMT is not an obligate pathway to metastases (Wicki et al., 2006), and invasive ductal breast carcinomas, which form the great majority of locally invasive breast lesions, retain epithelial characteristics and epithelial differentiation markers (Cleton-Jansen, 2002; Sarrio et al., 2008). Thus, a better understanding of EMT-independent mechanisms of tumour invasion and metastasis is critical for developing therapeutic strategies in ductal breast carcinoma in particular, but also other cancers in general.

By expressing exogenous *PODXL* in MCF7 cells, we have found that PC promotes altered morphogenesis, loss of adhesion and impaired tumour cell aggregation in 3D spheroid cultures (Graves and Roskelley, manuscript in preparation). Importantly, altered adhesion is not associated with disruption of cell-cell junctions, impaired expression or re-localization of cell-cell junction regulators (e.g., E-cadherin, β-catenin, ZO-1) or, disruption of extra-cellular matrix (ECM) adhesion complexes (e.g., β1-integrin, activated focal adhesion kinase (FAK)). Rather, expression of PC in polarized cells enhances the exclusion of β1-integrins from the free, apical surface of MCF7 and recruits F-actin from the basolateral ECM-adhesion complexes to an expanded apical membrane domain. We hypothesize that PC-mediated redistribution of F-actin and other cortical actin components weakens adhesion of the basolateral membrane domain to ECM. Moreover, when single cells are shed from the epithelial sheets, PC is redistributed in a global pattern that blocks homotypic adhesion. Expression of PC on shed, single cells may also promote chemotaxis, enhance adhesion-free survival or participate in the homing to distant tissue sites (**Fig. 2**). Thus, while PC expression in some epithelial tissues may help to define the apical domain and reinforce polarization, dysregulated mistargetting or overexpression of PC disrupts normal tissue architecture of epithelial sheets and spheroids and may promote invasive and metastatic characteristics.

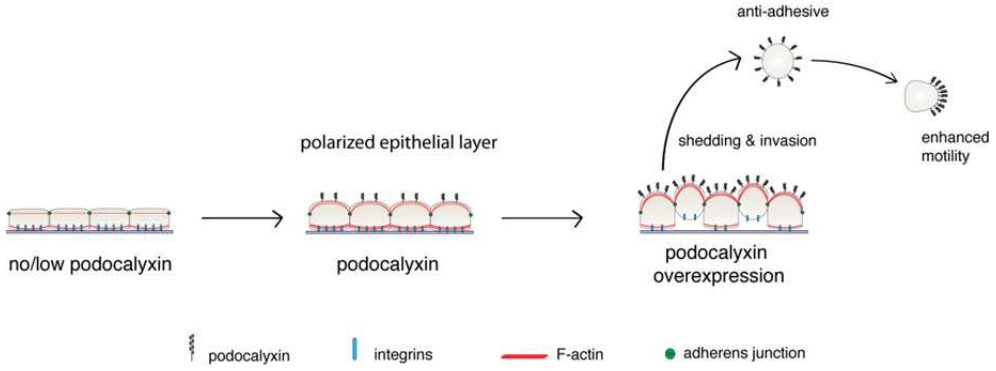


Fig. 2. Podocalyxin expression defines the apical domain of polarized epithelia but promotes non-EMT loss of adhesion and shedding when overexpressed.

Finally, to gain some insight into the *in vivo* function of PC in the growth, local invasion and metastatic potential of human breast cancers, we have used tumor xenograft models. In preliminary experiments, sub-cutaneous transplantation of MCF7 or PC-overexpressing MCF7 cells into immunodeficient mice (Rag2M) suggests that expression of high levels of

PC enhance tumour growth and density (Graves and Roskelley, manuscript in preparation). In addition, although the PC–positive MDA.MB.231 cell line forms lung tumors in NOD-SCID *Il2rγ* $^{-/-}$ (NSG) mice 6-8 weeks after intravenous injection, PC-deficient MDA.MB.231 cells fail to form lung tumours (Turvey and McColl, unpublished observations). Because equal numbers of PC-positive and PC-negative MDA.MB.231 tumor cells appear in the pulmonary parenchyma at early time points (3-14 days), we hypothesize that PC promotes survival in recipient lung tissue (Snyder, Hughes and McNagny, unpublished observations). We are continuing to use these models, and orthotopic mammary fat pad transplant breast tumour models, to fully characterize PC's role in breast tumour invasion and metastases. In summary, the data suggest PC plays a key role in enhancing breast carcinoma growth and invasiveness *in vivo*.

3.3 Ovarian carcinoma

Ovarian cancer remains the most lethal gynecological cancers and, like most epithelial carcinomas, systemic spread generally has a poor prognosis since there are currently no curative interventional therapies. PC is expressed in normal mesothelia-derived tissues that encase the ovaries and make up the free luminal surface of cells that line the oviducts and endometrium (Cipollone and Roskelley, manuscript submitted). In addition, PC protein is detectable in the tumor cells of approximately two-thirds of human ovarian carcinoma cases. Although the mere presence of PC protein in ovarian carcinomas does not correlate with disease outcome, expression of PC on the cell surface (as opposed to intracellular localization) is predictive of poor outcome for high-grade serous ovarian carcinoma, 87% of which express significant amounts of the mucin.

As with breast cancer cell lines, PC expression in ovarian cancer lines is variable (Cipollone and Roskelley, *ibid*). To evaluate PC's molecular functions in ovarian carcinoma, we ectopically expressed PC in the serous carcinoma-derived cell line (OVCAR-3) originally derived from malignant ascites (Hamilton et al., 1983)**.** OVCAR-3 cells express epithelial differentiation markers, form well-structured adherent epithelial sheets in culture, and are generally non-invasive and have low motility *in vitro* (Comamala et al., 2011). Forced expression of PC in OVCAR-3 cells reduced *in vitro* adhesion to extracellular matrices including; mesothelial-cell layers, immobilized β1-integrin antibodies and fibronectin. As with other epithelial cells, exogenously expressed PC is preferentially localized to the apical free surface of OVCAR-3 cell layers in 2D culture and spheroids in 3D culture. As observed in MCF7 cells, PC excludes β1-integrin from free apical domains. Thus, when expressed at the cell surface, PC may act as an anti-adhesin in serous ovarian carcinoma by sterically masking integrin-extracellular matrix interactions, and, by promoting cortical cytoskeletal rearrangements that weaken adhesion to ECM. This PC-induced morphogenesis may be of critical importance to ovarian carcinoma progression, as these tumors very often metastasize after small nodules are shed, in an anti-adhesive (non-EMT dependent) fashion, into the abdominopelvic cavity. Thus, while PC-driven non-EMT shedding and migration may be rare in some epithelial cancers (breast and renal), PC expression may be a common, and critically important molecular mechanism driving tumour metastasis in high-grade serous ovarian carcinoma.

3.4 Thyroid carcinoma

Thyroid carcinomas (non-lymphoma, squamous cell or sarcoma) are classified according to histological characteristics and origin. The most common "differentiated" subtypes (papillary and follicular) are highly treatable and responsive to therapy. The medullary and

undifferentiated (anaplastic) sub-types, although more rare, have a less favorable prognosis. Although PC is normally absent on thyroid epithelia and is not detected in well-differentiated thyroid carcinoma tumour subtypes or squamous cell carcinoma (thyroid), in immunohistological analyses of 238 thyroid tumours, PC was detected on tumours of over half the cases (n=69) of undifferentiated thyroid carcinoma (UTC). In addition, in cases where the thyroid tumours have a mixed histological type (UTC adjacent to differentiated tumour cells), PC was detected only on cells with UTC phenotype (Yasuoka et al., 2008). We note that the pattern of PC expression on thyroid carcinoma and small-cell lung carcinoma (**§3.5**) (correlates with a "de-differentiated" tumour phenotype consistent with an EMT transformation. Thus, although PC may not be sufficient to drive EMT, high expression on UTC and other mechanistic studies (**§3.5.1**) suggest that PC expression may be upregulated as part of an EMT program. If this is so, similar to its function in EMT-independent tumours, PC may help to promote a non-cohesive, invasive phenotype during EMT.

3.5 Small-cell lung carcinoma

Small-cell lung carcinomas (SCLC) (16% of lung cancers) are malignancies that typically arise from epithelial cells of the proximal airways (bronchus). SCLC tumours consist of multipotent epithelial ("stem-like") cells that form typically diffuse and non-cohesive clusters. Analysis of formalin-fixed, paraffin-embedded bronchial-tumour biopsies (and adjacent normal bronchial tissues) reveals that the majority of SCLC but not normal bronchial epithelia expresses PC and its expression in SCLC correlates with non-methlyated CpG islands in the *PODXL* gene (Koch et al., 2008).

Although PC is not detected in normal adult bronchial epithelium, clusters of PC-positive epithelial cells can be detected in deep pockets of developing proximal bronchi and trachea of fetal lung (Koch et al., 2008). The authors suggest the intriguing hypothesis that *PODXL* marks multipotent bronchial epithelial cells during lung development and that SCLC "cancer stem cells" may retain or acquire the characteristics of this stem-like population. There are currently no studies that correlate PC expression with patient outcome or response to chemotherapy in lung cancers. PC expression in non-small cell lung carcinomas (NSCLC) was not performed in this study so it is not yet known if PC also marks primary NSCLC tumours of the lung. For these reasons, the diagnostic value of PC expression in lung cancer is less certain than in other cancers. However, although many technical challenges remain, identification of tumour-specific glycoforms of PC (see following sections) may provide targets for adjuvant therapy in lung cancer. This is particularly important in SCLC where surgical resection of tumours is more difficult. Alternatively, if PC expression is consistently associated with type 3 EMT processes in lung cancer (see below), or other EMT-dependent cancer metastases, expression of PC (assessed by biopsy or *in vivo* imaging) may herald a switch from locally invasive to a more advanced disease stage.

3.5.1 Podocalyxin in TGFβ-mediated epithelial-to-mesenchymal transition (EMT)

Epithelial-mesenchymal transition (EMT) (type 3) is a paradigm of tumour invasion and metastasis whereby epithelial cells lose their apical and basal polarization and robust cell-cell contacts to become mesenchymal in morphology and adopt an invasive, migratory behaviour (Kalluri and Weinberg, 2009). Among many other alterations in gene expression patterns, EMT is associated with loss of the adherens-junction regulator E-cadherin (*CDH1*), stabilization and nuclear-localization of β-catenin (*CTNNB1*), and upregulation of the intermediate filament protein vimentin (*VIM*), a mesenchymal marker (Zeisberg and

Neilson, 2009). As we have noted above, PC does not appear to be capable of initiating EMT on its own. However, Wilkins et al. have recently demonstrated that it may contribute to the EMT that is initiated by transforming growth factor beta (TGFβ) (Meng et al., 2011).
Although TGFβ has tumour suppressing activity in some cancers, especially at the early stages of tumour progression, TGFβ, in the context of enhanced phosphatidylinositol-3-kinase (PI3K)/Akt and other survival signalling pathways can drive EMT processes (Ouyang et al., 2010). TGFβ-induced de-differentiation is not limited to malignant cells, as normal human and mouse podocytes undergo an EMT-like transition in response to TGFβ (Herman-Edelstein et al., 2011; Li et al., 2008; Wang et al., 2011). A549, a human lung adenocarcinoma cell line, undergoes EMT-like transformation in the presence of TGFβ, with prototypical EMT features including down regulation of E-cadherin, upregulation of vimentin, expression of MMP2 and fibronectin and, secretion of collagens (type I, III and IV) (Kasai et al., 2005). These alterations in gene expression pattern are accompanied with altered cell morphology, loss of cell-cell contact and enhanced migration (Kasai et al., 2005; Meng et al., 2011).

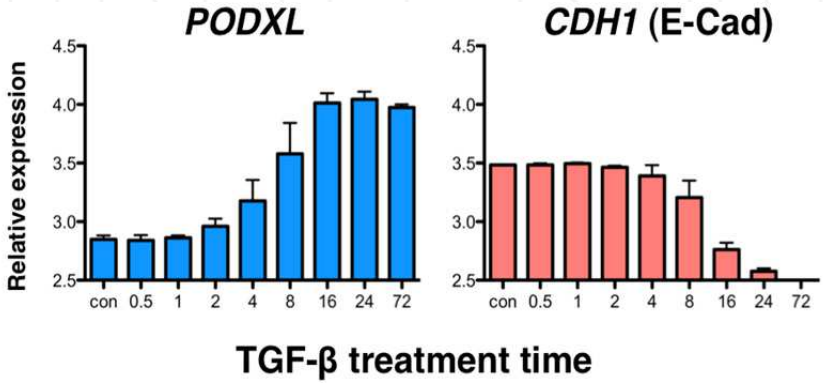


Fig. 3. *PODXL* expression is upregulated during TGFβ-induced EMT of a lung adeno-carcinoma cell line (A549). Expression profiles where generated by meta-analysis of publically available gene expression data using the Gene Expression Omnibus (GEO) dataset GDS3710 (Sartor et al., 2010).

Meng et al recently demonstrated that PC protein expression is upregulated in response to TGFβ1 (Meng et al., 2011) . A query of a publically available gene expression dataset of A549 cells undergoing TGFβ1-induced EMT corroborates their findings and shows that the kinetics of PODXL transcript expression are similar to EMT-associated down regulation of E-cadherin (*CDH1*) transcript (Sartor et al., 2010) **(Fig. 3)**. Intriguingly, using a gene-silencing approach (shRNA), Meng et al show that PC is required for at least some EMT-related processes including mesenchyme-like morphogenesis, cell migration and changes in expression of E-cadherin and vimentin (Meng et al., 2011). PC was also found to co-distribute with newly secreted collagen I at the leading edge of migrating TGFβ-treated A549 cells. In addition, collagen I could be co-precipitated with anti-PC antibodies in TGFβ-treated A549 cells. Notably, the collagen I-binding integrins (α1β1 and α2β1) were not identified in collagen I-PC complexes or detected in PC-collagen membrane domains. Thus, one possible role for PC in a collagen-complex could be the exclusion of collagen- or matrix-binding integrins from the leading edge of migrating cells and intercellular destabilization

of strong adhesion to ECM - themes consistent with our findings using breast and ovarian cancer cell lines (**§3.2.3 & 3.3**).

We note that in this example, PC expression is down-stream (or parallel) to TGFβ1-induced EMT. Thus, while high expression of PC in some tumour cells (e.g, breast carcinoma) can promote EMT-independent tumour progression, PC may also support the invasive and migratory functions of tumour cells that have undergone EMT.

3.6 Prostate carcinoma

A 1.1 Mb region of human chromosome 7q32-33 flanked by microsatellite markers D7S2452 and D7S684 strongly linked to prostate tumour aggressiveness contains the *PODXL* locus (Neville et al., 2002). In a sibling-pair study, a variable in-frame, germ-line deletion of one or two Ser-Pro repeats in the PC mucin domain (deletion of residues 23-24 or 23-26) was found to be associated with enhanced risk of developing prostate cancer (increased overall risk (OR)=2-2.5 fold) and increased tumour aggressiveness (OR=3-4.4 fold) (Casey et al., 2006). In addition, a missense single-nucleotide polymorphism (SNP, *G340A*) resulting in a Ser substitution at Gly114 (also in the mucin domain) was associated with a 50% increased risk of prostate cancer (Casey et al., 2006). Other SNPs in *PODXL* were detected but had lesser correlative significance to prostate cancer risk or tumour behaviour. We note that, unlike the other cancers discussed in this review, this is the only example of a germ-line mutation in *PODXL* associated with increased cancer risk. Although 7q32-33 is a region of high allelic imbalance in prostate cancer, this patient study did not specifically address PC protein or transcript expression levels in prostate tumours. Although we may not expect that deletion of one or two glycosylation sites arising from *PODXL* polymorphisms would significantly alter PC function, it is possible that such in-frame deletions may alter PC surface stability, apical localization or expression and therefore account for enhanced aggressiveness of prostate tumours. Alternatively, in-frame deletions of mucin domain Ser-Pro may indeed alter the functions of PC's extracellular domain or its association with unknown ligands. Regardless of the associated molecular mechanism, *PODXL* deletion polymorphisms and the *G340A* missense SNP may provide an additional genetic screening method to evaluate prostate cancer risk.

