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REVIEW

Antithetic roles of proteoglycans in cancer

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Abstract Proteoglycans (PGs), a family of complex post-translationally sculptured macromolecules, are fundamental regulators of most normal and aberrant cellular functions. The unparalleled structural–functional diversity of PGs endows them with the ability to serve as critical mediators of the tumor cells' interaction with the host microenvironment, while directly contributing to the organization and dynamic remodeling of this milieu. Despite their indisputable importance during embryonic development and in the adult organism, and their frequent dysregulation in tumor lesions, their precise involvement in tumorigenesis awaits a more decisive demonstration. Particularly challenging is to ascertain to what extent selected PGs may catalyze tumor progression and to what extent they may inhibit it, implying antithetic functions of individual PGs. Integrated efforts are needed to consolidate the routine use of PGs in the clinical monitoring of cancer patients and to broaden the exploitation of these macromolecules as therapeutic targets. Several PGs have the required attributes to be contemplated as effective antigens for immunotherapeutic approaches, while the tangible results obtained in recent clinical trials targeting the NG2/CSPG4 transmembrane PG urge further development of PG-based cancer treatment modalities.

Keywords Proteoglycans · Tumor growth · Metastasis formation · Tumor marker · Immunotherapeutics

Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
CLL-B	Chronic lymphocytic B-cell leukemia
CSPG4	Chondroitin sulfate proteoglycan-4
ECM	Extracellular matrix
EGF	Epidermal growth factor
FAK	Focal adhesion kinase
FGF	Fibroblast growth factor
GAG	Glycosaminoglycan
GPC	Glypican
HGF	Hepatocyte growth factor
HMW-MAA	High molecular weight melanoma-associated antigen
HS	Heparan sulfate
HSPG	Heparan sulfate proteoglycan
IGF	Insulin-like growth factor
MCSP	Melanoma cell surface proteoglycan
MLL	Mixed-lineage leukemia
MMP	Metalloproteinase
PG	Proteoglycan
SDC	Syndecan
TGFβ2	Transforming growth factor β2
VCAN	Versican
VEGF	Vascular endothelial growth factor

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Are proteoglycans involved in neoplastic transformation?

Transformation of cells causes overwhelming changes in their gene expression patterns and, implicitly, proteoglycans (PGs; and their GAGs, glycosaminoglycan side chains) fall within the classes of molecules affected by these genetic alterations [1–8]. The questions that remain unanswered are whether, and to what extent, PGs may directly influence the process of neoplastic transformation. It is also unknown to what extent modulation of PG expression is a mere consequence of this transformation and if this alone may impact upon the subsequent phases of tumorigenesis. Recent experimental evidence suggests that variations in the profile of PGs may be positively or negatively linked to the formation of tumors, but further data from animal models and patient material are required to conclusively establish such a link. Furthermore, it remains to be confirmed whether PGs affect neoplastic transformation at the single cell level (i.e., in a cell-autonomous fashion), or in the context of a cohort of interacting, transforming cells. For several discrete PGs, there have been remarkably perplexing observations concerning the ability of these molecules to influence tumorigenesis. While diversities may be associated with the tumor type, other differences may be attributable to the experimental design/set up through which data were generated. Coincidently, the currently reported observations on cancer patients are often inconsistent and/or are not sufficiently well elaborated to allow for definite conclusions to be drawn. One conclusion can nonetheless be made: namely, that certain PGs promote progression of a given tumor whereas others may inhibit and that, in some cases, the selfsame PG seems to exert converse effects in different tumors.

The first report on changes in PG expression during experimentally induced neoplastic transformation (revealed by monitoring changes in GAG synthesis) dates back to 1976 as recounted by the work of Michael Kalgsbrun [9]. A subsequent milestone paper of Erkki Ruoslahti's group [10], in which a similar experimental paradigm was employed, confirmed a transformation-dependent modulation of (unidentified) heparan sulfate (HS)-bearing PGs. These studies were paralleled by investigations revealing a marked malignancy-associated up-regulation of the high molecular weight melanoma-associated antigen/MCSP (now widely known as NG2/CSPG4) in various types of melanomas when compared to normal melanocytes [11–15].

Since publication of these early discoveries, innumerable studies have documented altered PG expression in all frequently occurring tumor types and, in these, virtually all currently known PGs have been implicated [Supplemental information] (Table 1). Detailed studies on discrete PGs have highlighted progressive modifications in the patterns of PG expression that closely follow the progressive phases of tumor development. This additional piece of information suggests that promotion of PG synthesis is subject to a delicate control imposed by the sequential genetic alterations ensuing in a cancer cell. Examples of this latter assumption are the in situ distribution patterns of versican in melanoma, the modulations of biglycan and lumican expression in this tumor [16] and in cholangiocarcinomas [17], and the acquired expression of opticin during the formation of tumors of the ciliary body [18]. In melanoma, versican has been found to be absent in benign melanocytic nevi, to be weakly expressed in dysplastic nevi, and to be abundantly expressed in advanced phases of the tumor and in metastatic lesions [19, 20]. Biglycan synthesis seems to accompany the "dysplasia–adenoma–carcinoma" transition, with the PG gradually increasing during this multistep carcinogenesis sequence [21].

Further examples of the fact that fluctuation of matrix PG synthesis may be intimately associated with the phenotypic changes that cancer cells undergo during malignant conversion are afforded by the neoplastic associations of lumican, endocan and bamacan [Supplemental information] (Table 1). In osteosarcomas, deposition of lumican seems to correlate with the differentiation status of the neoplastic cells, but to be inversely related to the growth of the primary tumor masses [22]. Differentiation-associated changes in PG expression have also been reported in mesothelioma, where they have been suggested to be instrumental in dictating the aggressive behavior of the neoplastic cells [23]. However, with the exception of a few sporadic cases, such as e.g., those of forced bamacan expression in NIH3T3 cells [24], or transduction of endocan into HEK293 human kidney cells [25], there is currently no firm support for a transformation-inducing role for PGs. Thus, collectively, current observations support the idea that PG expression may be a secondary event of neoplastic transformation, rather than a primary one, and do not convincingly enforce the idea that PGs may act as direct promoters of malignant conversion.

Bearing this in mind, we have recently addressed the putative tumor-initiating potential of NG2/CSPG4 using a proto-oncogene-based in vivo glioma model which entails retroviral transduction of PDGF into fetal brain of the mouse [26]. Given the well-established co-receptor role of NG2/CSPG4 in PDGF signaling [4, 27, 28], we hypothesized that NG2/CSPG4 could be essential glioma formation induced by the growth factor. Somewhat unexpectedly, PDGF transduction into NG2/CSPG4 null mouse brains yielded analogous glioma lesions to those detected in wild type animals. Furthermore, these tumors formed with the same frequency and timescale in the two mouse genotypes and were equally re-transplantable [29].

