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Tumor endothelium is characterized by a matrix remodeling signature

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1. ABSTRACT

Endothelial cells (EC) are attractive targets for therapeutic interference in diseases that are dependent on the formation of novel blood vessels, such as cancer. EC are readily accessible via the blood stream and are considered to be genetically stable, thus enabling efficient and effective drug delivery. However, for targeting of EC in blood vessels of the disease tissue, specific markers are needed. Though various studies have focused on the differences in gene expression in endothelial cells in different *in vitro* model systems, only few studies have focused on gene expression in EC derived from tumor tissues and corresponding normal tissues. Here, we review the gene expression data sets of EC isolated from tumors of the colon, breast, brain and ovaries. Gene ontology analysis reveals enrichment for genes involved in extracellular matrix turnover and adhesion. Several genes, including collagens 4A1, 4A2 and 1A1, SPARC, THY1 and MMP9 are overexpressed in the endothelium of more than one tumor type, whereas plexin domain containing 1 (PLXDC1), previously known as TEM7, is overexpressed in EC of all four tumor types.

2. INTRODUCTION

The acquisition of novel blood vessels, either by angiogenesis or by vasculogenesis, is pivotal to the progression of cancer. Inhibition of these processes is considered an attractive therapeutic strategy (1). Two general approaches can be employed to attack the tumor's blood supply. Either the formation of novel blood vessels can be inhibited - the narrow definition of angiogenesis inhibition -, or therapy can be aimed at the destruction of existing vessels - frequently referred to as vascular targeting. In practice, however, there is considerable overlap of these two strategies.

Endothelial cells (EC) readily respond to the metabolic needs of the surrounding tissue, and in tumors this frequently leads to a highly disorganized vascular system that is continuously being modified. In normal tissues, endothelial cells are in a resting state as angiogenesis in adult life only occurs during specific tissue remodeling processes such as e.g. wound healing. Differences in microenvironment result in alterations in gene and protein expression patterns in tumor EC compared to normal tissue EC.

Specific tumor endothelium markers are good molecular candidates for use in diagnosis, imaging and treatment of cancer. Though numerous studies have been undertaken to identify genes induced during angiogenesis *in vitro*, these expression profiles do not accurately reflect gene expression changes in tumors *in vivo* (2-4). The use of endothelial cells isolated from human tumor and normal human tissues is thus highly preferred for the selection of possible tumor vasculature markers with clinical potential.

3. GENE EXPRESSION ANALYSIS IN TUMOR ENDOTHELIAL CELLS

The number of reports on gene expression data of endothelial cells isolated from tumors and normal tissues is still rather limited. One of the major challenges is to obtain a pure population of endothelial cells, as EC comprise only a few percent of the total tissue mass. The preparation of single cell suspensions of fresh tissues by enzymatic digestion and the subsequent antibody labeling of EC for isolation using magnetic beads has been used most frequently (2, 3, 5-8). More recently, immunohistochemistry guided laser capture microdissection (immuno-LCM) of frozen tissue section has been employed to isolate endothelial cell material (9, 10).

Large-scale gene expression profiling techniques are widely used to detect changes in transcript expression levels and provide the tools to study molecular events in biological processes. Over the years, different techniques have been developed and employed. Currently, oligonucleotide and cDNA arrays are the most commonly used tools for gene expression profiling and are within reach of every laboratory. The completion of the sequence of the human genome has resulted in a high coverage of known genes on the arrays, though likely not all expressed sequences, including (tissue-specific) splice variants are present. Furthermore, depending on the platform, different genes are present that can be analyzed.

More technically demanding is SAGE, serial analysis of gene expression, a technique that provides a quantitative analysis of short, specific, concatenated tags derived from mRNA sequences. As SAGE is not dependent on known sequence information, this technique provides an unbiased analysis (11).

StCroix and colleagues (2) pioneered in the analysis of tumor endothelial gene expression. A combination of SAGE and the isolation of EC using antibody-coated magnetic beads was used to identify tumor endothelial markers (TEMs) for colorectal cancer. Nine different ESTs, TEM1 to TEM9, were identified that showed specific expression in tumor endothelial cells (TEC). In addition, a considerable number of collagens was overexpressed in TEC. Four of the TEMs (TEM1, TEM5, TEM7 and TEM8) were later shown to be cell surface proteins (12), and their mouse homologs were identified. Using the same combination of techniques, gene expression profiles of glioma EC and breast tumor EC were identified (7, 8). Several of the glioma endothelial markers that were identified had previously been associated with angiogenesis, including several collagens that were reported as TEMs in colon (7). Further comparison of the SAGE tags with normal tissue SAGE libraries identified five transcripts with specific expression in glioma endothelium: INSR, PV1, PLXA2, LAMC3 and an EST (7). Indeed, PV1 was expressed in glioma EC as well as in the vasculature of brain metastasis (7, 13). In addition to collagens and matrix metalloproteinases (MMPs), breast EC overexpressed a number of transcription factors that may drive the expression profile in these tumor EC (8). SNAIL1 and HEYL were expressed in tumor tissue and absent in normal tissues, and localized to the endothelial cells. Overexpression of HEYL in EC *in vitro* resulted in increased proliferation and protection from apoptosis (8).