3.6.1 Forced expression of podocalyxin enhances the motility of prostate cancer cells

PC3 cells are a human prostatic adenocarcinoma cell line derived from bone metastases (Kaighn et al., 1979). PC3 cells, which are considered to have a relatively high metastatic potential and invasive phenotype in comparison to other prostate cancer lines, do not express detectable levels of PC by western analysis (Sizemore et al., 2007). However, as for other epithelial tumour cell lines, forced expression of full-length PC in this prostate cell line enhanced cell motility *in vitro* (Sizemore et al., 2007). This enhanced motility may depend on the activation of Rac1 that, in turn, requires the association with two intracellular binding proteins, NHERF1 and ezrin. Thus, as for other carcinomas discussed in this review, expression of PC in prostate epithelial has the potential to impart a more aggressive phenotype with potential clinical importance. Further study of PC expression profiles in primary or secondary prostate tumors, coupled to tumour phenotype and outcome data, are required to evaluate whether expression of PC has diagnostic value in prostate cancer. Query of microarray gene expression data from normal prostate tissue and primary and secondary prostate cancer suggest that *PODXL* expression may not be altered in primary prostate tumors (**Fig. 4**). Conversely, metastatic tumours originating from the prostate may have increased *PODXL* expression (~1.4 fold) (**Fig. 4**). Therefore, it is unclear if the increased risk and tumour

aggressiveness associated with this *PODXL* polymorphism is related to altered PC function, expression or simply another marker that is linked to allelic imbalance of 7q32-33.

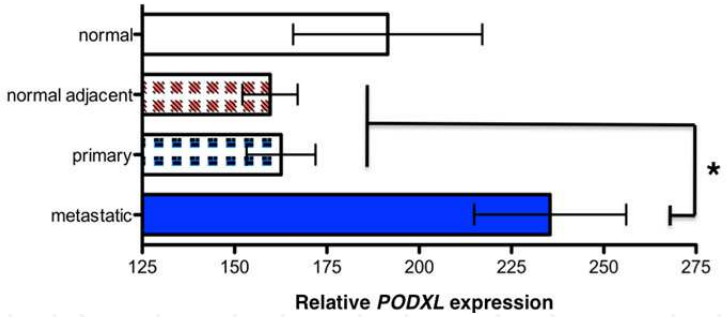


Fig 4. Expression of PODXL in normal human prostate tissue and prostate cancer (GEO dataset GSE6919) (Chandran et al., 2007; Yu et al., 2004). The metastatic tumour group is significantly different than the normal adjacent and primary tumour groups but not the normal donor group (*$p < 0.05$, Tukey one-way ANOVA analysis). All other group comparisons are not significantly different.

3.7 Testicular cancer and embryonal carcinoma

Testicular cancer, the most common cancer in young males, has a high cure rate even after systemic spread (reviewed in Winter & Albers, 2011). However, risk stratification and classification of the primary tumour by histological methods and determination of tumour marker levels is important for designing surveillance and systemic treatment strategy in the event of metastasis or re-lapses. The vast majority of testicular tumours (95%) can be broadly classified as either seminomatous- (SGCT) or non-seminomatous- (NSGCT) germ cell tumours. Seminomas are non-pluripotent, undifferentiated germ cell progenitors whereas non-seminomas tumours are comprised of several subtypes arising from embryonic or extraembryonic (e.g., yolk sac) lineages. Embryonal carcinomas and teratomas are examples of sub-types of NSGCT. Importantly, treatment strategies for SGCT and NSGCT cancers are different, with the major difference being that NSGCTs are generally resistant to radiotherapy.
PC is antigenically identical to tumour markers originally designated GCTM-2 (or gp200), TRA-1-60 and TRA-1-80 expressed on embryonal carcinoma (EC) cell lines and inner blastocyst-derived human embryonic stem cells (hESCs) (Schopperle et al., 2003; Schopperle & DeWolf, 2007). The TRA-1-60/81 epitopes are present only on a high molecular weight form of PC (200 kD) expressed by undifferentiated embryonal carcinoma or hESCs. Intriguingly, the antigenic sites detected by these antibodies disappear following retinoic acid (RA)-induced differentiation of ECs or following differentiation of ESCs into embryoid bodies (EBs) (Schopperle and DeWolf, 2007). However, EBs continue to express a lower molecular weight isoform (170 kD) of PC that can be detected using antibodies recognizing peptide epitopes, or by the terminal glycan, β-(D)-galactose (Galβ)-binding plant lectin, peanut agglutinin (PNA) (Schopperle and DeWolf, 2007). Thus, there is evidence to suggest that there are tumor and tissue type specific epitopes on PC. These epitopes are present on primitive or pluripotent germ-line tumours or hESCs.
GCTM-2 has been identified as a tumour marker of NSGCT and a soluble form of GCTM-2 has been detected in the sera in 7 of 20 patients diagnosed with NSGCT. In addition, TRA-1-

60 was present in the sera of over 75% of patients (n=42) with NSGCT and even in two-thirds of NSGCT patients negative for the "gold-standard" testicular cancer serum tumour markers, α-fetoprotein and chorionic gonadotrophin (Mason et al., 1991; Badcock et al., 1999; Gels et al., 1997 as cited in Schopperle et al, 2003). Although the mechanism has not yet been defined, serum-soluble forms of PC may arise from secreted isoforms or proteolytic cleavage (membrane shedding) of the membrane anchored protein. These findings suggest that PC; especially a modified, high molecular weight form preferentially expressed in undifferentiated embryonic lineages, may serve as a sensitive tumour marker detectable in patient serum. Thus, with further validation, the detection of soluble PC peptide fragments could aid in detection of testicular germ-line tumours and evaluating response to therapy.

3.7.1 Epigenetic regulation of podocalyxin by micro RNA in testicular cancer

In a screen of gene methylation patterns in the a human testicular tumour cell line (NT2) (an embryonic carcinoma), Cheung et al (Cheung et al., 2011) recently identified a hypermethylated region in intron 14 of the dynamin 3 locus (*DNM3*) located on chromosome 1(q24). This region codes for the expression of three micro RNAs (miR), including miR-199a2 (hsa-mir-199a-2) in an antisense orientation. miR-199a2, together with miR-199a-1 (located on hChr 19p13), encode the pre-microRNA species miR-199a. Reduced expression of miR-199a has previously been linked to poor prognosis in serous ovarian carcinoma (Nam et al., 2008) and pro-inflammatory and anti-apoptotic pathways in chemoresistant ovarian cancer stem cells (Yin et al., 2010). miR-199a, which is normally abundantly expressed in most human epithelial and non-epithelial tissues (with the notable exception of brain and peripheral blood mononuclear cells (Liang et al., 2007)**)**, targets a cluster of genes that include transcripts encoding PC, HIF-1α, IKKβ and SIRT1α, mTOR, SMAD1 and c-MET and several others (Cheung et al., 2011). Note that regulation of these targets by miR-199a and expression of the miR-199a-1 and miR-199a-2 loci is tissue specific. For example, reduced expression of miR-199a (along with other miRs) has also been linked to bladder cancer (Ichimi et al., 2009). Conversely, miR-199a expression is upregulated with a cluster of other miRs in metastatic uveal melanoma and gastric cancer (Ueda et al., 2010; Worley et al., 2008).

The miR-199a2 pre-microRNA is processed to yield two mature miR species, miR-199a-5p and miR-199a-3p, and both are down regulated in malignant testicular tumours in comparison to normal or benign tumour tissue (Cheung et al., 2011). miR-199a-5p and -3p have distinct RNA targets, and only miR-199a-5p is a direct negative regulator of *PODXL*. Expression of miR-199a-5p negatively correlates with PC protein expression in malignant testicular tumors (both seminomatous and non-seminomatous) (Cheung et al., 2011). Ectopic expression of miR199-a in NT2 cells attenuates migration and invasion *in vitro* and tumour growth and metastatic potential in xenograft transplant models. Suppression of *PODXL* in NT2 cells by RNAi also attenuates matrix invasion in an *in vitro* assay. These data suggest that epigenetic regulation of the ha-miR-199a-2 locus by CpG methylation results in repression of miR-199a2 expression and upregulation of *PODXL* and that these events correlate with tumour malignancy in testicular cancer. Other targets of miR-199a-5p and -3p are also likely involved in the invasive phenotype of testicular tumours.

3.8 Gastrointestinal cancers

3.8.1 Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a common adult liver malignancy associated with infection by hepatitis (HVB and HVC) and cirrhotic liver disease. Patients with cirrhosis

from a variety of causes are at an increased risk of developing HCC. Unfortunately, the majority of HCC patients (>80%) present with systemic or locally advanced tumours that cannot be cured surgically. Although recently, the application of anti-angiogenic drugs (e.g., sorafenib) has shown promise in slowing tumour growth in HCC patients, chemotherapy and radiotherapy are ineffective, and HCC has a very low five-year survival rate (~5%). Prompt detection and diagnosis of HCC in cirrhotic liver by imaging methods or histological assessment of biopsy is critical for designing an effective treatment strategy.

Although one group showed that PC was only weakly present in 9% of HCC tumours (i.e., neoplastic liver epithelial cells) (Ney et al., 2007), PC and CD34 were commonly upregulated on sinusoidal endothelia associated with primary HCC tumour tissue (Chen et al., 2004; Heukamp et al., 2006). In contrast, in normal liver tissue, PC is restricted to hepatic arterioles (Heukamp et al., 2006). PC and CD34 are also present (in a more punctuate pattern) on the sinusoidal endothelia of hyperplastic focal nodules and liver adenomas (Heukamp et al., 2006). Thus, expression of PC (and CD34) may provide an additional diagnostic marker for the evaluation of liver biopsies in HCC. PC and CD34 are widely expressed in vascular endothelia in adult tissues but their function remains ill-defined. Although PC has been shown to play a role in the formation of vascular lumens in the developing embryo, it is not essential for the homeostatic maintenance of vascular endothelia in adults (at least in mice). Nevertheless, mounting evidence suggests that PC and CD34 have a role in the formation of new vessels during development and vascularization of solid tumours; and, the maintenance of vessel integrity in inflamed tissues (Blanchet et al., 2008; Maltby et al., 2011; Strilic et al., 2009). Thus, further study of the role of CD34 and PC in neovascularization and vessel homeostasis may provide new targets for anti-angiogenic treatments for solid tumours.

3.8.2 Pancreatic ductal adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) is the most common pancreatic cancer, often detected at an advanced stage. Because there are no effective systemic therapies, PDAC diagnosis typically has a very poor prognosis (~3% 5-yr survival). Determining the primary source of adenocarcinoma is important in treatment strategies and prognosis. Neoplasms originating from the pancreatic duct, gall bladder, bile duct, ampulla of Vater and duodenum are difficult to distinguish by immunohistology alone since many express a similar profile of epithelial cytokeratin- and mucin-family proteins. Immunohistochemical analysis of primary adenocarcinoma and carcinoma tumours of gastrointestinal, pancreatic and extrapancreatic origin suggest expression of PC is associated with primary epithelial neoplasms of the pancreatic duct (44%) or ampullary origin (30%) (Ney et al., 2007). Although PC is expressed is less than half of these adenocarcinomas, it is not detected (or very weak) in normal or inflamed pancreas (chronic pancreatitis) or in most primary tumours from all other gastrointestinal sites including bile duct. Although these data suggest that expression of PC may be useful for the differential identification of primary adenocarcinoma, it is not known if PC expression at secondary tumour sites correlates with their primary origin. However, expression of PC does appear to correlate with more advanced histological tumour grade, as 53% of Grade 3, 48% of Grade 2 and 18% of Grade 1 tumours express PC (Ney et al., 2007). Thus, as with several other epithelial malignancies described in this review, expression of PC correlates with a more anaplastic, poorly differentiated phenotype and more aggressive (or advanced) cancers.

3.8.3 Colorectal cancer

Although the expression of PC has not been verified in primary or metastatic tumours in human colorectal cancer, two studies have identified PC expression in colon cancer cell lines, and PC protein as a shed, soluble component of colon cancer cell cultures (Ito et al., 2007; Thomas et al., 2009) In addition, Thomas et al (Thomas et al., 2009) demonstrated that PC, immunopurified from a colon cancer cell line (LS174T), can act as an E- and L-selectin ligand (but not P-selectin) using a PC-coated microbead flow adhesion assay. E/L-selectin binding was dependent on a sialofucosylated epitope of PC expressed in LS174T, similar to, but distinct from sulfated sLe^x epitopes present on sialomucins processed in HEV (see **§5.2**) (Thomas et al., 2009). Thus, tumor-specific modifications (glycosylation) of PC may allow for interaction with selectins.

Importantly, neither of these studies showed that PC protein is expressed on the surface of colon carcinoma cells (as opposed to intracellular), or that PC serves as an E/L-selectin adhesive ligand *in vitro* or *in vivo*. If PC were indeed shown to be a *bona fide* E/L-selectin ligand on these carcinomas, this would offer an attractive target for the development of adjuvant therapies. This is especially true if the unique modification of PC detected in human colon cancer cell lines is recapitulated in primary and metastatic colorectal adenomas and carcinomas. Retrospective analyses of tissue microarrays of primary colorectal carcinomas and adenocarcinomas (and perhaps metastatic, secondary tumour sites) similar to those performed for RCC and breast cancer are required to determine if expression of PC is associated with colorectal cancers and if expression correlates with patient outcome. At least one study (Ney et al., 2007) suggested that PC is not commonly expressed in gastrointestinal tumours, including colon-derived, with the exception of pancreatic cancers. Then again, this is also true for breast and renal cancer – the prognostic power of evaluating PC expression in human cancer appears to be in identifying rare, highly lethal sub-types.

3.9 AML, ALL and cutaneous myeloid sarcoma

PC expression has been reported on a human monocytic cell line (U937) after induced differentiation with vitamin D3 and transforming growth-factor beta (TGFβ) and on normal human monocytes treated with macrophage colony-stimulating factor (MCSF) for 3-6 days *in vitro* (Riccioni et al., 2006). In both cases, PC expression correlates with a more differentiated, monocytic cell-surface marker phenotype (e.g., $CD14^+CD11b^+$). However, PC expression has not been reported on human monocytes derived from normal bone morrow (BM) or peripheral blood (Kelley et al., 2005).