Table 1 Altered PG expression patterns in different tumor types

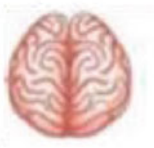
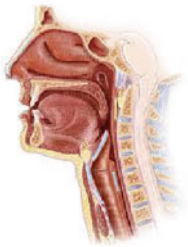
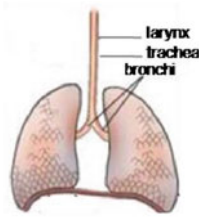
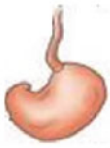
Organ	Tumour type	Proteoglycan[a]
	Glioblastoma	Agrin, Asporin, Biglycan, Brevican, Fibromodulin, GPC4, NG2, Mimecan, Osteomodulin, PRELP, SDC1
	Neuroblastoma	GPC3
	Head and neck carcinoma	Betaglycan, Biglycan, Decorin, Perlecan, SDC1, SDC3, VCAN
	Laryngeal carcinoma	Aggrecan, Decorin, SDC1, SDC1[b], VCAN
	Nasopharyngeal carcinoma	Serglycin, SDC1
	Salivary gland adenoma	Aggrecan
	Thyroid carcinoma	Asporin, Biglycan, GPC1, Lumican, NG2, Perlecan, VCAN
	Adenocarcinoma	GPC3, GPC5, Lumican, VCAN
	Esophageal carcinoma	Decorin[b]
	Non-small cell lung carcinoma	Betaglycan, Mimecan, SDC1
	Small cell lung carcinoma	Mimecan, SDC1[b]
	Squamous cell lung carcinoma	GPC3, SDC1
	Breast carcinoma	Asporin, Betaglycan, Decorin, Lumican, GPC1, GPC3[c], GPC4, SDC1, SDC1[c], SDC4[c], VCAN
	Pancreatic adenocarcinoma	Asporin, Betaglycan, Biglycan, Decorin, GPC1, Lumican, SDC1, VCAN
	Cholangiocellular carcinoma	Agrin, Agrin[c], Biglycan
	Hepatocellular carcinoma	Agrin, Betaglycan, Decorin, Endocan, GPC2, GPC3, GPC3[c], Perlecan, SDC1, VCAN
	Gastric carcinoma	Betaglycan, Decorin, GPC3, GPC3[c], SDC1, VCAN

Table 1 continued

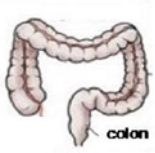
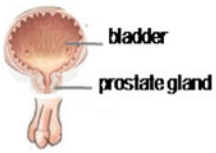
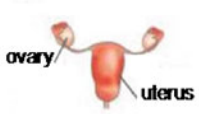
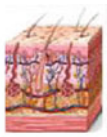
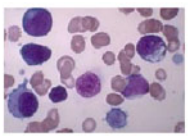
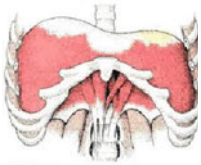
	Renal carcinoma	**Asporin**, **Betaglycan**, **GPC1**, **GPC2**, **Perlecan**
	Wilms' tumor	**GPC3**
	Colorectal carcinoma	**Asporin**, **Bamacan**, **Betaglycan**, **Biglycan**, **Decorin**, **Lumican**, **Mimecan**, **Perlecan**, **SDC1**, **VCAN**
	Prostate carcinoma	**Asporin**, **Betaglycan**, **Biglycan**, **Decorin**, **SDC1**, **SDC2**, **VCAN**
	Testicular germ cell tumour	**Decorin**, **VCAN**
	Urothelial carcinoma	**Asporin**, **Biglycan**, **Decorin**, **VCAN**
	Cervical/endometrial carcinoma	**Asporin**, **Biglycan**, **Decorin**, **Lumican**, **Perlecan**, **SDC1**, **SDC3**, **SDC4**, **VCAN**, **VCAN**[c]
	Ovarian carcinoma	**Asporin**, **GPC3**, **VCAN**
	Ovary clear cell carcinoma	**GPC3**
	Melanoma	**Asporin**, **GPC3**, **GPC3**[b], **NG2**, **Perlecan**, **VCAN**
	Fibrosarcoma	**NG2**
	Chondrosarcoma	**Aggrecan**
	Leiomyosarcoma	**Decorin**, **NG2**
	Liposarcoma	**Decorin**, **Decorin**[c], **NG2**
	Neurofibrosarcoma	**Decorin**, **Decorin**[c]
	Pleomorphic sarcoma	**Biglycan**, **Decorin**, **Decorin**[c], **NG2**
	Rhabdomyosarcoma	**Decorin**, **GPC3**, **GPC5**
	Synovial sarcoma	**Decorin**
	Multiple Myeloma	**Serglycin**, **SDC1**, **SDC1**[b]
	ALL(-B/-T)	**NG2**
	AML	**Serglycin**
	CLL-B	**Fibromodulin**, **SDC1**

Table 1 continued

	Hodgkin's lymphoma	SDC1
	Mantle cell lymphoma	Fibromodulin
	Non-Hodgkin's lymphoma	SDC1
	Mesothelioma	GPC3, SDC2

Data refer to PG expression at either mRNA and/or protein level in the indicated tumors when compared to healthy counterpart tissues or benign forms. Analyses performed at the molecular level did not contemplate prior microdissection of the neoplastic tissue, with the exception of syndecan-1 expression quantification in primary, recurrent and metastatic nasopharyngeal carcinoma specimens using the laser capture microdissection technique [*Supplementary information*]

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *CLL-B* chronic lymphocytic B-cell leukemia, *GPC1–5* glypican-1–5, *SDC1–4* syndecan-1–4, *VCAN* versican

[a] *Red* hyaluronan-binding PGs, *green* collagen-associated PGs, *purple* basement membrane PGs, *blue* cell surface PGs, *orange* Intracellular PG

[b] Refers to PGs detected in blood

[c] Refers to assessment of PG expression at either mRNA and/or protein level in metastatic versus primary lesions

Tumorigenesis-associated alterations of PG expression are functionally bivalent and may alternatively favor tumor growth or tumor suppression

The discovery that biglycan may become progressively down-regulated with the gradual acquisition of a malignant state underlines another crucial point: namely, that many PGs may be down-regulated rather than up-regulated and may exert antithetic roles during neoplastic transformation [Supplemental information] (Table 2). A pioneering discovery in this sense was the apparent silencing of the syndecan-1/CD138 gene in epithelial cells undergoing conversion to a cutaneous carcinoma cell [30, 31]. Since then, loss of syndecan-1 expression has been reinforced as a putative hallmark of tumorigenesis, not only in skin cancers [32–34], but also in the formation of a variety of other epithelial tumors [35–45, Supplemental information]. The outcome of these studies would assign to syndecan-1 an apparent tumor-suppressing role, at least in carcinomas. However, experimental data involving syndecan-1 null mice suggest that the PG could exert the opposite effect on cancer formation. Mice harboring deletion of the syndecan-1 gene were originally found to be refractory to Wnt-1-induced mammary carcinoma formation [46] and were subsequently discovered to be resistant to a variety of solid and hematopoietic tumors induced by treatment with carcinogens [47].

While a putative tumor-suppressing role of syndecan-1/CD138 in human cancer may have a significance in prognostic terms (see below), much more information is needed to understand the underlying molecular bases for its disappearance from the surface of neoplastic cells (not necessarily fully matched by a complete transcriptional shut-off of the gene). In particular, it remains to be fully understood whether loss-of-function of syndecan-1/CD138 is instrumental in the transformation process, or whether its disappearance is part of a globally altered gene profile characterizing the neoplastic cell. There is also the need for a better grasp of the biological consequences of this phenomenon, as well as just why deprivation of syndecan-1/CD138 should be so critically important in epithelial carcinogenesis. Especially considering that epithelial cells normally express multiple syndecans, which could potentially substitute for syndecan-1/CD138; none of the other syndecans has thus far been shown to possess tumor-suppressing properties.