We have recently conducted a gene expression survey for genes associated with tumor angiogenesis (3). By comparing gene expression of tumor endothelium (TEC) with both normal endothelium (NEC) of colon tissues, as well as with angiogenic endothelial cells of placenta (PLEC), seventeen different genes were identified that showed specific overexpression in TEC and were denoted tumor angiogenesis genes, or TAGs. Of the genes that were overexpressed in TEC vs NEC, the majority of genes were also overexpressed in PLEC vs NEC, suggesting a role in physiological angiogenesis (3). Thus, only a limited subset of the genes expressed in TEC appear to be uniquely induced in the tumor environment. The majority of the TAGs were related to extracellular matrix turnover, though the transcription factor HEYL was also specifically induced in TEC. Antibody targeting of two TAGs, HMGB1 and vimentin, successfully inhibited angiogenesis *in vivo* (3).

More recently, two papers were published describing glioblastoma endothelial cell expression profiles (5, 10), though by different methods. Beaty and colleagues used the previously described approach using a combination of SAGE analysis on EC isolated using beads (5). Stringent selection criteria were applied by which genes expressed in normal tissues (brain, liver, lung, kidney, heart and muscle), normal EC (colon, breast and liver) as well as bone marrow and white blood cells, were subtracted (5). Three genes showed highly specific expression in glioblastoma EC, including ANAPC10, PLXDC1 and CYP27B1. A completely different approach was employed by Pen and colleagues, who combined immuno-LCM with custom cDNA array analysis (10). EC from glioblastoma were compared to EC from non-malignant brain tissue. Among the highest differentially expressed genes were IGFBP7, SPARC and vimentin, that have previously been associated with tumor EC profiled using different methods (2, 3, 7, 8).

The profile of ovarian tumor EC was described in two recent publications (6, 9). Immuno-LCM on frozen tissues was used to isolate endothelial cells and profiling was performed on oligonucleotide arrays (9). Several selection criteria were applied to select the most relevant differentially expressed genes. Of the 12 selected genes for follow-up analysis, adlican (MXRA5), COL11A1, F2RL1,

Table 1. Gene expression data sets of isolated tumor endothelial cells

Tumor type	EC isolation	Method	Gene IDs	Reference
Breast	Beads	SAGE	24	8
Colon	Beads	SAGE	41	2
Colon	Beads	Array	17	3
Glioma	Beads	SAGE	11	5
Glioma	Beads	SAGE	106	7
Glioma	LCM	Array	21	10
Ovary	LCM	Array	68	9
Ovary	Beads	Array	28	6
		Total	316	

Table 2. Genes overexpressed in different TEC

Gene	Authors					N	Tumor types [1]					N^2	Method[3]					N^2
PLXDC1	Parker	StCroix	Beaty	Lu	Buckanovich	5	B	C	G	O	O	4	S	S	S	A	A	2
SPARC	Madden	Van Beijnum	Pen	Parker	StCroix	5	G	C	G	B		3	S	A	A	S	S	2
MMP9	Madden	Beaty	Lu	Parker		4	G	G	O	B		3	S	S	A	S		2
COL1A1	Madden	StCroix	Van Beijnum			3	G	C	C			2	S	S	A			2
COL4A1	Madden	StCroix	Van Beijnum			3	G	C	C			2	S	S	A			2
THY1	Madden	StCroix	Buckanovich			3	G	C	O			3	S	S	A			2
COL4A2	Parker	Madden	StCroix			3	B	G	C			3	S	S	S			1
IGFBP7	Van Beijnum	Pen	StCroix			3	C	G	C			2	A	A	S			2
COL3A1	Madden	StCroix				2	G	C				2	S	S				1
INSR	Madden	Van Beijnum				2	G	C				2	S	A				2
SERPINE1	Parker	Madden				2	B	G				2	S	S				1
STC1	Madden	Lu				2	G	O				2	S	A				2
VIM	Van Beijnum	Pen				2	C	G				2	A	A				1
ISG15	Parker	Lu				2	B	O				2	S	S				1
HEYL	Parker	Van Beijnum				2	B	C				2	S	A				2
CD248	Madden	StCroix				2	G	C				2	S	S				1
COL18A1	Parker	StCroix				2	B	C				2	S	S				1
COL6A2	Madden	StCroix				2	G	C				2	S	S				1
ADAM12	Lu	Buckanovich				2	O	O				1	A	A				1
VCAN	Lu	Buckanovich				2	O	O				1	A	A				1
LAMC3	Madden	Beaty				2	G	G				1	S	S				1
TNFAIP6	Lu	Buckanovich				2	O	O				1	A	A				1
FZD10	Lu	Buckanovich				2	O	O				1	A	A				1
FJX1	Lu	Buckanovich				2	O	O				1	A	A				1
TNFRSF21	Lu	Buckanovich				2	O	O				1	A	A				1
MXRA5	Lu	Buckanovich				2	O	O				1	A	A				1
EGFL6	Lu	Buckanovich				2	O	O				1	A	A				1
CENTA2	Lu	Buckanovich				2	O	O				1	A	A				1