Kelley et al (Kelley et al., 2005) found that PC is often expressed on leukemic blasts including 77% (n = 39) of acute myeloid leukemia (AML), 81% (n = 27) acute lymphoid leukemia (ALL) and 87% (n=15) cases of cutaneous myeloid sarcoma (Kelley et al., 2005). Expression of PC was detected in all clinical sub-types of AML (M1-M7) and both T and B-cell lineage ALL. A second study reported a much lower frequency of PC-positive blasts in 73 cases of AML with approximately 20% of cases presenting with a moderate frequency of PC-positive cells (20-50% positive blasts (+)) and 18% displaying high frequency (>50% positive blasts (++)) (Riccioni et al., 2006). Importantly, while Kelley et al used immunohistochemical analysis of formalin-fixed BM biopsy tissue and PC was observed in a punctuate, cytoplasmic distribution; Ricconini et al used freshly isolated BM or peripheral blood-derived leukemic blasts and flow cytometery analysis (i.e., cell-surface expression of PC). Thus, while PC is commonly expressed in leukemic blasts in the BM, cell-surface exposure of PC is likely more rare (at least in AML).

There are no studies that address the clinical consequences of PC expression in leukemia. Expression of PC in hematopoietic malignancies may reflect transformation of an immature progenitor that normally expresses PC or a "de-differentiation" gene expression pattern of a leukemic stem cell. We have found that expression of PC in mouse hematopoietic stem or progenitor cells (HSC/Ps) enhances CXCR4-induced chemotaxis and BM-homing/retention (**see §6.2.3**) (Nielsen & McNagny, 2009a) (and our unpublished observations). Thus, one possible consequence of PC overexpression in leukemia may be that PC enhances close contact to CXCL12-secreting cells and, subsequently, an advantage (over normal hematopoietic progenitors or PC-negative leukemia) with to respect retention, proliferation, survival or chemo-resistance of leukemic cells.

3.10 Malignant astrocytic tumours

Astrocytomas, tumours originating from astrocytes of the brain and spinal cord, are the most common neoplasm of the central nervous system (Zhu & Parada, 2002). Often presenting at an advanced stage, astrocytic tumours are classified into four histological grades that broadly include benign (I), diffuse (low-grade) (II), anaplastic astrocytoma (III) and glioblastoma (IV). The most aggressive, glioblastoma (GBM), is also the most common malignant astrocytic tumour with a 5-year survival rate of less than 3%. Although systematic metastasis of astrocytomas is rare, aggressive local invasion into surrounding tissue, rapid growth and resistance to apoptosis accounts for the lethality of these brain cancers – tumour behaviours supported by aggressive angiogenesis (Anderson et al., 2008).

In a study of 51 astrocytomas using immunohistochemical staining of frozen sections, immunoblotting of tumour lysates and quantitative real-time PCR, PC expression was found to be present in approximately half of the cases of anaplastic astrocytoma and GBM, but none of the lower-grade astrocytomas (Hayatsu et al., 2008). The pattern of staining in the anaplastic astrocytomas is consistent with cell-surface expression of PC; however, PC localization in GBM was more diffuse and PC-positive tumour cells were associated with regions of microvascular proliferation. Using a lectin-binding microarray combined with a mass spectrometry approach, another group detected PNA-reactive PC abundantly expressed on the surface of a "stem cell like" human GBM cell line (GBM1), but not on an adherent GMB line (U373) (He et al., 2010).

Notably, the sialomucin podoplanin (*PDPN*), which shares some striking structural and functional properties with PC, is also highly expressed in glioblastoma (Mishima et al., 2006). Podoplanin is expressed on the outer/invasive edge of some tumours and is thought to have a role in the anti-adhesive phenotype of EMT-independent tumour invasion (Wicki and Christofori, 2007). Invoking a similar paradigm to breast and ovarian carcinoma (**§3.2.3 & 3.3**), we posit that high expression of PC may promote an EMT-independent morphogenesis that, combined with other transforming pathways, promotes an invasive phenotype in astrocytomas.

4. Podocalyxin marks embryonal carcinomas and embryonic stem cells

PODXL transcript and protein is down regulated in embryoid bodies (EB) and lineage-restricted EB cultures compared to undifferentiated human embryonic stem cells (hESCs) (Brandenberger et al., 2004; Cai et al., 2006). PC-binding antibodies TRA-1-60 and TRA-1-81, together with membrane surface, stage-specific embryonic antigens (SSEA)-3 and SSEA-4; and, intracellular markers alkaline phosphatase, telomerase, Oct4 and Nanog are routinely

used to characterize undifferentiated hESC (De Miguel et al., 2010; Palecek, 2011). As mentioned previously, the TRA-1-60 and TRA-1-81 epitopes are present only on the high molecular weight form of PC detected in embryonal carcinoma (EC) cell lines and these markers are lost upon induced differentiation of EC or hESC. Thus, PC is a marker of undifferentiated human embryonal cells and PC is either alternately modified (e.g., glycosylation pattern) or its expression is reduced upon differentiation. In an effort to identify novel markers of undifferentiated hESC, Choo et al generated an anti-PC antibody (IgM isotype, mAb 84) cytotoxic to undifferentiated hESC (human embryonic stem (HES) cell lines HES-2, -3 and -4) and at least one embryonal carcinoma cell line (NCCIT) (Choo et al., 2008). Importantly, mAb 84 is not cytotoxic to mature human cell lines (HEK-293, HeLa) or hESC induced to differentiate by withdrawal of fibroblast growth factor 2 or following culture of hESC to form EB (day 22). Remarkably, mAb 84 rapidly (< 45 minutes) kills EC even at 4°C. The mechanism for mAb 84 cytotoxicity is not dependent on complement activation, hypercrosslinking of bound antibody or antibody internalization. Instead, mAb 84 mediates cell death by oncosis initiated by PC-mediated reassembly of cytoskeleton, degradation of cytoskeletal structural proteins (α-actinin, talin and paxillin), PC-aggregation and formation of large membrane pores (Tan et al., 2009). Since crosslinking other PC-specific antibodies bound to hESCs is not cytotoxic, the association of mAb 84 to a unique PC epitope (or potentially other additional unknown epitopes on hESCs) induces cytoskeletal reassembly in advance of PC aggregation and pore formation. Studies with antibody fragments revealed that cytotoxicity requires divalency with sufficient flexibility linking the antigen binding domains (Lim et al., 2011). Since pre-treatment of HES-3 hESC and NCCIT cultures with mAb 84 *in vitro* prevents teratoma formation in a severe combined immunodeficient (SCID) mouse xenograft model mAb 84 has potential applications in clearing tumour-forming undifferentiated hESCs from cultures of differentiated hESCs intended for cell-based, regenerative therapies (Choo et al., 2008; Tan et al., 2009). With respect to the treatment of cancer, a cytotoxic antibody like mAb 84 may also prove to be an effective oncolytic adjuvant therapy in germ-line tumours like testicular cancer (especially embryonal carcinoma subtype). This approach, of course, would require highly selective targeting of tumour-specific forms of PC to avoid renal toxicity. The encouraging example of mAb 84 suggests that highly selective targeting of PC expressing tumour cells *in vivo* may be possible.

5. Podocalyxin structure and function

In an effort to determine how expression of PC on cancer cells promotes tumour aggressiveness and metastasis, several groups, including our own, have performed structure-functional analyses in model cancer cell lines to delineate the specific roles of PC's protein domains in the regulation of cell morphology, motility and survival. The following sections summarize some of what is known of the molecular mechanisms regulated by PC. PC's highly charged and bulky extracellular domain imparts a biophysical anti-adhesive property that regulates cell morphology and cell-cell adhesion functions and, when appropriately modified, may also promote adhesion by binding to L- or E-selectin. The intracellular domain links apically-polarized PC to the cortical actin cytoskeleton. By recruiting ezrin and NHERF-1/2, PC's intracellular domain localizes signalling complexes at apical membrane domains. In general, expression of PC promotes cell motility and invasion functions in epithelial cells and promotes chemotaxis in hematopoietic cells.

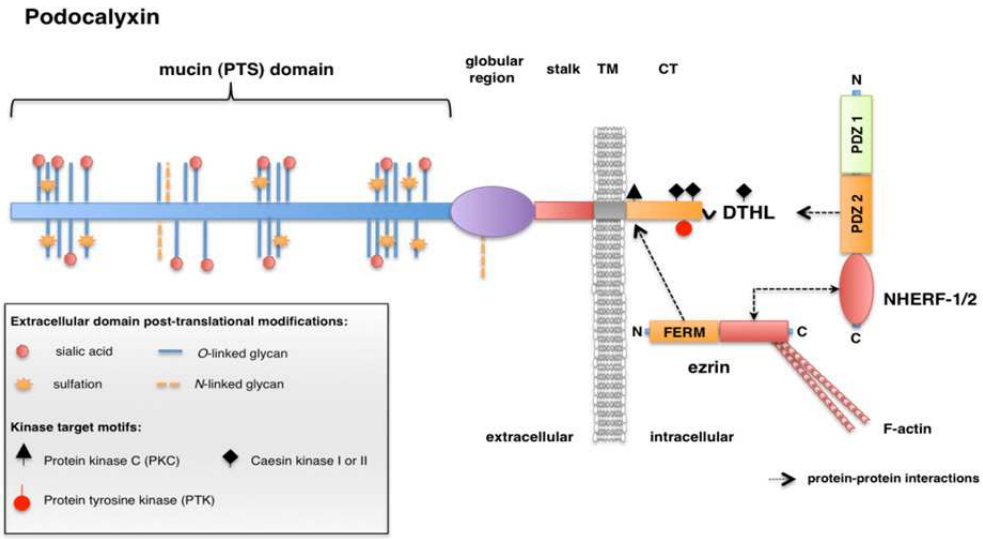


Fig. 5. Podocalyxin associates with NHERF-1/2 and ezrin to nucleate a variety of intracellular signalling complexes.

5.1 Podocalyxin is a CD34-family sialomucin

Podocalyxin is one of three members of the CD34-family of sialomucins that also includes CD34 and endoglycan (reviewed in Furness & McNagny, 2006). Mucins are members of a large class of secreted and type I transmembrane proteins that contain at least one extracellular domain rich in proline, serine and threonine residues (PTS domain). Mucin domains are extensively modified by post-translational addition of *O*-linked oligosaccharides (at S/T residues) and frequently modified by *N*-linked glycosylation and sulfation. Sialomucins, in particular, are also heavily modified by the addition of a terminal sialic acid to terminal *O*-linked glycans. PC is a sialomucin that, together with CD34 and endoglycan, makes up the CD34-family (reviewed in Furness and McNagny, 2006). The mature, cell surface expressed human PC protein is a 536 residue glycoprotein with a calculated peptide mass of 56 kilodaltons (kD); however, glycosylation and other post-translational modifications yield a product with an apparent molecular weight of 150-200 kD. The extracellular domain of PC consists of a heavily *O*-linked glycosylated, sialated and sulfated mucin domain, a globular domain containing a conserved four-cysteine (Cys) motif, and, a membrane-proximal stalk domain. Three or four sites of *N*-linked glycosylation also decorate the extracellular domain. Together, these modifications result in a highly negatively charged, sterically bulky glycoprotein (**Fig. 5**). Although the primary amino acid sequence of PC's extracellular domain is not well conserved by paralogs (or CD34-family homologs), the intracellular domain is highly conserved across species.

5.2 Extracellular ligands of podocalyxin

The only well-established extracellular ligand for CD34-family sialomucins is L-selectin (reviewed in (Furness & McNagny, 2006; Nielsen & McNagny, 2008)), however there are

also reports to suggest that, when appropriately modified, they may also bind E-selectin (Thomas et al., 2009). Selectins are a family of mammalian C-type lectin carbohydrate-binding proteins normally expressed by hematopoietic cells (L- and P-) or endothelia (P- and E-) (Laubli & Borsig, 2010). L-selectin is responsible for naïve homing of leukocytes to secondary lymphoid organs; whereas, P- and E-selectins play a predominant role in mediating homing of white blood cells to inflamed (or specialized) tissues. Recognition of CD34-family sialomucins by selectins requires an unusual carbohydrate post-translational modification (sulfated sialyl Lewis-X (sLe^X)). Although this modification is present on CD34-family sialomucins expressed by high endothelial venules (HEV) of lymph nodes, it is not typically found on CD34-type proteins expressed by non-lymphoid endothelia (the vast majority of all endothelia). Thus, although selectin-binding functions for CD34 sialomucins (including PC) have been demonstrated *in vitro*, the biological significance of this function is unclear for most endothelium *in vivo*. Nevertheless, the interaction of tumour cells with selectins (or expression of selectins or selectin ligands by tumour cells) is an important paradigm in cancer metastases (reviewed in Laubli & Borsig, 2010). Thus, aberrant modification (sLe^X) or enhanced expression of PC in malignant cells (Kannagi, 2004) may facilitate selectin-mediated tissue homing and trafficking.

5.3 Intracellular binding partners of podocalyxin

The intracellular domain of PC contains a membrane proximal ERM (ezrin, radixin, moesin)-protein association domain, as well as putative target sites for serine and threonine (S/T) kinases (e.g., casein kinases I/II (CKI/II) and protein kinase C (PKC)) and protein tyrosine kinases (PTK) (**Fig. 5**). In addition, the four C-terminal amino acids of the intracellular domain, aspartate-threonine-histidine-leucine (DTHL) is an interaction motif for proteins containing a PSD-95/Dlg/ZO-1 (PDZ)-domain.

The best-characterized intracellular binding partners for PC are ezrin and NHERF isoforms 1 and 2 (solute carrier family 9 (Na^+/H^+ exchanger), member 3 regulator -1 and -2) (**Fig. 5**). Notably, dysregulated expression, localization and mutants of ezrin and NHERF-1 (also called EBP50) and -2 have been implicated in human cancers (Dai et al., 2004; Brambilla & Fais, 2009; Mangia et al., 2009; Hayashi et al., 2010). As adaptor proteins with diverse binding partners, ezrin and NHERF-1/2 have the potential to link PC to several signalling pathways (see **Table 2**). Likewise, by targeting ezrin and NHERF-1/2 to select apical membrane domains, PC may regulate the localization, function and availability of these complexes.

5.3.1 NHERF-1 and NHERF-2

NHERF-1 and -2 both have two tandem PDZ domains - the centrally located PDZ class II domain associates with PC's C-terminal DTHL motif, leaving NHERF's N-terminal PDZ class I domain free to interact with other binding partners. **Table 2** provides a partial list of known NHERF-1/2 binding partners. Ezrin can either directly binds to PC via the membrane proximal ERM protein-binding motif or indirectly via the C-terminal ERM motif of NHERF-1/2. NHERF-1/2 have a wide range of binding partners that include ion channels, G protein coupled receptors, receptor tyrosine kinases (RTKs), intracellular signalling intermediates and β-catenin, a transcription factor (**Table 2**). NHERF-1/2 may also homodimerize or polymerize via PDZ-domain interactions, or bind other PDZ domain containing proteins like PDZ K1 (Garbett et al., 2010).