Several glypicans parallel syndecans in their surface reduction upon malignant transformation [Supplemental information] (Table 1). The most striking cases probably represented by inactivation of glypican-3 (GPC3) and glypican-5 (GPC5) genes in ovarian carcinomas, lung carcinomas, breast carcinomas, mesothelioma, and gastric cancers [48–54]. A remarkable finding in this context is that never smokers, who harbor specific polymorphisms in the GPC5 gene, are heavily prone to developing non-small cell lung carcinoma. Genetic variations within chromosome 13q31.3 of these individuals alter the expression of lung GPC5, normally present at a relatively high level in

Table 2 Antithetic roles of PGs in tumor formation and progression

Proteoglycan		
Aggrecan		Laryngeal carcinoma
Agrin	Hepatocellular carcinoma	Glioblastoma[§]
Asporin	Breast carcinoma	
Bamacan	Colorectal carcinoma*	
Betaglycan		Breast carcinoma Hepatocellular carcinoma Non-small cell lung carcinoma[#,§] Prostate carcinoma* Renal carcinoma*
Biglycan	Cholangiocellular carcinoma Colorectal carcinoma* Pancreatic adenocarcinoma[#] Pleomorphic sarcoma	Glioblastoma
Brevican		Glioblastoma[#,§]
Decorin	Head and neck carcinoma* Laryngeal carcinoma[#] Gastric carcinoma Pancreatic carcinoma[#] Rectum carcinoma Testicular germ cell tumor	Breast carcinoma*[,#,§] Colorectal carcinoma* Hepatocellular carcinoma* Lung adenocarcinoma[§] Sarcomas[§]
Endocan	Hepatocellular carcinoma*	
Fibromodulin	CLL-B Mantle cell lymphoma	Glioblastoma[§]

Table 2 continued

GPC1	Breast carcinoma Pancreatic carcinoma*	
GPC3	Hepatocellular carcinoma# Melanoma Neuroblastoma*,# Rhabdomyosarcoma Squamous cell lung carcinoma Wilms' tumor#	Breast carcinoma Gastric carcinoma Lung adenocarcinoma Mesothelioma# Ovarian carcinomas#
GPC5	Rhabdomyosarcoma#	Lung adenocarcinoma
Lumican	Breast carcinoma*,#,§ Colorectal carcinoma# Pancreatic carcinoma#	Invasive breast carcinoma§
NG2/CSPG4	ALL AML Glioblastoma Melanoma§ Sarcomas§	
Mimecan	Non-small cell lung carcinoma	Colorectal carcinoma Glioblastoma Small cell lung carcinoma
Osteomodulin		Glioblastoma
Perlecan	Colorectal carcinoma Head and neck carcinoma# Hepatocellular carcinoma Melanoma#	
PRELP		Glioblastoma
SDC1	Breast carcinoma*,§ Glioblastoma Multiple Myeloma#,§	Cervical/endometrial carcinoma*,§ Colorectal carcinoma§ Gastric carcinoma Head and neck carcinoma*

Table 2 continued

		Hepatocellular carcinoma[§]
		Laryngeal carcinoma
SDC1		Non-small cell lung carcinoma[§]
		Pancreatic adenocarcinoma*
		Prostate carcinoma*,[§]
		Squamous cell lung carcinoma[§]
SDC2	Mesothelioma	Prostate carcinoma*,[§]
	AML	
Serglycin	Multiple myeloma	
	Nasopharyngeal carcinoma[§]	
	Breast carcinoma	
	Endometrial carcinoma[§]	
	Gastric carcinoma	
	Head and neck carcinoma	
	Laryngeal carcinoma	
Versican	Melanoma*	
	Ovarian carcinoma[§]	
	Pancreatic carcinoma[#]	
	Prostate carcinoma	
	Rectum carcinoma	
	Testicular germ cell tumour[§]	

Refers to positive (*tumor-promoting*) or negative (*tumor-inhibitory*) effects caused by alterations in PG expression (i.e., caused by up- or down-regulation of PGs), wherever more solidly established by experimental means

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *CLL-B* chronic lymphocytic B-cell leukemia

* Involvement in tumor onset

[#] Involvement in tumor growth

[§] Involvement in tumor invasion/metastasis

this tissue. This seems in turn to cause a marked decrease in the in situ levels of the PG and the consequent propensity of the lung epithelial cells to undergo neoplastic transformation [55, Supplemental information] (Table 2). The finding builds upon the previous suggestion of a tumor-suppressing role for GPC3 (most closely related to GPC5) and provides the first indisputable evidence of a direct tumor-protecting function for a PG in humans. Thus, if confirmed on a larger scale, GPC5 polymorphism analysis would afford the first PG-related lung cancer risk assessment tool for routine clinical testing. On the other hand, a certain ambiguity remains regarding the effective biological role of GPC5 in tumorigenesis. This since genomic amplifications in the 13q31.3 chromosome have

been identified in mantle cell lymphomas [56, 57] and a variety of B-cell lymphoma lines [58]. Similar gains in GPC5 gene copy number have been disclosed in rhabdomyosarcomas [59] and have been suggested to be implicated in the pathogenesis of these pediatric tumors.

Cell surface PGs may not be the only ones negatively affecting tumorigenic events (Table 2). Enhanced expression of lumican suppresses v-Src- and v-K-Ras-induced cell transformation in vitro [60] and may also interfere with melanoma progression [61]. Mimecan is markedly down-regulated in colorectal carcinoma [62], whereas another PG of the small leucine-rich subfamily, decorin, is a potent tumor growth- and metastasis-inhibiting factor [63–69]. However, yet another antithetic condition is noted with this latter PG. Deletion of the decorin gene predisposes for intestinal tumors [70], confirming a possible link between decorin expression and oncogene-induced transformation as proposed early on by Kolettas and Rosenberger [71]. Furthermore, the experimentally demonstrated inter-relationship between decorin expression and the evolvement of tumors has a corollary in cancer patients. In fact, reduced levels of this PG are associated with a worse prognosis in soft-tissue sarcomas [72], carcinomas of the lung [73] and node-negative breast carcinoma patients [74].

Examples of more complex antithetic roles of PGs in the control of tumor progression are those related to perlecan and versican [Supplemental information] (Table 2). In a variety of tumor cells, antisense targeting of perlecan has simultaneously been reported to inhibit the growth and invasive potential of the cells in vitro and immunodeficient mice [75, 76] and to enhance the malignant behavior of the transduced cells [77, 78]. Discrepancies in the observed tumor growth-promoting effects of perlecan in vivo may be associated with tumor cell type-specific differences or with the involvement of the PG in the tumor angiogenic phenomena documented in knock-out mice [79].

Since perlecan is a constitutive basement membrane PG engaged in multivalent complexes with laminins, collagen type IV and nidogen, it is remarkable that loss of the endogenously produced macromolecule may interfere with cellular events such as proliferation, migration or invasion. One lead may be the observed cell surface retention of proteolytic fragments of perlecan, plausibly entrapped in that location through a binding to integrin $\alpha 2\beta 1$ [80–82]. In turn, cell surface sequestering of intact or fragmented perlecan may be instrumental for the PG's involvement in interactions with FGFs, FGF-binding proteins and FGF receptors [83–88], and thereby provide a mechanism by which tumor cells might augment their mitogenic FGF responses to increase their growth rates. Conversely, less clear is, again, just how this growth factor-docking function could intervene in cell motility and invasion phenomena. Another lead may be that intact and fragmented perlecan act differently. In fact, proteolytic release of the C-terminal domain of perlecan generates a cryptic anti-angiogenic factor denoted endorepellin [89, 90]. Thus, the idea is that in the abundant presence of matrix-degrading enzymes, perlecan may be converted from a tumor-promoting basement membrane component to a tumor-inhibitory angiogenesis antagonist. Based on this prevailing paradigm, overproduction of perlecan by the cancer cells may compensate for perlecan fragmentation to maintain high levels of intact (tumor-promoting) perlecan around the cells.

The effect of versican on tumor progression seems equally contrasting and is further confounded by differences observed between cellular and experimental models [Supplemental information] (Table 2). As versican synthesis increases in melanoma with increased malignancy [19, 20], abrogation of versican expression decreases the proliferation and migration capabilities of such cells [91]. Furthermore, overproduction of the V3 isoform induces a less aggressive behavior of cells by negatively modulating the CD44-assisted mitogenic responses of the cells [92–94]. However, attempts to dissect the individual functions of the G1 and G3 globular domains making entirely up this versican isoform have produced remarkably differing results. Forced G1 domain transduction in sarcoma cells [95] enhances growth of these cells in vivo and similar results have been obtained by expressing the full-length V1 versican isoform in carcinoma cells [96]. Over-expression of the G3 globular domain in breast carcinoma and astrocytoma cells mimics the EGF-dependent melanoma growth-promoting activity of the V3 isoform [97–99]. However, in glioma cells the fine-tuned control of EGF signaling exerted by the isolated G3 domain seems to counteract the in vivo growth-promoting activity of the full-length versions of the PG [100]. Thus, substantially more detailed and controlled studies are needed to assess to what extent versican domain-specific effects on tumor progression differ in diverse tumor types and to what extent these effects may be dependent upon distinct patterns of proteolysis of the PG. Similarly, we need to gain a deeper understanding of whether the consequences of enhanced V3 versican isoform expression are truly associated with its "non-PG" nature, or whether they are attributed to intracellular activities favored by an inefficient extracellular secretion of this versican variant. Of note is that there is currently no unequivocal distributional map of the V3 protein in tumor tissues that can support a prevalent intracellular or extracellular effect of this PG.

The identification of subsets of cancer stem/initiating cells, along with experimental evidence that tumors probably arise through an initial transformation of stem/progenitor cells (rather than of more mature phenotypes), complicates the functional interpretation of cancer-related

changes in PG expression manifested by neoplastic cells. It suggests that differences in PG expression observed between healthy tissues and neoplastic cells may be focalized in specific cancer cell subsets. Thus far, this has only been contemplated for NG2 in acute myeloid leukemia [101] and certain carcinomas [102]. Accurate four-way comparisons of the PG profiles of normal and cancer stem/progenitor cells versus mature cell phenotypes and the preponderant bulk of tumor cells making up the lesions have not yet been achieved. Consequently, it remains unclear whether the normal stem/progenitor-cancer stem/initiating cell-axis is characterized by overlapping PG repertoires, or whether defined PG expression patterns may pre-delineate highly malignant subpopulations of tumor cells.

Elimination of experimental- and study-design caveats is the key to firmly establishing the potential of PGs as diagnostic and/or prognostic factors

Aberrant expression of PGs in neoplastic tissues provides a rationale for evaluating their potential as diagnostic and/or prognostic factors and for considering them as a valuable parameter in therapeutic response prediction. However, only in a few instances are PGs currently contemplated as routine diagnostic markers and in virtually no documented situation is altered PG expression accepted as a decisive tumor classification parameter [Supplemental information] (Table 3). This by no means infers that expression patterns of PGs in neoplasia lack important diagnostic information. For instance, syndecan-1/CD138 is a widely recognized plasma cell marker and is generally adopted in the routine diagnosis of Hodgkin and non-Hodgkin's lymphomas, as well as in differential diagnosis of a number of more infrequent virally-mediated lymphomas [43, 103–106]. De novo/ectopic expression of NG2 in certain variants of infant acute lymphoblastic and myeloid leukemia with MLL rearrangements distinguishes unique disease entities with hybrid phenotypic traits [107–114].

Enhanced expression of serglycin may further add to the differential diagnosis of acute myeloid leukemia [115] and may have a prognostic impact in multiple myeloma [116], whereas GPC3 is a firmly recognized hepatocellular carcinoma marker [51, 117–122], whose expression levels seem to correlate with malignancy degree [123]. Lastly, up-regulated mimecan expression is proposed as a key element for the diagnostic discrimination of non-small cell and small-cell lung carcinomas [124]. Overall, it is plausible to assume that the scanty utilization of diverse PG expression patterns as an adjunct in routine cancer diagnosis may reflect the paucity of proper supportive data. This incites the need to pay closer attention to the potential of PGs in tumor classification efforts and reconsider their putative value as cues in differential cancer diagnosis. In this context, our preliminary observations on soft-tissue sarcomas and head and neck tumors suggest that it is fundamental to consider variations in the global pattern of PG expression rather than modulations of the single PGs. Our findings also emphasize the need to take into account the differential distributions of glycanation isoforms of the molecules, as consistent with the recurrent theme of alterations in post-translational modifications of PGs in cancer cells.

While the diagnostic potential of PGs seems presently somewhat limited, their perturbed expression may have a much stronger impact on disease course prediction. Accordingly, in many tumor types, perturbed expression of single or combinations of PGs has been suggested as being of prognostic relevance (Table 3). For instance, augmented expression of versican, decorin, lumican, biglycan, serglycin, NG2 and syndecan-1/CD138 have been proposed as relevant in the prognostication of the disease course in a plethora of tumors [74, 106, 107, 116, 125–134, Supplemental information]. However, only in a few cases have these PG alterations been documented to be bona fide "independent prognostic factors", as specifically referred to disease-free or overall survival prediction [112, 114, 135–138].

PG alterations often fail to reach the stringency level required to adopt them as independent prognostic indicators. This may in part be due to the scarce attention that has been given to more accurately evaluating the independence of the disclosed PG aberrations from other recognized clinical parameters of prognosis. Secondarily, a recurrent caveat is that studies in this field have involved heterogeneous cohorts of patients (e.g., surgical and post-surgical treatment modalities/regimens, risk factors, or parameters associated with intrinsic characteristics of the tumors), or have failed to make precise comparisons between primary, metastatic, and relapsing lesions from the same individual. In one of our recent study on the role diagnostic role of NG2 in adult soft-tissue sarcomas [137], and its extension [Benassi et al., unpublished], we have specifically contemplated this latter point. In fact, we ascertained the prognostic (disease-free and overall survival) significance and metastasis formation-predicting potential of the PG when comparing primary and metastatic lesions from the same individuals.

Overall, the unsatisfactory experimental and study design of many published investigations leaves it rather uncertain to what extent modulation of PG expression in neoplastic versus healthy tissues and in primary versus metastatic/relapsing lesions might effectively be exploited to advance the clinical management of cancer patients. Furthermore, apart from some sporadic pieces of

Table 3 Prospected clinical potential of PGs

Proteoglycan	Diagnostic	Predictive/Prognostic	Therapeutic[a]
Aggrecan	Chondroblastoma Chondrosarcoma Chordoma	Not known	Not known
Agrin	Hepatocellular carcinoma	Not known	Not known
Biglycan	Cholangiocarcinoma	Pleomorphic sarcoma	Not known
Brevican	Not known	Not known	Not known
Decorin	Not known	Breast carcinoma Esophageal squamous cell carcinoma Ovarian carcinoma Pancreatic carcinoma Sarcomas	Not known
Endocan	Not known	Hepatocellular carcinoma Non-small cell lung carcinoma Renal cell carcinoma	Not known
Fibromodulin	CLL-B	Not known	Not known
GPC1	Not known	Ovarian carcinoma Pancreatic carcinoma	Not known
GPC2	Not known	Not known	Not known
GPC3	Hepatocellular carcinoma	Hepatocellular carcinoma Ovarian carcinoma	Hepatocellular carcinoma?
GPC4	Not known	Not known	Not known
GPC5	Not known	Not known	Not known
GPC6	Not known	Not known	Not known
Keratocan	Not known	Not known	Not known

Table 3 continued

Lumican	Not known	Breast carcinoma Colorectal cancer	Not known
Mimecan	Non-small cell lung carcinoma	Not known	Not known
Neurocan	Not known	Not known	Not known
Neuroglycan-C	Not known	Not known	Not known
NG2/CSPG4	ALL AML Chordoma?	AML Glioma Sarcomas	Melanoma Sarcomas?
Perlecan	Not known	Not known	Not known
Serglycin	AML	Nasopharyngeal carcinoma	Not known
SDC1	Multiple myeloma Pleural effusion lymphoma	Breast carcinoma Cervical carcinoma Cholangiocarcinoma Endometrial carcinoma Head and neck carcinoma Hodgkin's lymphoma Mesothelioma Multiple Myeloma Nasopharyngeal carcinoma Ovarian carcinoma Pancreatic carcinoma Prostate carcinoma Small cell lung carcinoma	Lymphomas? Multiple Myeloma?
SDC2	Colon carcinoma Mesothelioma	Prostate carcinoma	Not known
SDC3	Not known	Not known	Not known
SDC4	Not known	Not known	Not known
Versican	Melanoma	Breast carcinoma Head and neck carcinoma Ovarian carcinoma Prostate carcinoma	

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *CLL-B* chronic lymphocytic B-cell leukemia

[a] Refers to immunotherapeutic approaches

information available for syndecans and lumican [139–143], surprisingly little is known about how single or groups of PGs might be exploited as predictors of therapeutic response [Supplemental information] (Table 3). Thus, even in this case, a closer attention needs to pay to how PG expression profiles (both in situ and in

circulation) may vary over the course of treatment of cancer patients. This would better resolve the therapeutic response predicting value of PGs (a clinical issue now recognized to be of paramount importance for personalized medicine).