NHERF-1/2 binding partners	Ezrin binding partners
Sialomucins Podocalyxin, endoglycan (but not CD34) ***Receptor tyrosine kinases*** Epidermal growth factor receptor (EGFR), Platelet derived growth factor receptor (PDGFR) ***G protein coupled receptors*** β2-adrenergic receptor, κ-opiod receptor, Frizzled isoforms (Wheeler et al., 2011), Parathyroid hormone receptor, P2Y1 purinergic receptor ***Ion channels*** Cystic fibrosis transmembrane conductance regulator (CTFR), Na^+/H^+ exchanger member 3 (NHE3) TRPC4 (transient receptor potential cation channel 4) (Lee-Kwon et al., 2005) Mrp2 (multidrug resistance-associated protein 2) (Li et al., 2010) ***Transcription factors*** β-catenin ***PDZ domain proteins*** PDZ K1 (Garbett et al., 2010), NHERF-1/2 (homodimer or polymer) ***ERM proteins*** Ezrin, radixin, moesin, merlin ***PI3K pathway regulators*** PTEN (Takahashi et al., 2006), Akt (Wang et al., 2008) ***Rho-family regulators or downstream effectors*** ARHGEF7 (Hsu et al., 2010), RACK1 (Liedtke et al., 2004)	***Sialomucins*** Podocalyxin, CD44, CD43, PSGL-1 ***Non-receptor tyrosine kinases*** Focal adhesion kinase (FAK), Src-family kinase (SFKs) ***Integrin-binding adhesion molecules*** Intercellular-adhesion molecules (ICAMs) -1, -2 & -4 ***Serine/threonine kinase*** Protein kinase A (PKA) ***Phosphatidylinositol 3-kinases*** p85 of class I PI3Ks ***Cytoskeleton/adaptors*** F-actin, NHERF-1/2 ***Membrane phospholipids*** PI (4,5) P_2 ***Ca^{2+} signaling effectors*** S100P (Austermann et al., 2008) ***Rho-family regulators or downstream effectors*** RhoGDI (Schmieder et al., 2004)

Table 2. A partial list of reported binding partners of NHERF-1/2 and ezrin. Specific binding references are shown in the table. For reviews of NHERF-1/2 binding interactions see references Weinman et al., 2006; Georgescu et al., 2008; Nielsen and McNagny, 2009 & Hayashi et al., 2010. Ezrin binding interactions were recently reviewed in Bambilla & Fais, 2009.

5.3.2 Ezrin

Ezrin acts as an intracellular adaptor to provide linkage of integral membrane proteins with the cytoskeleton. Ezrin contains an N-terminal FERM protein-interaction domain capable of binding the intracellular domain of several type I transmembrane proteins, NHERF-1/2, RhoGDI and the membrane lipid, phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$), and a C-terminal domain that bind polymerized actin (F-actin) (**Table 2**). The C-terminal domain of ERM folds over to associate with its N-terminus in an inactive conformation and

phosphorylation of a conserved Thr residue in the C-terminus, downstream of Rho kinase, protein kinase C isoforms (and other S/T kinases), induces an open conformation that permits ERM proteins to link complexes to polymerized actin (F-actin) (Fehon et al., 2010). Phosphorylation of Tyr residues (perhaps by RTKs or Src-family kinases) further enhances ezrin activation or activation of ezrin-dependent signalling events. By using NHERFs as an adaptor, ezrin can link several other transmembrane proteins to the cytoskeleton including ion channels and GPCRs. We note that PC is one of few transmembrane proteins that can bind ezrin both directly and indirectly through NHERF-1/2.

6. The molecular basis for podocalyxin function in tumour metastasis

At least some of PC's molecular functions in normal and neoplastic tissues can be explained by viewing PC as a key electrostatic anti-adhesive force between extracellular membrane surfaces (Gelberg et al., 1996; Takeda et al., 2000; Galeano et al., 2007; Strilic et al., 2009). However, since the intracellular domain of PC is phosphorylated on Ser/Thr and Tyr residues and PC assembles signalling complexes in response to cell activation, PC undoubtedly influences the behaviour of tumour cells by regulating intracellular signalling pathways. Some of these intracellular signalling complexes likely reinforce the localization of PC to the apical membrane of polarized cells. However, they may also enhance (or otherwise regulate) downstream signalling events important in the rearrangement of cytoskeleton and adhesion-independent survival. We propose the extracellular domain of PC is necessary and sufficient for PC's homotypic anti-adhesive functions and morphogenesis of apical membranes. However, stable apical localization of PC, and its effects on the migration and, potentially, survival-promoting functions depends on the intracellular domain and assembled signalling complexes. In the next section we will review the proposed molecular signalling mechanisms regulated by PC and relate these signalling functions to PC's role as a promoter of tumour progression and metastases.

6.1 Podocalyxin regulates adhesion and membrane morphogenesis in epithelial cells

Many of the molecular functions of PC, including signalling mechanisms mediated via ezrin and NHERF-1/2, have been elucidated using the human breast cancer cell line MCF7, the human prostate cancer line PC3 and the canine renal tubule cell line, Madin-Darby canine kidney (MDCK). Since human RCC arise from renal tubule cells, the MDCK model cell line may offer valuable insights into the molecular mechanisms of tumour progression and metastasis in RCC. Although renal tubules in human and rabbit do not express detectable levels of PC protein, MDCK cells and adult canine kidney tubular cells have detectable PC (Cheng et al., 2005; Meder et al., 2005). As mentioned in previous sections, MCF7 and PC3 cells are derived from metastatic breast and prostate tumours, respectively. Both of these cell lines express low to moderate levels of PC and are therefore useful for *in vitro* and *in vivo* assays using methods to force expression of PC or structural mutants of PC. Conversely, RNAi methods can be used to study the functions of endogenous PC in tumour cells or cell lines. Although there are discrepancies in the assignment of functional roles to PC's structural domains (see details below), taken together, these studies show that high expression of PC results in enhanced motility, invasiveness and destabilization of epithelial cell morphology in 2D- and 3D-cultures. These phenotypes are characteristics of aggressive epithelial tumours with propensity to metastasize.

6.1.1 Podocalyxin's function in epithelial architecture and morphogenesis

Although MCF7 cells express moderate levels of *PODXL* transcript (Kao et al., 2009) **(Fig. 1)**; by western blotting and immunofluorescence confocal microscopy, expression of PC is low or not detected, respectively (Nielsen et al., 2007; Sizemore et al., 2007; Somasiri et al., 2004). In order to study the molecular function of PC in breast epithelial cells, others and we have ectopically expressed full-length PC and a series of structural mutants in MCF7 (**Fig. 6**). Overexpression of full-length PC (FL) in MCF7 results in apical bulging of monolayers, reduced transepithelial resistance (i.e., disrupted cell junctions), delamination and shedding of cells from monolayers *in vitro* (Nielsen et al., 2007; Somasiri et al., 2004). Strikingly, expression of PC in MCF7 (and MDCK) potently induces formation of apical microvilli. Epithelial microvilli are common features in specialized epithelial layers involved in the secretion or absorption of nutrients, vesicles and proteins from the extracellular space (Lange, 2011).

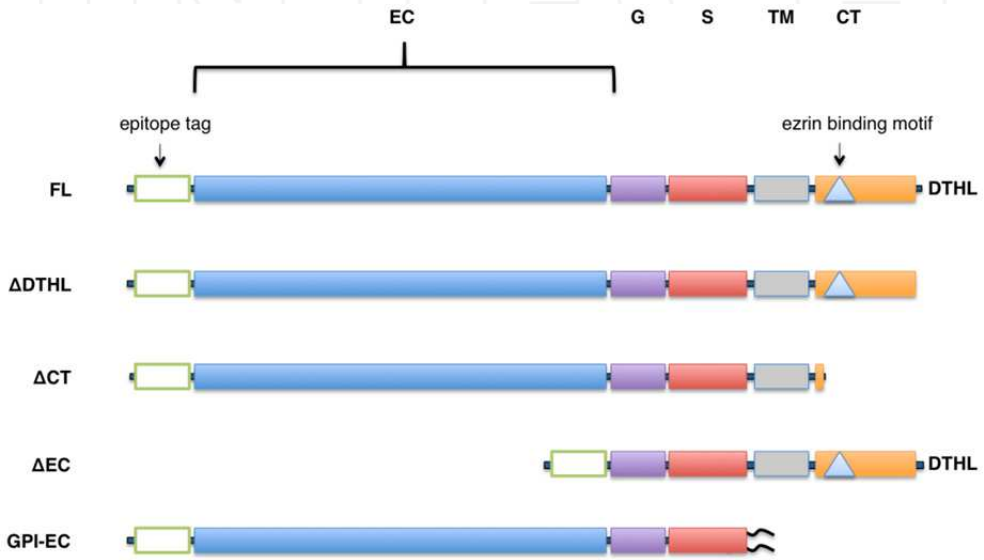


Fig. 6. Schematic of podocalyxin protein structural mutants used to delineate biological functions of specific protein domains. EC- extracellular (*O*-glycosylated mucin) domain, G – globular domain, S- stalk domain, TM- transmembrane domain, CT –cytoplasmic domain. Triangle in membrane proximal CT domain denotes ezrin-binding motif. Adapted from Meder et al, 2005 & Nielsen et al, 2007.

As we anticipated, full-length, exogenously expressed PC recruits NHERF-1, ezrin and F-actin to apical membrane domains and forms microvilli in MCF7 cells. However, surprisingly, the intracellular domain of PC is dispensable for the formation of microvilli since the transmembrane-anchored extracellular domain alone (ΔCT) is sufficient to drive morphogenesis and the recruitment of ezrin and F-actin to apical membranes and microvilli in MCF7 (Nielsen et al., 2007). Notably, NHERF-1 is not recruited to apical domains or microvilli in the absence of PC's C-terminal DTHL domain (ΔDTHL or ΔCT). This result suggests that the extracellular domain, likely by a biophysical charge- and steric-based intermolecular repulsion mechanism, can drive membrane morphogenesis and

rearrangement of the cytoskeleton (including nucleation of ezrin and F-actin in microvilli) to support stable microvilli. We note that this biophysical mechanism requires high expression of PC since endogenous levels of PC in MDCK and MCF7 are not sufficient to drive microvillus formation. However, such high expression of PC may have relevance in some human epithelial cancers, including the rare sub-sets of high PC-positive metastatic breast cancer and renal cell carcinomas mentioned previously.

Although many investigators agree that PC, NHERF-1/2 and ezrin form a complex that marks apical membrane domains, the mechanism of PC's apical targeting is not yet certain. We have found that expression of full-length PC (FL), and PC mutants ΔCT, ΔDTHL and ΔEC (**Fig. 6**) in MCF7 result in sorting of these exogenously expressed proteins to the apical membrane domain. Conversely, using a similar series of PC mutants expressed in MDCK cells, two other laboratories show that precise apical targeting of PC requires its intracellular domain (Cheng et al., 2005; Meder et al., 2005). In these studies, although the majority of intracellular-deletion PC mutants were still sorted to the apical domain, only full-length PC with an intact *O*-linked glycosylation extracellular domain plus an intracellular domain was targeted to apical domains in a way that precisely co-localized with endogenous PC. A GPI-linked PC extracellular domain mutant (GPI-EC, **Fig. 6**) displayed the most non-restricted membrane localization, suggesting that the transmembrane region of PC may play a role in excluding PC from basal lateral membranes and cell-cell contacts. However, Meder et al show that a non-membrane anchored construct of PC is predominantly secreted from the apical face, thus suggesting that the extracellular domain dictates initial apical sorting of PC in epithelial cells (Meder et al., 2005). Thus, although the extracellular domain of PC may be sufficient for initial apical distribution, the ezrin and NHERF-1/2 binding determinants in the intracellular domain reinforce and stabilize the apical targeting of PC in polarized epithelial sheets.

The above studies were performed by expressing PC constructs in epithelial cell lines that express low to moderate levels of endogenous PC. Using a siRNA-mediated gene-silencing approach to knockdown *PODXL* in MDCK cells, Meder et al show that endogenous PC is required for appropriate polarization of epithelial sheets and lumen formation in MDCK clusters in a 3D semi-solid culture medium containing extracellular matrix components (Meder et al., 2005). While the parental MDCK line formed organized spheres with a central lumen when suspended in matrix, deletion of PC disrupts this architecture, and MDCK fail to form an organized lumen (Meder et al., 2005). Correspondingly, using siRNA-mediated knockdown of endogenous PC in MDCK, Cheng et al show that endogenous PC is required for hepatocyte growth factor (HGF)-induced tubulogenesis (Chen et al., 2005). Transfection with siRNA-resistant PC (FL) and mutant constructs reveals that tubule formation of MDCK requires the intracellular domain. However, PC's extracellular *O*-linked glycosylated mucin domain has a role in refining the architecture of tubule lumens. Finally, enforced expression of PC in MDCK cells inhibits cell-cell adhesion and disrupts junctional complexes. This effect is dependent on sialylation of PC, suggesting a charge-repulsion mechanism.

6.1.2 NHERF-herding function of podocalyxin's intracellular domain

Although the mechanisms responsible for the apical localization of PC require further investigation, it is clear that one of PC's functional roles is the recruitment of adaptor proteins to apical domains. By binding ezrin and NHERF-1/2, PC has the potential to impact the activity or compartmentalization of a variety of signalling pathways. While some

of PC's functions can be explained purely by invoking a biophysical charge/steric-repulsion paradigm, several biological functions of PC undoubtedly require recruitment of ezrin and NHERF into apical-domain signalling complexes. Since PC can recruit and restrict NHERF and ezrin (and potentially their numerous binding partners (**Table 2**)) to apical membrane domains, we posit that many of PC's functions stem from the concentration of signalling complexes – either down regulating signals in other membrane domains (e.g., adherens junctions or focal adhesions) or enhancing signals in the apical domains.

Cortactin (*CTTN*) is an F-actin- and Arp2/3-binding adaptor involved in the branching and nucleation of the cortical actin cytoskeleton and formation of membrane protrusions (reviewed in Weaver, 2008). Cortactin's localization and functional activation is regulated by a large number of binding partners. Importantly, cortactin is enriched in cell protrusions called "invadopodia" and has a role in the invasion of tumour cells through extracellular matrix (Weaver, 2008). PC was found to co-localize with cortactin (and perhaps directly or indirectly bind) at the apical domain of rat podocytes (Kobayashi et al., 2009). Effacement of podocytes caused phosphorylation of cortactin, dissociation of cortactin from PC/apical domains and translocation of cortactin to the basolateral face (Kobayashi et al., 2009). Although the interaction of cortactin and PC in invasive tumours has not yet been examined, PC localizes to "invadopodia"-like protrusions in *in vitro* assays. Furthermore, cortactin is known to regulate the secretion of MMPs at invadopodia. In this way, and by recruitment of NHERFs and ezrin, PC may regulate changes in cortical actin assembly associated with tumour invasion and metastasis. This idea is supported by an experiment showing that forced expression of PC in MCF7 enhances expression and secretion of matrix metalloproteinases (MMP)-1 and MMP-9 (Sizemore et al., 2007).

6.2 Podocalyxin's role in the regulation of tumour growth, adherence-independent survival and cell motility

6.2.1 Regulation of Rho-family GTPases

In MDCK cells, formation of a PC-NHERF-1-ezrin ternary complex regulates RhoA activation (Schmieder et al., 2004). As part of a regulatory cycle controlling activation state of RhoA, RhoGDI maintains RhoA in an inactive GDP-bound state. Redistribution of RhoGDI to the apical membrane domain complex PC-NHERF-1-ezrin frees RhoA for activation by guanine exchange factors. Although sequestration of RhoGDI is not dependent on the interaction of PC and NHERF-1 (but does depend on ezrin), activation of RhoA requires NHERF-1 association with PC's C-terminal DTHL motif (**Fig. 7**).