Contribution and clinical impact of PGs in the organization of the tumor stroma

Interaction of tumor cells with the intra-lesional stroma is becoming widely accepted as a primary mediator of local tumor growth and is likewise believed to be indirectly implicated in the formation of metastases. In particular, it is believed that the tumor stroma may trigger the escape of pro-metastatic cells from primary lesions. Consequently, compositional and quantitative analyses of the stromal compartment of tumor lesions are considered as fundamental not only for the understanding of the biology of cancer, but also for clinical intervention. In most tumor types, the intra-lesional stroma is abounding in PGs suggesting that up-regulation of these macromolecules may be directly associated with the formation and maintenance of this compartment. A primary PG of the tumor stroma is versican [128, 140, 144–149], as would be expected of a loose connective tissue composed mainly of fibroblastic cells and hematopoietic precursors. It is therefore doubtful whether intra-lesional accumulation of this PG truly is a specific trait of the stroma of certain tumor types.

One wonders if enhanced levels of versican are not simply a measure of a more abundant stromal content in a lesion. As such, versican would not be a superior tumor progression factor than other stromal components. As a corollary, the prognostic significance attributed to stromal versican has not consistently been found as absolute and thereby capable of surmounting the importance of other clinical–pathological predictors of patient survival [45, 127, 128, 136, 144–146, 150–152]. Versican may not be the "hyalectan" PG associated with tumor stroma. In fact, in pleomorphic adenoma of the salivary gland (the most common epithelial neoplasm in these glands), aggrecan lines ECM microfibrils of the myxoid tumor stroma and it can be immunolocalized in association with neoplastic myoepithelial cells [153]. Along with versican, two smaller PGs are frequently detected in the stroma of numerous tumor types where they exhibit distribution patterns that mirror those of versican. While decorin may be down-regulated, lumican, fibromodulin (see below) and biglycan are often up-regulated. This may be the key explanation as to why these PGs are reported as having contrasting effects on tumor progression. In particular, because of the documented involvement of decorin in TGF signaling [63, 154–157], reduced levels of this PG might be causing a reduction in the magnitude of stimuli transduced by members of this growth factor superfamily. Thus, down-regulation of decorin may serve the purpose to maintain the stromal compartment in a rather plastic, undifferentiated state to better adapt to the tumor's 'needs'.

Paradoxically, while retention of syndecan-1 on the surface of cancer cells may prevent progression towards a more malignant phenotype, and can thereby be exploited as a positive prognostic indicator, up-regulation and/or enhanced shedding of the same PG in the tumor stroma appears to be a clinically negative factor [45, 126, 158–161]. The most representative such case is probably that of bone marrow-derived stroma of multiple myeloma. There a complex pattern of expression of syndecan-1 on the neoplastic plasma cells and on the bone marrow-resident stromal cells appears to govern the biology of the disease [162, 163]. Despite the elusive role of stromal PGs in the control of tumor growth and expansion, enhanced or de novo expression of fibromodulin has been a certain significance by experimental means. In fact, augmented levels of this PG seem to induce a rise in osmotic pressure within primary tumor masses and thereby promote the creation of a particularly hostile microenvironment impermeable to cytotoxic drugs [164]. Thus, "fibrotic" cross-linking of collagenous fibers by fibromodulin, rather than by decorin, appears to counteract the hydrating role of versican-hyaluronan complexes in stromal tissues to induce compaction of tumor nodules. The seemingly widespread deposition of fibromodulin in the stroma of several tumor types [165] (our own unpublished observations) also suggest that this PG may effectively contribute to the stromal influence on the aggressiveness of discrete tumors.

Multivalency of the PG promotion of local tumor growth

Being divided between the extracellular milieu, as constituents of the ECM or as cell surface shedded components, and the outer membrane of cancer cells, PGs may invariably take part in the control of multiple oncogenic events; PGs may do so in a multivalent manner [Supplemental information] (Table 4). While it is axiomatic that cell division and cell movement are the cardinal drivers of tumor progression, it may not be so implicit that PGs influence both cellular phenomena. It is similarly often overlooked that PGs may be controlling these cellular events at multiple levels, i.e., with multiple PGs cooperating with one another in some cases while counteracting each other's individual function in other cases.

Cell surface-associated PGs, such as syndecans and glypicans, are widely recognized co-receptors for a number

Table 4 Antithetic involvement of PGs in the regulation of cellular events associated with tumor progression

Proteoglycan	Cellular event
Biglycan	↑ invasion
Brevican	↑ motility, ↑ invasion
Decorin	↓ proliferation, ↓ motility, ↓ metastasis formation
Endocan	↑ proliferation, ↑ angiogenesis
Fibromodulin	↓ motility, ↑ T cell activation, ↓ drug resistance
GPC1	↑ proliferation, ↑ invasion, ↑ angiogenesis
GPC3	↑ metastasis formation, ↑ proliferation, ↑ apoptosis
Lumican	↓ proliferation, ↓ motility
NG2/CSPG4	↑ motility, ↑ proliferation, ↑ metastasis formation, ↑ T cell activation, ↑ immune response, ↑↓ apoptosis
Perlecan	↑↓ proliferation, ↓ invasion, ↑↓ angiogenesis
Serglycin	↑ motility, ↑metastasis formation
SDC1	↑↓ proliferation, ↑differentiation, ↑angiogenesis, ↑ motility, ↑ apoptosis, ↓ drug resistance, ↓ invasion
SDC2	↑ proliferation, ↑ angiogenesis, ↑ motility, ↑ apoptosis
SDC4	↑ motility, ↑ invasion, ↑ angiogenesis, ↑ proliferation
Versican	↑ proliferation, ↑ motility, ↓ apoptosis, ↑ invasion, ↑ angiogenesis, ↑ T cell activation

of heparin-binding growth factors and signaling molecules, including those of the FGF family, HGF, PDGFs, IGFs, VEGF, Hedgehogs and Wnts [6, 8, 138, 166–171]. Investigations into their co-receptor activity have focused heavily on the role of their HS side chains and, accordingly, a vast literature describes in great detail the various aspects of the HS-growth factor interaction and its significance for the down-stream signal transductions [Supplemental information] (Table 4; Fig. 1). This means that the kinetics, magnitude, sequence specificity, and structural–functional dynamics of the HS–FGF interplays are well elaborated at the molecular level. In contrast, considerably more veiled is how different HS-bearing PGs, expressed simultaneously on the cell surface, may be orchestrating their discrete co-receptor activities. Hence, there is little or no understanding of how diverse patterns of cell surface PGs, putatively carrying similar or even identical HS chains (since GAG synthesis is proper of the cell type and its specific state), may diversify the cells' growth factor responses. It therefore remains unresolved to what degree specificities of growth factor signaling are determined by particular growth factor-PG core protein combinations (Fig. 1). At present, there is also scant or no experimental evidence that surface HS-PGs necessarily cooperate with one another to potentiate the cancer cells' response to signaling molecules. There is similarly no convincing data supporting the idea that they could alternate their activities spatio-temporally to diversify these responses.