A recent study corroborates and extends this finding by showing that the PC-NHERF-ezrin ternary complex also regulates activation of Rac1 (a Rho-family member) by a mechanism whereby the N-terminal PDZ1 domain of NHERF-1 recruits the Rho guanine nucleotide exchange factor 7 (*ARHGEF7*) to this complex via ARHGEF7's C-terminal ENTL (a PDZ-binding motif) (**Fig. 7**) (Hsu et al., 2010). Cell motility requires a coordinated reorganization of the cytoskeleton, a process regulated in part by Rho-family GTPases. By regulating the activity and apical localization of RhoGDI, and ARHGEF7, PC may promote motility in some circumstances. Thus, in addition to dramatic morphogenic changes in the apical domain of epithelial cells resulting in reduced cell-cell and cell-matrix adhesion, inappropriate or unregulated expression of PC may promote cell motility by regulating the activity of small GTPases. Although the above studies were conducted in MDCK cells, others have shown that Rac1 mediates PC-enhanced invasion and motility of PC3 and MCF7 cells (Sizemore et al., 2007).

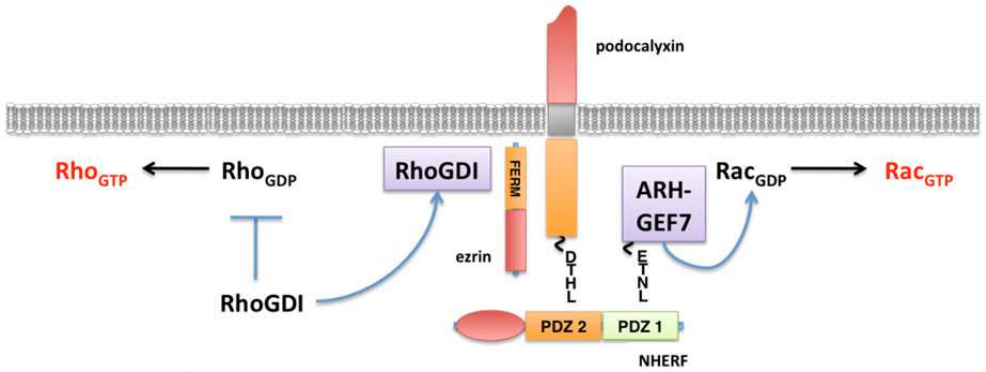


Fig. 7. Podocalyxin enhances activation of Rho-family GTPases by sequestration of RhoGDI and recruitment of a guanine exchange factor (ARHGEF7) to a PC-ezrin-NHERF complex assembled at membrane domains.

6.2.2 ERK and PI3K pathways

Forced expression of PC in MCF7 and PC3 cells induces ezrin-dependent activation of the Akt and Erk1/2 pathways (Sizemore et al., 2007). Moreover, activation of PI3K/Akt and Erk1/2 is required for PC-enhanced invasiveness of MCF7 and PC3. Invasiveness of PC-expressing cells was mediated in part by expression and secretion of matrix metalloproteinase -9 (MMP-9) and MMP-1, which in turn, required expression of ezrin and activation of PI3K and Erk1/2 (Sizemore et al., 2007).

Tyrosine phosphorylation of ezrin at central residue (Y353) has been shown to bind the regulatory subunit (p85) of class I PI3Ks and this association, in turn, induces activation of the PI3K pathway (Fievet et al., 2004; Gautreau et al., 1999). In addition, tyrosine phosphorylation of a residue (Y567) in ezrin's C-terminal domain disrupts the active conformation of ezrin and promotes intermolecular interactions with F-actin and other binding partners (Brambilla & Fais, 2009). Expression of PC in MCF7 and PC3 enhances phosphorylation of both the PI3K-activating Y353 and Y567 residues of ezrin (perhaps by Src-family kinases) (Sizemore et al., 2007). Activation of Erk1/2 via ezrin may be more indirect, proceeding through activation of Rho-family GTPases (Sahai and Marshall, 2002).

6.3 Podocalyxin enhances CXCL12-mediated migration of hematopoietic cells

CXCL12 (formerly known as stromal derived factor 1 (SDF1)) is a critical chemotatic factor for the maintenance of hematopoietic cells in the BM niche and also for the maintenance of secondary lymphoid organ architecture. CXCL12 is a ligand for two GPCR chemokine receptors, CXCR4 and CXCR7 (reviewed in Teicher and Fricker, 2010). CXCR4 has a well-established role in mediating CXCL12 chemotatic cues in cells, whereas CXCR7 does not appear to be a competent signalling receptor on its own, but may regulate CXCR4 activity. Importantly, CXCR4 and CXCR7 expression or function is dysregulated in many human cancers, including (but not limited to) myleloid and lymphoid leukemias and many of the carcinomas discussed in this review. Expression or dysregulated signalling of CXCR4 and CXCR7 promote the metastasis and survival of cancers to (or within) tissues rich in CXCL12 (Sun et al., 2010).

In our efforts to understand the function of PC expression on hematopoietic stem and progenitor cells (HSC/Ps), we have used an RNAi approach to knockdown *Podxl* expression

in a mouse myeloid-progenitor, factor dependent cell line, FDCP-1. We have previously shown that PC is highly expressed in FDCP-1 and, upon stimulation with interleukin-3 (IL3), PC is redistributed from a global membrane expression pattern to a polarized membrane domain that also recruits NHERF-1 (Tan et al., 2006). Recently, we have found that silencing *Podxl* in FDCP-1 inhibits CXCL12-mediated migration (manuscript submitted). In corroboration of this finding, primary hematopoietic cells derived from *Podxl*$^{-/-}$ fetal liver (day 15.5 embryos) also display impaired migration to CXCL12 in an *in vitro* assay. Although we have not yet uncovered the molecular mechanisms underlying PC-enhanced migration to CXCL12, we have found that stimulation of FDCP-1 (or mouse fetal liver) with stem cell factor induces enhanced surface exposure CXCR4 that then becomes distributed with PC to a common polarized membrane domain. This distribution suggests that PC and CXCR4 can physically associate at the cell surface. Indeed, CXCR4 can be co-precipitated with PC antibodies under some conditions (unpublished observations). We hypothesize that PC, as exemplified by the related sialomucins CD34, PSGL-1 and CD164 (endolyn) (Forde et al., 2007; Veerman et al., 2007; Blanchet et al, 2011), has an active role in hematopoietic cell migration and tissue homing by stabilizing or enhancing chemokine receptor signalling. Although we currently favour a mechanism by which PC associates with, and physically stabilizes, CXCR4 at a polarized membrane domain, PC may also enhance intracellular CXCR4 signalling pathways including activation of Rho GTPase, PI3K and Erk1/2 (**see §6.2.1 & 6.2.2**).

6.4 Stabilization of the glucose-3-transporter (GLUT3)

GLUT3 (*SLC2A3*), one of 14 glucose transporter family members (GLUT1-14), was originally identified as a neuronal-specific glucose transporter but is also expressed in other tissues with high-energy demands, including cancer cells (Macheda et al., 2005; Thorens & Mueckler, 2010; Cairns et al., 2011). PC was recently found to form a stable complex with GLUT3 in human embryonal carcinoma cells (Tera-1 and NCITT) since GLUT3 can be co-precipitated from cell lysates using PNA or anti-PC antibody (and, inversely, podocalyxin is co-precipitated with anti-GLUT3 antibody) (Schopperle et al., 2010). Suppression of *PODXL* expression via siRNA also reduced GLUT3 protein concentrations in NCITT (Schopperle et al., 2010) – suggesting that PC may stabilize GLUT3 protein or regulate expression of GLUT3.

7. Conclusion

7.1 Podocalyxin drives tissue morphogenesis and promotes tumour invasion, metastasis and survival

By expressing full-length and mutant forms of PC in cancer cell lines and assessing cell morphology, apical targeting of proteins, motility and invasion, we and others begun to assign specific functions to PC structural domains. An emerging theme is that the highly charged and glycosylated extracellular domain of PC helps to define the apical domain of epithelial sheets – perhaps purely by a biophysical charge- and steric-repulsion mechanism. However, when overexpressed at high concentration, PC can drive epithelial cell morphogenesis and trigger intracellular cytoskeletal rearrangements that support formation of microvilli (possibly as a mechanism for coping with apical domain expansion), reduce adhesion to extracellular matrix and adjacent cells; and, facilitate cell shedding from monolayers. In this way, overexpression of PC promotes EMT-independent tumour invasion and metastasis. **Table 3** summarizes these and other tumour-promoting mechanisms regulated by PC.

Mechanism of invasion & metastasis	Podocalyxin functions in tumour cells
Morphogenesis & detachment from basement membranes	• Inter- and intra-cellular biophysical charge-repulsion • Recruitment/sequestration of cortical actin complexes to apical membrane and weakening of ECM adhesion • Exclusion of integrins from the apical domain
Invasion of surrounding tissue	• PC association with collagen at leading edge • Recruitment and apical localization of cortactin and induced expression and secretion of MMPs at the leading edge
High motility	• Enhanced CXCL12/CXCR4 axis signalling and chemotaxis • Activation and localization of Rho-family GTPases • Enhanced activation of PI3K and ERK1/2
Homing and to distant tissue sites	• Potential E/L-selectin ligand in some tumours • CXCL12/CXCR4 mediated engraftment and survival in secondary tissue
Adherence-independent survival	• Enhanced activation of PI3K/Akt and Erk 1/2 pathways • Promote expression/stabilization of GLUT3

Table 3. Mechanisms of podocalyxin-enhanced tumour progression.

Although the initial apical domain-sorting of PC may be, in part, a property of extracellular or transmembrane determinants, the prevailing evidence suggests that association of PC's intracellular domain with ezrin and NHERF-1/2 (and consequently to F-actin and the cytoskeleton), is responsible for fine-tuning or stabilizing the apical localization of PC in epithelial cells. In other words, NHERF-1/2 and ezrin enhance the efficient and functionally appropriate apical targeting of PC. Subsequently, PC engagement of NHERF-1/2 and ezrin can serve to nucleate signalling complexes at these apical domains and regulate the spatial signaling by these complexes. For example, NHERF-1/2 and ezrin promote activation of Rho-family GTPases, and cooperate to promote the activation of the Erk1/2 and PI3K/Akt pathways. In addition, since NHERF-1/2 and ezrin themselves have multiple protein-protein interaction domains, regulatory motifs and associate with numerous signalling intermediates (**Table 2**), the potential function of PC in regulating apical signalling events is likely much more extensive. By recruiting NHERF-1/2 and ezrin, PC has a role in the temporal and spatial localization of signalling intermediates that contribute to enhanced invasion, motility and adhesion-independent survival of tumor cells.

PC recruits cortactin, to apical membrane domains by an as-yet undetermined mechanism that may be independent of NHERF-1/2 and ezrin. Cortactin is commonly localized to leading edge tumour cell protrusions, called invadopodia, where it promotes invasive functions, including the secretion of matrix metalloproteinase and regulates cortical actin rearrangements that promote cell morphogenesis and motility. In addition, by stabilizing GLUT3, PC may not only support a metabolic switch to oxygen-poor glycolysis, but also alter downstream glycosylation machinery. Both of these events have the potential to promote adhesion-independent survival and metastasis of tumour cells.

Since some sialomucins, including endolyn (CD164) and PSGL-1, have been implicated in enhanced chemokine-mediated migration of hematopoietic cells (Forde et al., 2007; Veerman et al., 2007), we have also examined PC's role in mediating the migration of hematopoietic precursor cells to specific chemokines. Our preliminary work suggests that PC promotes the

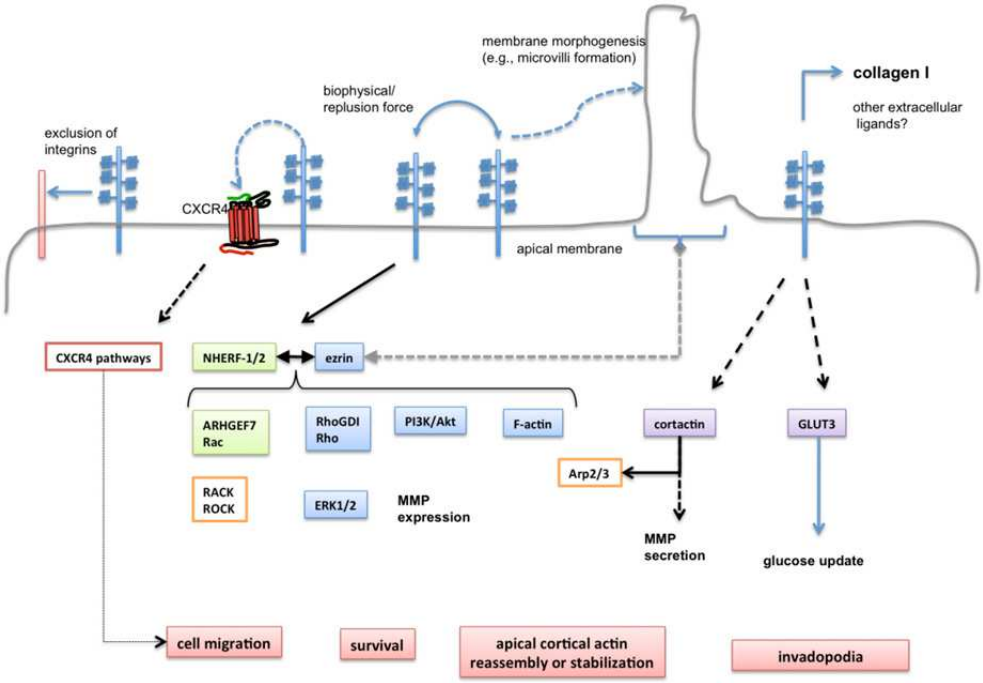


Fig. 8. Summary of podocalyxin-mediated molecular mechanisms promoting tumour growth, invasion, migration and adhesion-independent survival.

migration of hematopoietic cells and possibly tumor cells to CXCL12, perhaps by stabilizing the surface expression (and localization) of CXCR4. Thus, upregulation of PC on tumors may herald the acquisition of a great capacity for migration to CXCL12 rich tissues including bone, lung and brain.

7.2 Podocalyxin poorly differentiated tumours and promotes EMT and EMT-independent invasion and metastasis in human cancers

Evaluation of PC expression in human cancers, coupled with tumour characteristics and patient outcome data, reveals four cancer-indication "profiles" where high PC expression in primary tumours provides important diagnostic and prognostic information (**Table 4 (I-III)**) or polymorphisms in *PODXL* exons predicts cancer risk and tumour aggressiveness (**Table 4, IV**). These tumour-expression profiles, combined with *in vitro* and *in vivo* mechanistic studies, support the contention that PC promotes tumour invasion and metastases. In some human cancers, high PC expression is commonly detected in poorly differentiated, anaplastic tumour subtypes but not in lower grade tumours that retain differentiation markers and characteristics (Profile I). PC expression is also commonly detected in testicular cancer, a germ-line tumour. In these cases, PC may serve as a marker of immature or "de-differentiated" cells that have undergone EMT or retained a more primitive gene expression profile (perhaps derived from tissue stem cells). However, a causal link between PC expression and tumour progression and invasion is not proven in these profile I cancers. Nevertheless, drawing on *in vitro* studies, we hypothesize that PC may contribute to (but not

initiate) the invasive or aggressive tumour phenotype in some of these cancers. For this reason, cancers of this type may benefit by the advent of PC diagnostics as a supplemental approach to the staging or differential diagnosis of primary tumor origin. We note that the clinical application of PC expression profiling for cancer diagnostics is achievable with existing reagents and technology, requiring only a concerted effort of validation.

Profile I. Podocalyxin is commonly expressed in high-grade or poorly differentiated tumours
• Thyroid carcinoma (undifferentiated)
• Pancreatic ductal/ampullary carcinoma
• Small-cell lung carcinoma
• Acute myeloid and lymphoid leukemia and myeloid sarcoma
• Anaplastic nephroblastoma
• Testicular cancer (germ cell)
• Malignant astrocytoma
Profile II. Podocalyxin is overexpressed in rare subsets of highly aggressive tumours and predicts poor prognosis
• Breast carcinoma
• Ovarian carcinoma
• Renal cell carcinoma
Profile III. PC expression as potential serum marker but clinical significance expression profile is undetermined
• Testicular cancer
• Colon cancer (cell line)
Profile IV. *PODXL* polymorphisms and SNPs associated with cancer risk and tumor aggressiveness
• Prostate carcinoma

Table 4. Podocalyxin expression profiles in human cancer.

In some cancers, PC overexpression in rare, highly aggressive tumour subtypes clearly correlates with enhanced tumour invasion, risk of metastasis and poor patient outcome (Profile II). In these cases, we propose that PC expression directly promotes EMT-independent invasion and metastases. We anticipate that further detailed analyses of aggressive tumor sub-types, especially for epithelial carcinomas, will expand the examples of rare, PC expressing, aggressive tumours in profile II. For instance: Because forced expression of PC in a prostate tumour cell line enhances invasive and migration potential, and PC is expressed in malignant prostate tumours (but not primary or normal adjacent tumours) (**Fig. 4**), we predict that a subset of high PC-expressing primary prostate tumours will display high risk of metastases. Evaluation of PC expression in primary tumours of profile II has the most value for prognostic assessment and the design of treatment and surveillance strategy. Furthermore, we hope that it will be possible to exploit PC expression in these indications for the development of targeted adjuvant therapies (**see §7.3**).

The detection of PC (or fragments) in serum may also provide a noninvasive biomarker of tumour progression or treatment progress in some cancers (profile III). The case is strongest for testicular cancer where serum PC (aka GCTM-2 or TRA-1-60) may be a more robust marker than current "gold-standard" biomarkers used in testicular cancer diagnostics. We suspect that cancers that commonly express high levels of PC (profile I) may also shed PC

fragments into serum and continued research will likely confirm PC's utility as diagnostic serum marker. If so, PC detection in serum may be a generally useful for detecting disease or monitoring tumour progression and treatment efficacy.

Finally, genetic profiling of the *PODXL* locus reveals a fourth profile where polymorphisms in *PODXL* exons are associated with increase prostate cancer risk and tumor aggressiveness (**Table 4 (IV)**). Although it will be important to evaluate whether these genetic markers correlate with PC expression in prostate cancer or have any role in PC's functions in these tumours, screening for *PODXL* polymorphisms has potential theranostic applications in designing treatment strategies for prostate cancer patients.

7.3 Podocalyxin-targeted therapies for the treatment of metastatic cancer

There are several challenges to overcome in the development of PC-targeted adjuvant therapies for the treatment of high-risk primary tumours or oncolytic treatment of systemic cancers. First, we do not yet know if the high PC expression detected on primary tumours in the case of aggressive breast, renal cell and ovarian carcinoma is maintained on metastatic tumour cells or following engraftment at a secondary tissue site. However, evaluation of PC expression in breast and ovarian carcinoma cell lines, many of which were derived from metastatic tumours, suggests that PC expression might be a common feature of metastatic breast and ovarian cancer – especially, basal-type breast carcinomas (**Fig. 1**). Second, any PC-targeted therapies must carefully consider potential renal and vascular toxicity since PC is highly expressed on glomerular podocytes and on most vasculature. Fortunately, there are several examples of uniquely modified, tumour-specific forms of PC. These provide feasible targets for antibody-based drugs that are either directly oncolytic or block PC-mediated functions without affecting the function of normal cells.

8. Acknowledgement

The authors would foremost like to thank the patients that have donated tissues, genetic material and sacrificed some of their privacy to support the studies described in this review. KMM is a Senior Scholar of the Michael Smith Foundation of Health Research. KMM and CDR receive operating research funds for the study of podocalyxin's role in cancer biology from the Canadian Institutes of Health Research.

9. References

Anderson, J.C., McFarland, B.C. & Gladson, C.L. (2008). New molecular targets in angiogenic vessels of glioblastoma tumours. *Expert Reviews in Molecular Medicine.* Vol. 10, (Aug 7), pp. e23, ISSN 1462-3994

Austermann, J., Nazmi, A.R., Muller-Tidow, C., and Gerke, V. (2008). Characterization of the Ca2+ -regulated ezrin-S100P interaction and its role in tumor cell migration. *The Journal of Biological Chemistry.* Vol. 283, No. 43, (Oct 24), pp. 29331-29340, ISSN 0021-9258

Blanchet, M-R., Bennett J.L., Gold M., Levantini, E., Tenen, D.G., Girard M., Cormier Y. & McNagny, K.M. (2011). CD34 is required for dendritic cell trafficking and pathology in murine hypersensitivity pneumonitis. *American Journal of Respiratory and Critical Care Medicine*. In press, ISSN 1073-449X

Brambilla, D. & Fais, S. (2009). The Janus-faced role of ezrin in "linking" cells to either normal or metastatic phenotype. *International Journal of Cancer.* Vol. 125, No. 10, (Nov 15), pp. 2239-2245, ISSN 1097-0215

Brandenberger, R., Wei, H., Zhang, S., Lei, S., Murage, J., Fisk, G.J., Li, Y., Xu, C., Fang, R., Guegler, K., Rao, M.S., Mandalam, R., Lebkowski, J., and Stanton, L.W. (2004). Transcriptome characterization elucidates signaling networks that control human ES cell growth and differentiation. *Nature Biotechnology.* Vol. 22, No. 6, (Jun), pp. 707-716, ISSN 1087-0156

Butta, N., Larrucea, S., Alonso, S., Rodriguez, R., Arias-Salgado, E., Ayuso, M., Gonzalez-Manchon, C. & Parrilla, R. (2006). Role of transcription factor Sp1 and CpG methylation on the regulation of the human podocalyxin gene promoter. *BMC Molecular Biology.* Vol. 7, (Jan), pp. 17-29, ISSN 1471-2199

Cai, J., Chen, J., Liu, Y., Miura, T., Luo, Y., Loring, J.F., Freed, W.J., Rao, M.S., and Zeng, X. (2006). Assessing self-renewal and differentiation in human embryonic stem cell lines. *Stem Cells.* Vol. 24, No. 3, (Mar), pp. 516-530, ISSN 1066-5099

Cairns, R.A., Harris, I.S. & Mak, T.W. (2011). Regulation of cancer cell metabolism. *Nature Reviews.Cancer.* Vol. 11, No. 2, (Feb), pp. 85-95, ISSN 1474-1768

Casey, G., Neville, P.J., Liu, X., Plummer, S.J., Cicek, M.S., Krumroy, L.M., Curran, A.P., McGreevy, M.R., Catalona, W.J., Klein, E.A. & Witte, J.S. (2006). Podocalyxin variants and risk of prostate cancer and tumor aggressiveness. *Human Molecular Genetics.* Vol. 15, No. 5, (Mar 1), pp. 735-741, ISSN 0964-6906

Chandran, U.R., Ma, C., Dhir, R., Bisceglia, M., Lyons-Weiler, M., Liang, W., Michalopoulos, G., Becich, M. & Monzon, F.A. (2007). Gene expression profiles of prostate cancer reveal involvement of multiple molecular pathways in the metastatic process. *BMC Cancer.* Vol. 7, (Apr 12), pp. 64, ISSN 1471-2407

Chauhan, S.C., Kumar, D. & Jaggi, M. (2009). Mucins in ovarian cancer diagnosis and therapy. *Journal of Ovarian Research.* Vol. 2, (Dec 24), pp. 21-30, ISSN 1757-2215

Chen, X., Higgins, J., Cheung, S.T., Li, R., Mason, V., Montgomery, K., Fan, S.T., van de Rijn, M. & So, S. (2004). Novel endothelial cell markers in hepatocellular carcinoma. *Modern Pathology.* Vol. 17, No. 10, (Oct), pp. 1198-1210, ISSN 0893-3952

Cheng, H.Y., Lin, Y.Y., Yu, C.Y., Chen, J.Y., Shen, K.F., Lin, W.L., Liao, H.K., Chen, Y.J., Liu, C.H., Pang, V.F. & Jou, T.S. (2005). Molecular identification of canine podocalyxin-like protein 1 as a renal tubulogenic regulator. *Journal of the American Society of Nephrology.* Vol. 16, No. 6, (Jun), pp. 1612-1622, ISSN 1046-6673

Cheung, H.H., Davis, A.J., Lee, T.L., Pang, A.L., Nagrani, S., Rennert, O.M. & Chan, W.Y. (2011). Methylation of an intronic region regulates miR-199a in testicular tumor malignancy. *Oncogene.* (Mar 7), pp. 1-12, ISSN 1476-5594

Choo, A., Tan, H., Ang, S., Fong, W., Chin, A., Lo, J., Zheng, L., Hentze, H., Philp, R., Oh, S. & Yap, M. (2008). Selection against undifferentiated human embryonic stem cells by a cytotoxic antibody recognizing podocalyxin-like protein-1. *Stem Cells.* Vol. 26, No. 6, (Jun), pp. 1454-1463, ISSN 1066-509

Cleton-Jansen, A.M. (2002). E-cadherin and loss of heterozygosity at chromosome 16 in breast carcinogenesis: different genetic pathways in ductal and lobular breast cancer? *Breast Cancer Research.* Vol. 4, No. 1, pp. 5-8, ISSN 1465-5411

Cohen, H.T. & McGovern, F.J. (2005). Renal-cell carcinoma. *The New England Journal of Medicine.* Vol. 353, No. 23, (Dec 8), pp. 2477-2490, ISSN 1533-4406

Comamala, M., Pinard, M., Theriault, C., Matte, I., Albert, A., Boivin, M., Beaudin, J., Piche, A. & Rancourt, C. (2011). Downregulation of cell surface CA125/MUC16 induces epithelial-to-mesenchymal transition and restores EGFR signalling in NIH:OVCAR3 ovarian carcinoma cells. *British Journal of Cancer.* Vol. 104, No. 6, (Mar 15), pp. 989-999, ISSN 1532-1827

Dai, J.L., Wang, L., Sahin, A.A., Broemeling, L.D., Schutte, M. & Pan, Y. (2004). NHERF (Na+/H+ exchanger regulatory factor) gene mutations in human breast cancer. *Oncogene.* Vol. 23, No. 53, (Nov 11), pp. 8681-8687, ISSN 0950-9232

De Miguel, M.P., Fuentes-Julian, S. & Alcaina, Y. (2010). Pluripotent stem cells: origin, maintenance and induction. *Stem Cell Reviews.* Vol. 6, No. 4, (Dec), pp. 633-649, ISSN 1558-6804

Doyonnas, R., Kershaw, D., Duhme, C., Merkens, H., Chelliah, S., Graf, T. & McNagny, K. (2001). Anuria, omphalocele& perinatal lethality in mice lacking the CD34-related protein podocalyxin. *The Journal of Experimental Medicine.* Vol. 194, No. 1, (Jul), pp. 13-27, ISSN 0022-1007

Doyonnas, R., Nielsen, J., Chelliah, S., Drew, E., Hara, T., Miyajima, A. & McNagny, K. (2005). Podocalyxin is a CD34-related marker of murine hematopoietic stem cells and embryonic erythroid cells. *Blood.* Vol. 105, No. 11, (Jun), pp. 4170-4178, *ISSN* 0006-4971

Fehon, R.G., McClatchey, A.I. & Bretscher, A. (2010). Organizing the cell cortex: the role of ERM proteins. *Nature Reviews. Molecular Cell Biology.* Vol. 11, No. 4, (Apr), pp. 276-287, ISSN 1471-0080

Fievet, B., Gautreau, A., Roy, C., Del Maestro L, Mangeat, P., Louvard, D., and Arpin, M. (2004). Phosphoinositide binding and phosphorylation act sequentially in the activation mechanism of ezrin. *The Journal of Cell Biology.* Vol. 164, No. 5, (Mar), pp. 653-659, ISSN 0021-9258

Forde, S., Tye, B., Newey, S., Roubelakis, M., Smythe, J., McGuckin, C., Pettengell, R. & Watt, S. (2007). Endolyn (CD164) modulates the CXCL12-mediated migration of umbilical cord blood CD133+ cells. *Blood.* Vol. 109, No. 5, (Mar), pp. 1825-1833.

Furness, S.G. & McNagny, K. (2006). Beyond mere markers: functions for CD34 family of sialomucins in hematopoiesis. *Immunologic Research.* Vol. 34, No. 1, pp. 13-32, ISSN 0257-277X

Galeano, B., Klootwijk, R., Manoli, I., Sun, M., Ciccone, C., Darvish, D., Starost, M.F., Zerfas, P.M., Hoffmann, V.J., Hoogstraten-Miller, S., Krasnewich, D.M., Gahl, W.A. & Huizing, M. (2007). Mutation in the key enzyme of sialic acid biosynthesis causes severe glomerular proteinuria and is rescued by N-acetylmannosamine. *The Journal of Clinical Investigation.* Vol. 117, No. 6, (Jun), pp. 1585-1594, ISSN 0021-9738

Garbett, D., LaLonde, D.P. & Bretscher, A. (2010). The scaffolding protein EBP50 regulates microvillar assembly in a phosphorylation-dependent manner. *The Journal of Cell Biology.* Vol. 191, No. 2, (Oct 18), pp. 397-413, ISSN 1540-8140

Gautreau, A., Poullet, P., Louvard, D., and Arpin, M. (1999). Ezrin, a plasma membrane-microfilament linker, signals cell survival through the phosphatidylinositol 3-kinase/Akt pathway. *Proceedings of the National Academy of Sciences of the United States of America.* Vol. 96, No. 13, (Jun 22), pp. 7300-7305, ISSN 0027-8424

Gelberg, H., Healy, L., Whiteley, H., Miller, L.A. & Vimr, E. (1996). In vivo enzymatic removal of alpha 2-->6-linked sialic acid from the glomerular filtration barrier

results in podocyte charge alteration and glomerular injury. *Laboratory Investigation.* Vol. 74, No. 5, (May), pp. 907-920, ISSN 0023-6837

Georgescu, M.M., Morales, F.C., Molina, J.R. & Hayashi, Y. (2008). Roles of NHERF1/EBP50 in cancer. *Current Molecular Medicine.* Vol. 8, No. 6, (Sep), pp. 459-468, ISSN 1566-5240

Hamilton, T.C., Young, R.C., McKoy, W.M., Grotzinger, K.R., Green, J.A., Chu, E.W., Whang-Peng, J., Rogan, A.M., Green, W.R. & Ozols, R.F. (1983). Characterization of a human ovarian carcinoma cell line (NIH:OVCAR-3) with androgen and estrogen receptors. *Cancer Research.* Vol. 43, No. 11, (Nov), pp. 5379-5389, ISSN 0008-5472

Hayashi, Y., Molina, J.R., Hamilton, S.R. & Georgescu, M.M. (2010). NHERF1/EBP50 is a new marker in colorectal cancer. *Neoplasia.* Vol. 12, No. 12, (Dec), pp. 1013-1022, ISSN 1476-5586