One of our recent studies shows that NG2 may substitute for, or cooperate with, HSPGs to mediate the cells' responses to various FGF ligands [172]. The co-receptor activity of NG2 is exclusively mediated by the core protein, as was previously demonstrated in the case of its contribution to PDGF signaling [27, 28, 173]. This finding is also consistent with a lack of chondroitin sulfate involvement in FGF-induced mitogenesis. Most importantly, NG2 binds FGF ligands with a higher affinity than that displayed by HSs, while simultaneously modulating the FGF receptor binding abilities at the extracellular level. This would imply that the alternative engagement of different cell surface PGs in co-receptor activities may be instrumental in the perception of growth factor gradients and the diversification of cellular responses to these gradients. One intriguing aspect in this context is that both syndecans and glypicans may additionally exert a complex intracellular co-receptor function through their endocytic recycling patterns. Intracellular vesicular transport of these PGs may occasionally involve a heparanase-dependent nuclear import route [174–178].

While cell surface PGs preferentially potentiate promitogenic stimuli, decorin has a unique ability to act as a negative regulator of tumor growth through its intervention in EGF-related signaling events and down-stream activation of cyclin-dependent kinase inhibitors. A study by De Luca and collaborators [179] first hinted at the existence of such an EGF-decorin relationship while subsequent studies by the same group clarified a number of facets of the phenomenon. Briefly, decorin antagonizes EGF signaling by competing for binding to the EGF receptor and thereby induces the sustained down-regulation of the receptor [64, 69, 179–182]. This in turn causes a unique up-regulation of p21 and the internalization of decorin–EGF complexes via caveolin-mediated endocytosis, which channels them to lysosomic degradation [183]. This may, however, not be a generalized mechanism of tumor suppression since enhanced levels of decorin in osteosarcoma cells do not result in growth-arresting effects, but tend to counteract the cytostatic effects of TGFβ2 [184]. The tumor-suppressing function of decorin appears preponderant when considering its ability to negatively influence at least three primary tumor-associated signaling systems, TGFβ, EGF and the c-Met-β-catenin axis [185].

The multifaceted involvement of PGs in signal transduction phenomena is ultimately underscored by the well-established modulation of Wnt (canonical) and Hedgehog signaling cascades exerted by glypicans [3, 138, 169, 186] (Fig. 1). However, it remains unknown how an increased glypican-mediated Wnt and IGF responsiveness can be reconciled with a loss of Hedgehog responsiveness to affect tumor growth. A further intriguing point emerging in this context is that the indirect (or more direct) contribution of

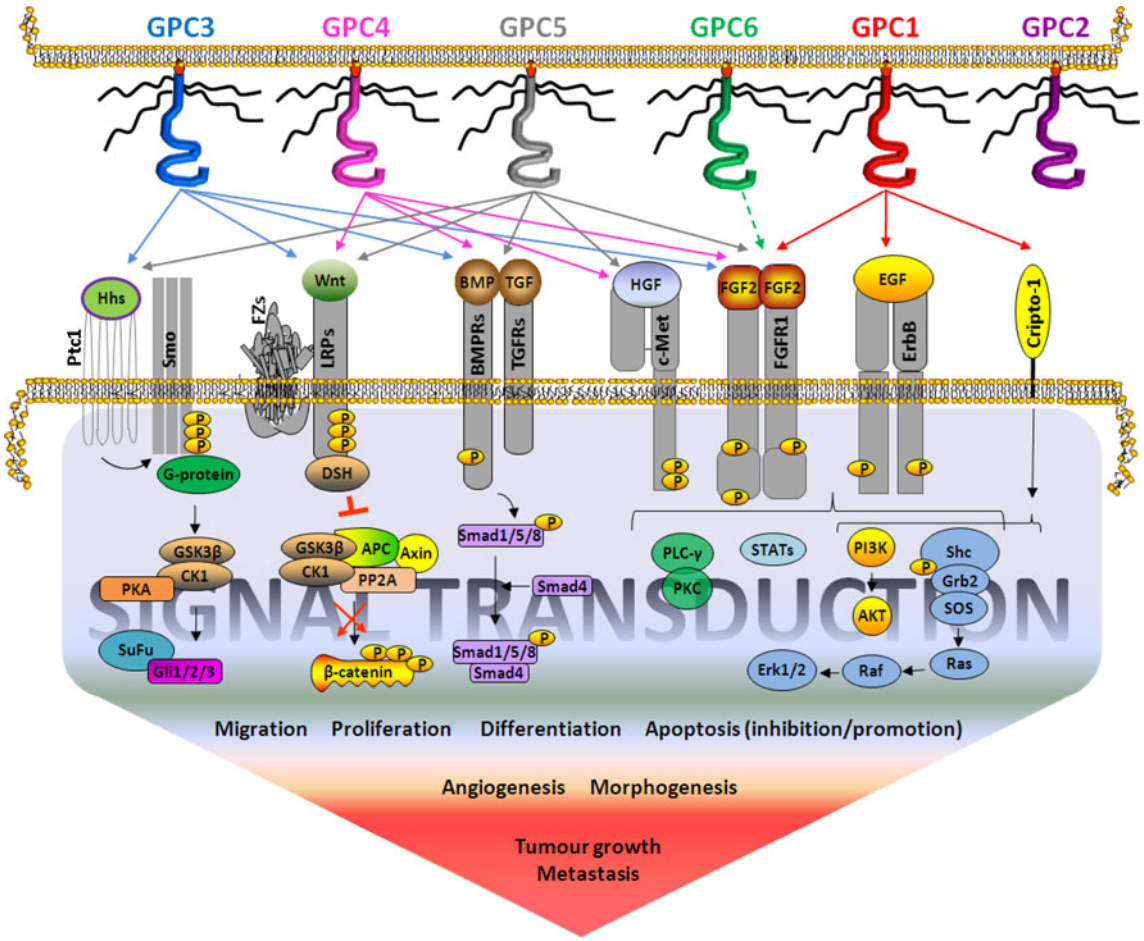


Fig. 1 Schematic overview of the documented molecular interactions exhibited by glypicans at the cellular and subcellular level and their down-stream consequences. Information is primarily available for GPC1, GPC3, GPC4, and GPC5 hints at agonizing co-receptor functions affecting at least five different signaling systems. This in turn implies that glypicans may be capable of influencing a number of biological events that are instrumental for tumor progression. In many cases, the signaling molecule docking effects of glypicans are mediated by their HS side chains, leaving to be unfolded the actual contribution of the core proteins in determining the specificity of these molecular interactions. Yet another obscure issue is modes through which GPC3 and GPC5 may influence multiple signaling pathways critically involved in tumor growth and dissemination and more precisely how they may counterbalance the up-stream regulation of these signal transductions to eventually elicit tumor-promoting or tumor-inhibiting outputs

certain cell surface PGs in a specific signaling cascade affects the expression of other PGs implicated as co-receptors in other signaling cascades. This would create PG-governed positive and negative feedback signaling loops, the biological significance of which is difficult to understand at the present time. The proposed involvement of glypicans in multiple agonizing or counterbalancing signaling cascades also provides a representative example of the complex, multivalent role of PGs in the perception of stimuli controlling the behavior of cancer cells [Supplemental information] (Table 4). This, in turn, prompts the need to investigate in more detail how combinations of PGs may dictate the cellular response to microenvironmental cues by either synergizing or antagonizing each others' functions.

A unique and highly intriguing antithetic role of PGs in the control of tumor cell-host microenvironment is that exhibited by fibromodulin in CLL-B. The completely unpredictable production of this PG by leukemic B cells generates sets of HLA-A2 peptides capable of selecting for $CD8^+$ autologous tumor-specific T cells through antigen presentation [187]. Coincidently, knock-down of fibromodulin in isolated CLL-B cells induces apoptosis, suggesting that the PG is required for survival of these neoplastic cells [188]. Thus, in CLL-B fibromodulin seems to alternatively sustain tumor cell survival and promote indirect cell killing. Most remarkable is that both biological effects seems to be exerted intracellularly because very little or no fibromodulin is actually secreted by these leukemic cells.