Hayatsu, N., Kaneko, M., Mishima, K., Nishikawa, R., Matsutani, M., Price, J. & Kato, Y. (2008). Podocalyxin expression in malignant astrocytic tumors. *Biochemical and Biophysical Research Communications.* Vol. 374, No. 2, (Sep), pp. 394-398, ISSN 0006-291X

He, J., Liu, Y., Xie, X., Zhu, T., Soules, M., DiMeco, F., Vescovi, A.L., Fan, X. & Lubman, D.M. (2010). Identification of cell surface glycoprotein markers for glioblastoma-derived stem-like cells using a lectin microarray and LC-MS/MS approach. *Journal of Proteome Research.* Vol. 9, No. 5, (May 7), pp. 2565-2572, ISSN 1535-3907

Herman-Edelstein, M., Thomas, M.C., Thallas-Bonke, V., Saleem, M., Cooper, M.E. & Kantharidis, P. (2011). Dedifferentiation of Immortalized Human Podocytes in Response to Transforming Growth Factor-{beta}: A Model for Diabetic Podocytopathy. *Diabetes.* (Apr 26), pp. 1-10, ISSN 1939-327X

Heukamp, L.C., Fischer, H.P., Schirmacher, P., Chen, X., Breuhahn, K., Nicolay, C., Buttner, R. & Gutgemann, I. (2006). Podocalyxin-like protein 1 expression in primary hepatic tumours and tumour-like lesions. *Histopathology.* Vol. 49, No. 3, (Sep), pp. 242-247, ISSN 1365-2559

Hsu, Y., Lin, W., Hou, Y., Pu, Y., Shun, C., Chen, C., Wu, Y., Chen, J., Chen, T. & Jou, T. (2010). Podocalyxin EBP50 ezrin molecular complex enhances the metastatic potential of renal cell carcinoma through recruiting Rac1 guanine nucleotide exchange factor ARHGEF7. *The American Journal of Pathology.* Vol. 176, No. 6, (Jun), pp. 3050-3061, ISSN 0002-9440

Huff, V. (2011). Wilms' tumours: about tumour suppressor genes, an oncogene and a chameleon gene. *Nature Reviews. Cancer.* Vol. 11, No. 2, (Feb), pp. 111-121, ISSN 1474-1768

Ichimi, T., Enokida, H., Okuno, Y., Kunimoto, R., Chiyomaru, T., Kawamoto, K., Kawahara, K., Toki, K., Kawakami, K., Nishiyama, K., Tsujimoto, G., Nakagawa, M. & Seki, N. (2009). Identification of novel microRNA targets based on microRNA signatures in bladder cancer. *International Journal of Cancer.* Vol. 125, No. 2, (Jul 15), pp. 345-352, ISSN 1097-0215

Ito, T., Maki, N., Hazeki, O., Sasaki, K. & Nekooki, M. (2007). Extracellular and transmembrane region of a podocalyxin-like protein 1 fragment identified from colon cancer cell lines. *Cell Biology International.* Vol. 31, No. 12, (Dec), pp. 1518-1524, ISSN 1065-6995

Jacobson, E.M., Hugo, E.R., Tuttle, T.R., Papoian, R. & Ben-Jonathan, N. (2010). Unexploited therapies in breast and prostate cancer: blockade of the prolactin receptor. *Trends in Endocrinology and Metabolism.* Vol. 21, No. 11, (Nov), pp. 691-698, ISSN 1879-3061

Kaighn, M.E., Narayan, K.S., Ohnuki, Y., Lechner, J.F. & Jones, L.W. (1979). Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Investigative Urology.* Vol. 17, No. 1, (Jul), pp. 16-23, ISSN 0021-0005

Kalluri, R. & Weinberg, R.A. (2009). The basics of epithelial-mesenchymal transition. *The Journal of Clinical Investigation.* Vol. 119, No. 6, (Jun), pp. 1420-1428, ISSN 1558-8238

Kannagi, R. (2004). Molecular mechanism for cancer-associated induction of sialyl Lewis X and sialyl Lewis A expression-The Warburg effect revisited. *Glycoconjugate Journal.* Vol. 20, No. 5, pp. 353-364, ISSN 0282-0080

Kao, J., Salari, K., Bocanegra, M., Choi, Y.L., Girard, L., Gandhi, J., Kwei, K.A., Hernandez-Boussard, T., Wang, P., Gazdar, A.F., Minna, J.D. & Pollack, J.R. (2009). Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery. *PloS One.* Vol. 4, No. 7, (Jul 3), pp. e6146, ISSN 1932-6203

Kasai, H., Allen, J.T., Mason, R.M., Kamimura, T. & Zhang, Z. (2005). TGF-beta1 induces human alveolar epithelial to mesenchymal cell transition (EMT). *Respiratory Research.* Vol. 6, (Jun 9), pp. 56, ISSN 1465-993X

Kelley, T.W., Huntsman, D., McNagny, K.M., Roskelley, C.D. & Hsi, E.D. (2005). Podocalyxin: a marker of blasts in acute leukemia. *American Journal of Clinical Pathology.* Vol. 124, No. 1, (Jul), pp. 134-142, ISSN 0002-9173

Kobayashi, T., Notoya, M., Shinosaki, T. & Kurihara, H. (2009). Cortactin interacts with podocalyxin and mediates morphological change of podocytes through its phosphorylation. *Nephron. Experimental Nephrology.* Vol. 113, No. 3, pp. e89-96, ISSN 1660-2129

Koch, L., Zhou, H., Ellinger, J., Biermann, K., Holler, T., von Rucker A, , Buttner, R. & Gutgemann, I. (2008). Stem cell marker expression in small cell lung carcinoma and developing lung tissue. *Human Pathology.* (Jul), pp. 1597-1605, ISSN 0046-8177

Kufe, D.W. (2009). Mucins in cancer: function, prognosis and therapy. *Nature Reviews. Cancer.* Vol. 9, No. 12, (Dec), pp. 874-885, ISSN 1474-1768

Lacroix, M. & Leclercq, G. (2004). Relevance of breast cancer cell lines as models for breast tumours: an update. *Breast Cancer Research and Treatment.* Vol. 83, No. 3, (Feb), pp. 249-289, ISSN 0167-6806

Lange, K. (2011). Fundamental role of microvilli in the main functions of differentiated cells: Outline of an universal regulating and signaling system at the cell periphery. *Journal of Cellular Physiology.* Vol. 226, No. 4, (Apr), pp. 896-927, ISSN 1097-4652

Laubli, H. & Borsig, L. (2010). Selectins promote tumor metastasis. *Seminars in Cancer Biology.* Vol. 20, No. 3, (Jun), pp. 169-177, ISSN 1096-3650

Lee-Kwon, W., Wade, J.B., Zhang, Z., Pallone, T.L., and Weinman, E.J. (2005). Expression of TRPC4 channel protein that interacts with NHERF-2 in rat descending vasa recta. *American Journal of Physiology. Cell Physiology*, Vol. 288, No. 4, (Apr 1), pp. C942-C949, ISSN 0363-6143

Li, L. & Davie, J.R. (2010). The role of Sp1 and Sp3 in normal and cancer cell biology. *Annals of Anatomy.* Vol. 192, No. 5, (Sep 20), pp. 275-283, ISSN 1618-0402

Li, M., Wang, W., Soroka, C.J., Mennone, A., Harry, K., Weinman, E.J., and Boyer, J.L. (2010). NHERF-1 binds to Mrp2 and regulates hepatic Mrp2 expression and function. *The Journal of Biological Chemistry.* Vol. 285, No. 25, (Jun 18), pp. 19299-19307, ISSN 1083-351X

Li, Y., Kang, Y.S., Dai, C., Kiss, L.P., Wen, X. & Liu, Y. (2008). Epithelial-to-mesenchymal transition is a potential pathway leading to podocyte dysfunction and proteinuria. *The American Journal of Pathology.* Vol. 172, No. 2, (Feb), pp. 299-308, ISSN 0002-9440

Liang, Y., Ridzon, D., Wong, L. & Chen, C. (2007). Characterization of microRNA expression profiles in normal human tissues. *BMC Genomics.* Vol. 8, (Jun 12), pp. 166, ISSN 1471-2164

Liedtke, C.M., Raghuram, V., Yun, C.C., and Wang, X. (2004). Role of a PDZ1 domain of NHERF1 in the binding of airway epithelial RACK1 to NHERF1. *American Journal of Physiolology. Cell Physiology.* Vol. 286, No. 5, (May), pp. C1037-44, ISSN 0363-6143

Lim, D.Y., Ng, Y.H., Lee, J., Mueller, M., Choo, A.B. & Wong, V.V. (2011). Cytotoxic antibody fragments for eliminating undifferentiated human embryonic stem cells. *Journal of Biotechnology.* Vol. 153, No. 3-4, (May 20), pp. 77-85, ISSN 1873-4863

Macheda, M.L., Rogers, S. & Best, J.D. (2005). Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *Journal of Cellular Physiology.* Vol. 202, No. 3, (Mar), pp. 654-662, ISSN 0021-9541

Maltby, S., Freeman, S., Gold, M.J., Baker, J.H., Minchinton, A.I., Gold, M.R., Roskelley, C.D. & McNagny, K.M. (2011). Opposing Roles for CD34 in B16 Melanoma Tumor Growth Alter Early Stage Vasculature and Late Stage Immune Cell Infiltration. *PloS One.* Vol. 6, No. 4, (Apr 11), pp. e18160, ISSN 1932-6203

Maltby, S., Hughes, M., Zbytnuik, L., Paulson, R. & McNagny, K. (2009). Podocalyxin selectively marks erythroid-committed progenitors during anemic stress but is dispensable for efficient recovery. *Experimental Hematology.* Vol. 37, No. 1, (Jan), pp. 10-18, ISSN 0301-472X

Mangia, A., Chiriatti, A., Bellizzi, A., Malfettone, A., Stea, B., Zito, F.A., Reshkin, S.J., Simone, G. & Paradiso, A. (2009). Biological role of NHERF1 protein expression in breast cancer. *Histopathology.* Vol. 55, No. 5, (Nov), pp. 600-608, ISSN 1365-2559

McNagny, K.M., Pettersson, I., Rossi, F., Flamme, I., Shevchenko, A., Mann, M. & Graf, T. (1997). Thrombomucin, a novel cell surface protein that defines thrombocytes and multipotent hematopoietic progenitors. *The Journal of Cell Biology.* Vol. 138, No. 6, (Sep 22), pp. 1395-1407, ISSN 0021-9525

Meder, D., Shevchenko, A., Simons, K. & Fullekrug, J. (2005). Gp135/podocalyxin and NHERF-2 participate in the formation of a preapical domain during polarization of MDCK cells. *The Journal of Cell Biology.* Vol. 168, No. 2, (Jan 17), pp. 303-313, ISSN 0021-9525

Meng, X., Ezzati, P. & Wilkins, J.A. (2011). Requirement of podocalyxin in TGF-beta induced epithelial mesenchymal transition. *PloS One.* Vol. 6, No. 4, (Apr 12), pp. e18715, ISSN 1932-6203

Miettinen, A., Solin, M.L., Reivinen, J., Juvonen, E., Vaisanen, R. & Holthofer, H. (1999). Podocalyxin in rat platelets and megakaryocytes. *The American Journal of Pathology.* Vol. 154, No. 3, (Mar), pp. 813-822, ISSN 0002-9440

Mishima, K., Kato, Y., Kaneko, M.K., Nishikawa, R., Hirose, T. & Matsutani, M. (2006). Increased expression of podoplanin in malignant astrocytic tumors as a novel

molecular marker of malignant progression. *Acta Neuropathologica.* Vol. 111, No. 5, (May), pp. 483-488, ISSN 0001-6322

Nam, E.J., Yoon, H., Kim, S.W., Kim, H., Kim, Y.T., Kim, J.H., Kim, J.W. & Kim, S. (2008). MicroRNA expression profiles in serous ovarian carcinoma. *Clinical Cancer Research.* Vol. 14, No. 9, (May 1), pp. 2690-2695, ISSN 1078-0432

Neve, R.M., Chin, K., Fridlyand, J., Yeh, J., Baehner, F.L., Fevr, T., Clark, L., Bayani, N., Coppe, J.P., Tong, F., Speed, T., Spellman, P.T., DeVries, S., Lapuk, A., Wang, N.J., Kuo, W.L., Stilwell, J.L., Pinkel, D., Albertson, D.G., Waldman, F.M., McCormick, F., Dickson, R.B., Johnson, M.D., Lippman, M., Ethier, S., Gazdar, A. & Gray, J.W. (2006). A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell.* Vol. 10, No. 6, (Dec), pp. 515-527, ISSN 1535-6108

Neville, P.J., Conti, D.V., Paris, P.L., Levin, H., Catalona, W.J., Suarez, B.K., Witte, J.S. & Casey, G. (2002). Prostate cancer aggressiveness locus on chromosome 7q32-q33 identified by linkage and allelic imbalance studies. *Neoplasia.* Vol. 4, No. 5, (Sep-Oct), pp. 424-431, ISSN 1522-8002

Ney, J.T., Zhou, H., Sipos, B., Buttner, R., Chen, X., Kloppel, G. & Gutgemann, I. (2007). Podocalyxin-like protein 1 expression is useful to differentiate pancreatic ductal adenocarcinomas from adenocarcinomas of the biliary and gastrointestinal tracts. *Human Pathology.* Vol. 38, No. 2, (Feb), pp. 359-364, ISSN 0046-8177

Nielsen, J.S. & McNagny, K.M. (2009a). CD34 is a Key Regulator of Hematopoietic Stem Cell Trafficking to Bone Marrow and Mast Cell Progenitor Trafficking in the Periphery. *Microcirculation.* (May 27), pp. 1-10, ISSN 1549-8719

Nielsen, J.S. & McNagny, K.M. (2009b). The role of podocalyxin in health and disease. *Journal of the American Society of Nephrology.* Vol. 20, No. 8, (Aug), pp. 1669-1676, ISSN 1533-3450

Nielsen, J.S. & McNagny, K.M. (2008). Novel functions of the CD34 family. *Journal of Cell Science.* Vol. 121, No. Pt 22, (Nov 15), pp. 3683-3692, ISSN 0021-9533

Nielsen, J., Graves, M., Chelliah, S., Vogl, A., Roskelley, C. & McNagny, K. (2007). The CD34-related molecule podocalyxin is a potent inducer of microvillus formation. *PLoS One.* Vol. 2, No. 2, (Jan), pp. e227, ISSN 1932-6203

Ouyang, G., Wang, Z., Fang, X., Liu, J. & Yang, C.J. (2010). Molecular signaling of the epithelial to mesenchymal transition in generating and maintaining cancer stem cells. *Cellular and Molecular Life Sciences.* Vol. 67, No. 15, (Aug), pp. 2605-2618, ISSN 1420-9071

Palecek, S.P. (2011). Pluripotent Stem Cells: Sources and Characterization, In: *Tissue Engineering: From Lab to Clinic*, Pallua N. & Suschek, C.V. (eds.), pp. (69-82), Springer-Verlag, ISBN 978-3-642-02824-3, Berlin-Heidelberg.

Palmer, R.E., Kotsianti, A., Cadman, B., Boyd, T., Gerald, W. & Haber, D.A. (2001). WT1 regulates the expression of the major glomerular podocyte membrane protein Podocalyxin. *Current Biology.* Vol. 11, No. 22, (Nov 13), pp. 1805-1809, ISSN 0960-9822

Riccioni, R., Calzolari, A., Biffoni, M., Senese, M., Riti, V., Petrucci, E., Pasquini, L., Cedrone, M., Lo-Coco, F., Diverio, D., Foa, R., Peschle, C. & Testa, U. (2006). Podocalyxin is expressed in normal and leukemic monocytes. *Blood Cells, Molecules & Diseases.* Vol. 37, No. 3, (Nov-Dec), pp. 218-225, ISSN 1079-9796

Sahai, E., and Marshall, C.J. (2002). RHO-GTPases and cancer. *Nature Reviews. Cancer.* Vol. 2, No. 2, (Feb), pp. 133-142, ISSN 1474-175X

Sarrio, D., Rodriguez-Pinilla, S.M., Hardisson, D., Cano, A., Moreno-Bueno, G., and Palacios, J. (2008). Epithelial-mesenchymal transition in breast cancer relates to the basal-like phenotype. *Cancer Research.* Vol. 68, No. 4, (Feb 15), pp. 989-997, ISSN 1538-7445

Sartor, M.A., Mahavisno, V., Keshamouni, V.G., Cavalcoli, J., Wright, Z., Karnovsky, A., Kuick, R., Jagadish, H.V., Mirel, B., Weymouth, T., Athey, B. & Omenn, G.S. (2010). ConceptGen: a gene set enrichment and gene set relation mapping tool. *Bioinformatics.* Vol. 26, No. 4, (Feb 15), pp. 456-463, ISSN 1367-4811

Sathyanarayana, P., Menon, M., Bogacheva, O., Bogachev, O., Niss, K., Kapelle, W., Houde, E., Fang, J. & Wojchowski, D. (2007). Erythropoietin modulation of podocalyxin and a proposed erythroblast niche. *Blood.* Vol. 110, No. 2, (Jul), pp. 509-518, ISSN 0006-4971

Schmieder, S., Nagai, M., Orlando, R.A., Takeda, T. & Farquhar, M.G. (2004). Podocalyxin activates RhoA and induces actin reorganization through NHERF1 and Ezrin in MDCK cells. *Journal of the American Society of Nephrology.* Vol. 15, No. 9, (Sep), pp. 2289-2298, ISSN 1046-6673

Schopperle, W.M. & DeWolf, W.C. (2007). The TRA-1-60 and TRA-1-81 human pluripotent stem cell markers are expressed on podocalyxin in embryonal carcinoma. *Stem Cells.* Vol. 25, No. 3, (Mar), pp. 723-730, ISSN 1066-5099

Schopperle, W.M., Kershaw, D.B. & DeWolf, W.C. (2003). Human embryonal carcinoma tumor antigen, Gp200/GCTM-2, is podocalyxin. *Biochemical and Biophysical Research Communications.* Vol. 300, No. 2, (Jan 10), pp. 285-290, ISSN 0006-291X

Schopperle, W.M., Lee, J.M. & Dewolf, W.C. (2010). The human cancer and stem cell marker podocalyxin interacts with the glucose-3-transporter in malignant pluripotent stem cells. *Biochemical and Biophysical Research Communications.* Vol. 398, No. 3, (Jul 30), pp. 372-376, ISSN 1090-2104

Singh, A.P., Senapati, S., Ponnusamy, M.P., Jain, M., Lele, S.M., Davis, J.S., Remmenga, S. & Batra, S.K. (2008). Clinical potential of mucins in diagnosis, prognosis& therapy of ovarian cancer. *The Lancet Oncology.* Vol. 9, No. 11, (Nov), pp. 1076-1085, ISSN 1474-5488

Sizemore, S., Cicek, M., Sizemore, N., Ng, K.P. & Casey, G. (2007). Podocalyxin increases the aggressive phenotype of breast and prostate cancer cells in vitro through its interaction with ezrin. *Cancer Research.* Vol. 67, No. 13, (Jul 1), pp. 6183-6191, ISSN 0008-5472

Somasiri, A., Nielsen, J.S., Makretsov, N., McCoy, M.L., Prentice, L., Gilks, C.B., Chia, S.K., Gelmon, K.A., Kershaw, D.B., Huntsman, D.G., McNagny, K.M. & Roskelley, C.D. (2004). Overexpression of the anti-adhesin podocalyxin is an independent predictor of breast cancer progression. *Cancer Research.* Vol. 64, No. 15, (Aug 1), pp. 5068-5073, ISSN 1538-7445

Soule, H.D., Vazguez, J., Long, A., Albert, S. & Brennan, M. (1973). A human cell line from a pleural effusion derived from a breast carcinoma. *Journal of the National Cancer Institute.* Vol. 51, No. 5, (Nov), pp. 1409-1416, ISSN 0027-8874

Stanhope-Baker, P., Kessler, P.M., Li, W., Agarwal, M.L. & Williams, B.R. (2004). The Wilms tumor suppressor-1 target gene podocalyxin is transcriptionally repressed by p53. *The Journal of Biological Chemistry.* Vol. 279, No. 32, (Aug 6), pp. 33575-33585, ISSN 0021-9258

Strilic, B., Kucera, T., Eglinger, J., Hughes, M.R., McNagny, K.M., Tsukita, S., Dejana, E., Ferrara, N. & Lammert, E. (2009). The molecular basis of vascular lumen formation

in the developing mouse aorta. *Developmental Cell.* Vol. 17, No. 4, (Oct), pp. 505-515. ISSN 1878-1551

Sun, X., Cheng, G., Hao, M., Zheng, J., Zhou, X., Zhang, J., Taichman, R.S., Pienta, K.J. & Wang, J. (2010). CXCL12 / CXCR4 / CXCR7 chemokine axis and cancer progression. *Cancer Metastasis Reviews.* Vol. 29, No. 4, (Dec), pp. 709-722, ISSN 1573-7233

Takahashi, Y., Morales, F.C., Kreimann, E.L., and Georgescu, M.M. (2006). PTEN tumor suppressor associates with NHERF proteins to attenuate PDGF receptor signaling. *The EMBO Journal.* Vol. 25, No. 4, (Feb 22), pp. 910-920, ISSN 0261-4189

Takeda, T., Go, W.Y., Orlando, R.A. & Farquhar, M.G. (2000). Expression of podocalyxin inhibits cell-cell adhesion and modifies junctional properties in Madin-Darby canine kidney cells. *Molecular Biology of the Cell.* Vol. 11, No. 9, (Sep), pp. 3219-3232, ISSN 1059-1524

Tan, H.L., Fong, W.J., Lee, E.H., Yap, M. & Choo, A. (2009). mAb 84, a cytotoxic antibody that kills undifferentiated human embryonic stem cells via oncosis. *Stem Cells.* Vol. 27, No. 8, (Aug), pp. 1792-1801, ISSN 1549-4918

Tan, P., Furness, S., Merkens, H., Lin, S., McCoy, M., Roskelley, C., Kast, J. & McNagny, K. (2006). Na+/H+ exchanger regulatory factor-1 is a hematopoietic ligand for a subset of the CD34 family of stem cell surface proteins. *Stem Cells.* Vol. 24, No. 5, (May), pp. 1150-1161, ISSN 1066-5099

Teicher, B.A. & Fricker, S.P. (2010). CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clinical Cancer Research.* Vol. 16, No. 11, (Jun 1), pp. 2927-2931, ISSN 1078-0432

Thomas, S., Schnaar, R. & Konstantopoulos, K. (2009). Podocalyxin-like protein is an E-/L-selectin ligand on colon carcinoma cells: comparative biochemical properties of selectin ligands in host and tumor cells. *American Journal of Physiology. Cell Physiology.* Vol. 296, No. 3, (Mar), pp. 505-513, ISSN 0363-6143

Thorens, B. & Mueckler, M. (2010). Glucose transporters in the 21st Century. *American Journal of Physiology. Endocrinology and Metabolism.* Vol. 298, No. 2, (Feb), pp. E141-5, ISSN 1522-1555

Ueda, T., Volinia, S., Okumura, H., Shimizu, M., Taccioli, C., Rossi, S., Alder, H., Liu, C.G., Oue, N., Yasui, W., Yoshida, K., Sasaki, H., Nomura, S., Seto, Y., Kaminishi, M., Calin, G.A. & Croce, C.M. (2010). Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. *The Lancet Oncology.* Vol. 11, No. 2, (Feb), pp. 136-146, ISSN 1474-5488

Veerman, K., Williams, M., Uchimura, K., Singer, M., Merzaban, J., Naus, S., Carlow, D., Owen, P., Rivera-Nieves, J., Rosen, S. & Ziltener, H. (2007). Interaction of the selectin ligand PSGL-1 with chemokines CCL21 and CCL19 facilitates efficient homing of T cells to secondary lymphoid organs. *Nature Immunology.* Vol. 8, No. 5, (May), pp. 532-539, ISSN 1529-2908

Vitureira, N., Andres, R., Perez-Martinez, E., Martinez, A., Bribian, A., Blasi, J., Chelliah, S., Lopez-Domenech, G., De Castro, F., Burgaya, F., McNagny, K. & Soriano, E. (2010). Podocalyxin is a novel polysialylated neural adhesion protein with multiple roles in neural development and synapse formation. *PloS One.* Vol. 5, No. 8, (Aug 10), pp. e12003, ISSN 1932-6203

Vitureira, N., McNagny, K., Soriano, E. & Burgaya, F. (2005). Pattern of expression of the podocalyxin gene in the mouse brain during development. *Gene Expression Patterns.* Vol. 5, No. 3, (Feb), pp. 349-354, ISSN 1567-133X

Wang, B., Yang, Y., and Friedman, P.A. (2008). Na/H exchange regulatory factor 1, a novel AKT-associating protein, regulates extracellular signal-regulated kinase signaling through a B-Raf-mediated pathway. *Molecular Biology of the Cell.* Vol. 19, No. 4, (Apr), pp. 1637-1645, ISSN 1939-4586

Wang, D., Li, Y., Wu, C. & Liu, Y. (2011a). PINCH1 is transcriptional regulator in podocytes that interacts with WT1 and represses podocalyxin expression. *PloS One.* Vol. 6, No. 2, (Feb 24), pp. e17048, ISSN 1932-6203

Weaver, A.M. (2008). Cortactin in tumor invasiveness. *Cancer Letters.* Vol. 265, No. 2, (Jul 8), pp. 157-166, ISSN 0304-383

Weinman, E.J., Hall, R.A., Friedman, P.A., Liu-Chen, L.Y., and Shenolikar, S. (2006). The association of NHERF adaptor proteins with g protein-coupled receptors and receptor tyrosine kinases. *Annual Review of Physiology.* Vol. 68, pp. 491-505, ISSN 0066-4278

Wheeler, D.S., Barrick, S.R., Grubisha, M.J., Brufsky, A.M., Friedman, P.A., and Romero, G. (2011). Direct interaction between NHERF1 and Frizzled regulates beta-catenin signaling. *Oncogene.* Vol. 30, No. 1, (Jan 6), pp. 32-42, ISSN 1476-5594

Wicki, A. & Christofori, G. (2007). The potential role of podoplanin in tumour invasion. *British Journal of Cancer.* Vol. 96, No. 1, (Jan 15), pp. 1-5, ISSN 0007-0920

Wicki, A., Lehembre, F., Wick, N., Hantusch, B., Kerjaschki, D., and Christofori, G. (2006). Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell.* Vol. 9, No. 4, (Apr), pp. 261-272, ISSN 1535-6108

Winter, C. & Albers, P. (2011). Testicular germ cell tumors: pathogenesis, diagnosis and treatment. *Nature Reviews. Endocrinology.* Vol. 7, No. 1, (Jan), pp. 43-53, ISSN 1759-5037

Worley, L.A., Long, M.D., Onken, M.D. & Harbour, J.W. (2008). Micro-RNAs associated with metastasis in uveal melanoma identified by multiplexed microarray profiling. *Melanoma Research.* Vol. 18, No. 3, (Jun), pp. 184-190, ISSN 0960-8931

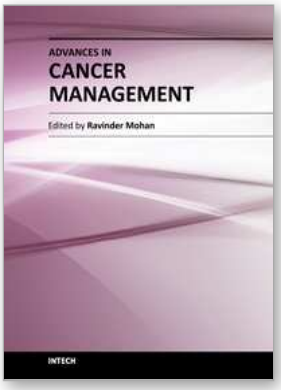
Yasuoka, H., Tsujimoto, M., Hirokawa, M., Tori, M., Nakahara, M., Miyauchi, A., Kodama, R., Sanke, T. & Nakamura, Y. (2008). Podocalyxin expression in undifferentiated thyroid carcinomas. *Journal of Clinical Pathology.* Vol. 61, No. 11, (Nov), pp. 1228-1229, ISSN 1472-4146

Yin, G., Chen, R., Alvero, A.B., Fu, H.H., Holmberg, J., Glackin, C., Rutherford, T. & Mor, G. (2010). TWISTing stemness, inflammation and proliferation of epithelial ovarian cancer cells through MIR199A2/214. *Oncogene.* Vol. 29, No. 24, (Jun 17), pp. 3545-3553, ISSN 1476-5594

Yu, Y.P., Landsittel, D., Jing, L., Nelson, J., Ren, B., Liu, L., McDonald, C., Thomas, R., Dhir, R., Finkelstein, S., Michalopoulos, G., Becich, M. & Luo, J.H. (2004). Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy. *Journal of Clinical Oncology.* Vol. 22, No. 14, (Jul 15), pp. 2790-2799, ISSN 0732-183X

Zeisberg, M. & Neilson, E.G. (2009). Biomarkers for epithelial-mesenchymal transitions. *The Journal of Clinical Investigation.* Vol. 119, No. 6, (Jun), pp. 1429-1437, ISSN 1558-8238

Zhu, Y. & Parada, L.F. (2002). The molecular and genetic basis of neurological tumours. *Nature Reviews. Cancer.* Vol. 2, No. 8, (Aug), pp. 616-626, ISSN 1474-175X



Advances in Cancer Management

Edited by Prof. Ravinder Mohan

ISBN 978-953-307-870-0
Hard cover, 278 pages
Publisher InTech
Published online 27, January, 2012
Published in print edition January, 2012

Cancer is now the most common cause of death in the world. However, because of early diagnosis, better treatment, and advanced life expectancy, many cancer patients frequently live a long, happy, and healthy life after the diagnosis- and often live as long as patients who eventually do not die because of cancer. This book presents newer advances in diagnosis and treatment of specific cancers, an evidence-based and realistic approach to the selection of cancer treatment, and cutting-edge laboratory developments such as the use of the MALDI technique and computational methods that can be used to detect newer protein biomarkers of cancers in diagnosis and to evaluate the success of treatment.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Kelly M. McNagny, Michael R. Hughes, Marcia L. Graves, Erin J. DeBruin, Kimberly Snyder, Jane Cipollone, Michelle Turvey, Poh C. Tan, Shaun McColl and Calvin D. Roskelley (2012). Podocalyxin in the Diagnosis and Treatment of Cancer, Advances in Cancer Management, Prof. Ravinder Mohan (Ed.), ISBN: 978-953-307-870-0, InTech, Available from: http://www.intechopen.com/books/advances-in-cancer-management/podocalyxin-in-the-diagnosis-and-treatment-of-cancer

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821