Implication of PGs in the optimization of the tumor cells' interaction with the microenvironment and their tissue dissemination

The abundant expression of multiple cell surface PGs and the complex patterns of ECM PG synthesis and secretion by neoplastic cells imply a direct involvement of these macromolecules in the control of the tissue interactions sustaining tumor growth and dissemination. In fact, PGs are recognized as being implicated in the two principle cellular events conducive to metastasis formation: cell motility and tissue invasion (Table 2; Fig. 2). To contribute to these cellular phenomena, cell surface-associated PGs may either directly link to the ECM or modulate the activity of integrins [189] and other matrix receptors. However, with the exception of the well-documented interaction of NG2 with collagen type VI [190–193] and the possible binding of syndecan-1 to collagen type I [194–196], there is currently no incontrovertible evidence of a direct ECM interaction of the core proteins of cell surface PGs. Notably, myeloma cells lacking NG2 take advantage of the HS chains of their syndecan-1 to interact with collagen type VI of the bone marrow stroma [197].

Overall, cell surface PGs may either act as promoters of tissue invasion and metastasis formation (Fig. 2), as demonstrated for syndecan-1, GPC1 and NG2 [198–200], or as inhibitors of these processes, as in the case of GPC3 [201, 202]. In the case of syndecans, alternative promoting or inhibiting functions may depend upon their differential ability to serve as co-receptors with ligand specificities dictated by their GAG chain diversity [203, 204]. This idea has its grounds in the fact that syndecan-1 has been reported to induce intracellular signal transduction independently of integrins [205] and that binding of a variety of tumor cell types to laminins requires a syndecan-1-substrate interaction [206–209]. A similar putative interaction

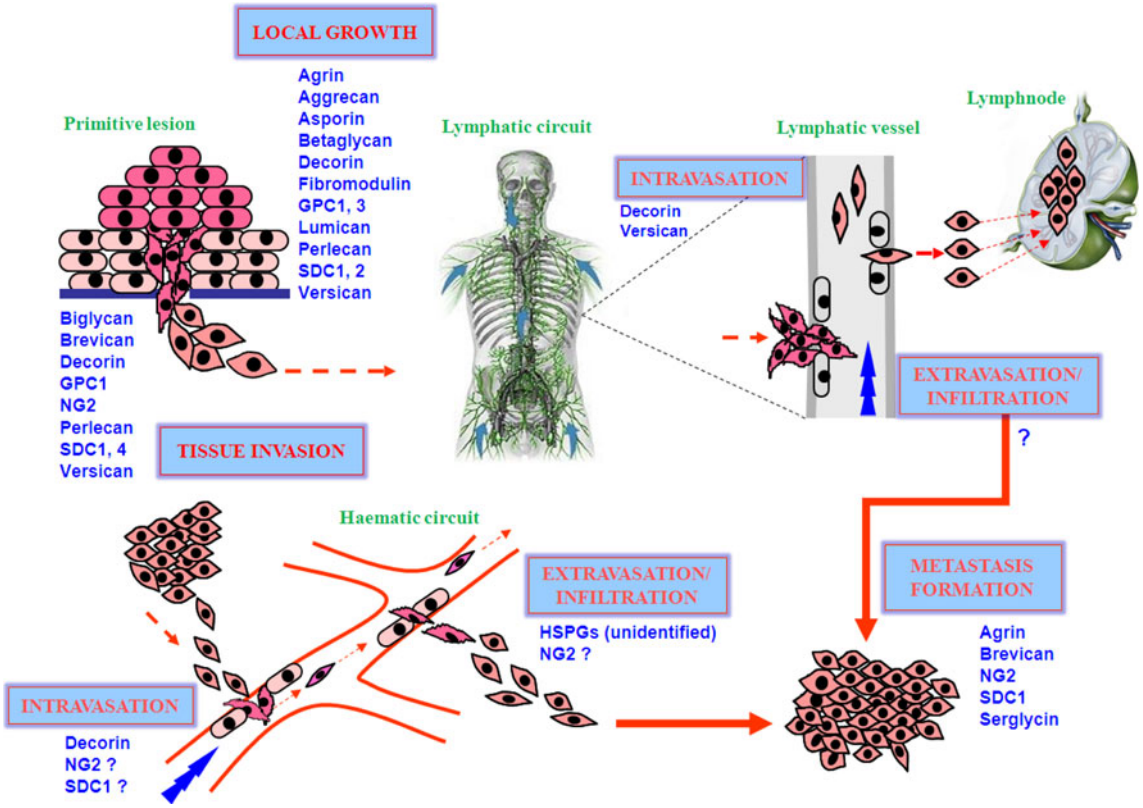


Fig. 2 Schematic overview of the putative PG involvement in the different phases of tumorigenesis. A number of PGs expressed by the tumor cells themselves or by the intralesional stroma promote local growth (and may also affect intra-lesional angiogenesis). By contrast, very little is known about to what extent PGs may contribute to local tissue invasion, the entrance (intravasation) of disseminating cells into the hematic and lymphatic circuits, and their exit from these circuits in target organs (extravasation). HS-bearing PGs have been proposed to be implicated in the process of leukocyte/lymphocyte extravasation (which is thought to be regulated by mechanisms analogous to those governing tumor cell trafficking), but the identity of these PGs has not yet been disclosed. Cell surface PGs, in their membrane-associated or shedded form, are similarly believed to the implicated in this phenomenon and our preliminary findings assigns a pivotal role to NG2 in this process and the recycling disseminating cancer cells through the circulation. A number of PGs have also been demonstrated to be associated with metastasis formation in various experimental models and most of these PG are accordingly found to be up-regulated in human metastatic lesions

of syndecan-1 with ECM components involves thrombospondin-1 [210]. More recently, it has became clear that syndecan-1 operates as an immediate regulator of the activity of $\alpha v\beta 3$, $\alpha v\beta 5$, and $\alpha 6\beta 4$ on breast carcinoma cells [211, 212], presumably through a linkage of its cytoplasmic tail with the intracellular portion of integrins [170, 213].

It has further been suggested that syndecan-1, -2, and -4 act in concert with multiple integrins (e.g., $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha 6\beta 1$ and αv-containing integrins) in different tumor cell lines to specifically affect the cells' fibronectin-, vitronectin-, collagen- and laminin-binding. These syndecans have also been suggested to influence tumor cell invasion through collagenous fibrillar matrices, in part by intervening in lamellopodia formation through Tiam-1 and Rac1 activation [214–218]. However, it remains to be ascertained whether cooperation of syndecans with integrins affects cell adhesion and migration in a positive or negative fashion [34, 69, 208, 218, 219, Supplemental information] (Table 4). It would be equally crucial in this context to determine the precise syndecan–Rho–lamellopodia–filopodia interplay relationship [194–196, 220–227]. NG2 also serves as a mediator of membrane-ECM interactions taking place in moving cells and may also be critical in establishing a continuum between the microenvironment and the actin cytoskeleton. In fact, NG2 is abundant in the advancing front of migrating cells, with a preferential accumulation in filopodia and in juxtaposition to $\alpha 3\beta 1$ [228] and $\alpha 4\beta 1$ [229, 230] integrins. By an as yet concealed mechanism, NG2 seems to modulate the (binding-)activity of these integrins and act as a substrate for FAK/PCKα-/ERK-dependent signaling cascades [231] leading to phosphorylation of two threonines within its cytoplasmic tail [232, 233]. Through its intimate connection with the cytoskeleton, NG2 may additionally activate Rac1 and p130CAS to transduce shape modulating and polarizing signals in locomoting cells [234, 235].

Matrices rich in certain PGs such as versican, decorin, biglycan (see above) and lumican [236] may also act non-permissively for cell movement and invasion and do so by engaging selected integrins as mediators of this invasion-restricting effect. This highlights the fact that a fine balance between relative levels of PGs and migration-permissive integrin ligands are required to allow effective displacement of tumor cells. PG regulation of cancer tissue invasion also takes place by a mechanism distinct from a direct PG-ECM binding, or modulation of integrin binding activities, and may alternatively involve the activation of MMPs. In fact, both syndecan-2 and NG2 possess the ability to modify the activity of MT1-MMP, MMP2 and MMP7 [218, 237–239]. Even in this context there is still too little information on how these PGs actually control MMP activity. This deficit of knowledge makes it unable to draw more definite conclusions regarding the significance for tumor biology of MMP-associated PG activities.

A unique and highly intriguing antithetic role of PGs in the control of tumor cell-host microenvironment is that exhibited by fibromodulin in CLL-B.

Exploitation of PGs for therapeutic tumor targeting

Tumor targeting through the use of PGs lags behind [Supplemental information] (Table 3), partly because of the difficulty in designing effective PG function-blocking agents, especially ones that might target ECM-associated PGs. On the other hand, cell surface-bound PGs appear as readily accessible antigens for antibody-based anti-neoplastic approaches. Because of its multifunctional properties and its large size, experimental data strongly support NG2 as an ideal PG to be neutralized through specific antibodies in the context of immunotherapeutic approaches. Having access to immunological reagents (with which NG2 was originally discovered) it was implicit to attempt exploitation of such agents in vivo. Pioneering approaches in this sense entailed the use of monoclonal antibodies to immunolocalize, and visualize by radioimaging, occult secondary lesions and metastases in melanoma patients [240, 241]. These imaging procedures were rapidly followed by pre-clinical testing of a variety of anti-NG2 antibodies as putative anti-neoplastic agents, either when used alone or following conjugation to cytotoxic compounds [242–247]. Refinement of such immunotargeting strategies has been perpetuated to recent times and it appears to be a field of interest for numerous laboratories around the world seeking immunological methods to eradicate (NG2-positive) tumors.

In early studies of NG2 it was discovered that the PG harbored unique immunological properties that could also be exploited for adaptive immunotherapeutic approaches in tumor patients [248–250, Supplemental information] (Table 3). This has recently been confirmed by a survey evaluating the relative efficacy of 75 primary cell surface and intracellular cancer antigens as putative immunotherapeutic targets. This investigation placed NG2 as the 65th most potent tumor vaccination agent [251]. Prevailing vaccination strategies based upon NG2 as an immunological target include those involving mimotope peptides, or anti-idiotypic antibodies in their constitutive Ig or chimeric forms [252–257, Supplemental information] (Table 3). Certain alternative approaches have also been elaborated and experimented pre-clinically [258]. For instance, in a breast tumor model, immunization with Lm-LLO-HMW-MAA-C, (a recombinant *Listeria monocytogenes* that expresses and secretes a fragment of NG2 fused to the first 441 residues of the listeriolysin O protein), has been found

to cause CD8(+) T-cell infiltration in the tumor stroma and a significant decrease in the number of pericytes in the intralesional tumor vessels [259]. It is not yet clear why re-evoked anti-NG2 immune response would be so effective in the treatment of melanoma, and possibly other tumors, such as triple-negative breast carcinoma [102], while ablation of pericytes, especially angiogenic ones, is a recurrent theme of immunotherapeutic approaches targeting NG2 [260–262]. In breast and other carcinomas, NG2 targeting could prove to be a particularly effective anti-tumor approach because of the potential to selectively target cancer stem/initiating cells through this PG [213].

The potency of NG2 as a putative anti-tumor vaccine antigen has been unequivocally demonstrated by Phase I/II clinical trials involving immunization of advanced, stage IV melanoma patients with the anti-idiotypic antibody MK2-23. When a KLH-conjugated form of mAb MK2-23 was administered to melanoma patients, 60% of the immunized melanoma patients developed a marked anti-NG2 immune response, which prolonged their survival. Pre-clinical investigations [263] suggested later on that this effect was associated with an antigen-specific T-cell activation [264, 265]. Even more striking is the outcome of a Phase II trial recently completed by Dr. Michele Maio's group at the National Cancer Institute of Aviano. In more than 100 metastatic melanoma patients who were immunized with antibody MK2-23 over a period of 12 years, more than 10% showed complete remission of the disease while approximately a further 20% of them manifested a partial remission (M. Maio, personal communication). As in the case of previous clinical studies, responding patients had high titers of endogenous anti-NG2 antibodies in circulation and mounted up a robust T cell-mediated anti-NG2 response as demonstrated by in vitro tests. These exceptionally promising clinical results urge the pursuit of larger immunotherapeutic multicenter studies on patients affected by melanoma and other NG2-expressing tumors in which treatment approaches are still rather limited (e.g., soft-tissue sarcomas and cerebral tumors). The successful immunotherapeutic targeting of NG2 opens the way for more determined attempts to transfer the approach to other PGs and, in particular, GPC3 has recently been contemplated for the development of just such a therapeutic intervention [266].

Transferring PGs to the clinics

Accruing experimental evidence suggests that, independently of the composition of their GAG side chains (i.e., through core protein-mediated effects), PGs are critically involved in distinct facets of tumor progression, including metastasis formation. Conversely, there is currently no tangible evidence of their direct and essential involvement of PGs in tumor relapse and very little is known about whether PGs may alone drive metastasis formation. In several instances, altered PG expression might serve as an indicator of disease course, but a clinical transfer of this biomarker potential awaits the outcome of larger and multicenter-based case studies. Along with this, there is a need to define accurate and standardized methods to employ in molecular diagnostics, such as to permit the highlighting of relevant PG alterations in individual patients. Furthermore, care should be exerted when interpreting the prognostic value of PG alterations because of the numerous pitfalls and caveats. First and most importantly, it has to be assured that the implicated PG alterations are representing genuine *independent* prognostic factors. Secondly, PGs may be modulated in the neoplastic cells (with differences in different subsets of cells), may be components of the tumor stroma, or may be both. Which cellular entity of the tumor lesion exhibits diversities in PG expression is not always trivial to pinpoint, leaving a "biological doubt" about the relevance of the PG modulation.

If the data obtained thus far on the prognostic importance (in terms of disease-free or overall survival) of dysregulation of certain PGs would be strictly matched with the way patients responded to treatments, pivotal correlations between PG expression profiles in primary or secondary lesions and therapeutic response would probably be disclosed. Parallel to studies on the expression patterns of single PGs in different tumors, larger efforts should be made to address how the overall expression pattern of ECM and surface-associated PGs may vary during tumorigenesis, and what the biological and clinical implication of such variation may be. This is particularly applicable to PGs with similar functions (i.e., growth factor/signaling molecule binding, ECM organization/remodeling, promotion of motility/tissue invasion, apoptosis/drug resistance, etc.) and in cases where there is an indication that different PGs may potentiate or antagonize each other's effects.

Genomic alterations in discrete PG genes, such as those encoding certain glypicans, are emerging as incisive predictors of tumor formation. This opens the unique potential of exploiting PGs for genetic testing of individual predisposition for cancer. Since other PGs, else than glypicans, seem also to be down-regulated in tumors, it is likely that the "tumor suppressing" role of certain glypicans may be extended to selected PGs then acquiring a clinical cancer prevention connotation. It seems presently difficult to conceive PGs as direct targets for therapeutic intervention. This is in part due to the fact that no PG has yet been found to act as a true "oncogene", a prerequisite for investing efforts into the design and testing of drugs electively antagonizing that PG. In part, the limited interest in finding

compounds that would neutralize the function PGs derives from the difficulty in elaborating such specific agents.

Peptide-based anti-PG cancer drugs could be an option, but more alluring would certainly be the approach of targeting cytoplasmic tails of cell surface-associated PGs contributing to vital signal transduction events in cancer cells. A better understanding of the importance of such protumorigenic PG contribution could incite the future evolvement of targeted therapeutic agents specifically directed against PGs. Immunotherapeutic targeting of PGs is becoming a fruitful area, both when considering direct immunotargeting and when contemplating adaptive (vaccination-based) immunotherapeutic strategies. The successful outcome of recent clinical trials in which NG2 was used as an immunotherapeutic target confirms this potential and provides a lead for the future development of PG-directed modalities to treat cancer.

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