[1] B, breast; C, colon; G, glioma; O, ovary. [2] Number of *different* tumor types or methods. [3] A, array; S, SAGE.

GPM6B and STC2 also showed higher expression in tumor as compared to corpus luteum, proliferative endometrium and placenta, indicating their specificity for tumor angiogenesis. Comparisons with publicly available data sets additionally confirmed overexpression of several of these markers in diverse types of tumors (9). In a second study, EC isolated from 5 normal ovaries and 10 ovarian tumors using antibody-coated magnetic beads were also subject to oligonucleotide array analysis (6). Of the 625 genes that were upregulated more than 2-fold, 35 genes were reported to be upregulated more than 6-fold. EGFL6, TNFAIP6, TWIST1, STC1, HOP and PLXDC1 were overexpressed in tumor EC more than 10-fold as compared to normal ovarian EC (6).

3.1 Tumor endothelial cells are enriched in ECM related gene expression

To gain a comprehensive insight in the expression profiles of tumor endothelial cells, we collected the available data sets published in the above described studies (Table 1). Only the overexpressed genes (fold change ≥2; N=316) were included in the analysis as complete data sets were not in all cases publicly available. In addition, the different expression profiling platforms

used (Table 1) do not allow straightforward comparison of raw data. Using BioMart (http://www.biomart.org/), Ensembl databases were mined for additional information including gene ontology and chromosomal localization (Supplementary Table 1). Gene annotations were categorized to eight different categories and gene expression data of the different tumor types (breast, colon, brain, ovarian) were compared (Figure 1A). Categories were chosen in such a way that all basic cellular functions are represented. Gene ontology (GO) annotations for all genes were retrieved from Ensembl and NCBI databases, sorted for GO accession number and categorized independently of the genes (Supplementary Table 2A; column GO classification). Subsequently, per gene, the ontology terms were grouped and the individual genes were assigned to one or two groups reflecting biological function and cellular localization (Supplementary Table 2B). However, as GO annotations of genes are the result of interpretation of e.g. literature and are manually assigned to genes, GO descriptions will be subjective to a certain extent, depending on the information that is publicly available.

The distributions of gene function correlate relatively well between the different tumor types. Unfortunately, only one breast tumor EC data set is available, in which several functional groups are not represented (Figure 1A). Genes associated with ECM function and synthesis, as well as cell surface molecules involved in cell-cell and cell-matrix adhesion are highly represented in all tumor types examined. ECM related genes are most abundant in colon TEC, whereas breast TEC express a relatively large proportion of genes involved in protein regulation, such as heat shock proteins ((Figure 1A, B). Nuclear proteins were generally underrepresented in TEC, whereas extracellular, cytoplasmic and cell surface proteins were more abundant ((Figure 1C).

As the gene sets were derived from different gene expression platforms, no normal gene set enrichment analysis could be performed. Therefore, the genes were loaded in DAVID (http://david.abcc.ncifcrf.gov/) for functional annotation clustering against a background of the whole genome. This tool classifies genes in functionally related groups based on gene ontology, protein data and pathway data, and calculates enrichment scores for groups of genes with a related biological function. A large number of genes were functionally linked to extracellular matrix organization and biogenesis (N=100 genes; enrichment score 9.43), as well as to cell motility (N=11 genes; enrichment score 2.71) and cell communication (N=66 genes; enrichment score 1.82) using the default algorithm using the whole genome as background ((Figure 1D and data not shown). Interestingly, only a few genes were functionally linked with angiogenesis (N=3 genes, enrichment score 0.6) ((Figure 1D). This is most likely due to missing annotation data as e.g. SPARC has been associated with angiogenesis for years (14). Furthermore, many collagens have been reported to be involved in blood vessel formation, either as basement membrane components or as proteolytic fragments with angiomodulatory function (15), but only three of them (COL18A1, COL4A2 and COL15A1) have a GO annotation relating them to angiogenesis (Supplementary Table 2A, B) and only the latter one is recognized during functional annotation clustering by DAVID ((Figure 1D). Thus, though this annotation clustering is highly informative, one should realize that the information contained in the GO databases is not exhaustive and therefore needs to be interpreted with caution. Future progress in the (automated) extraction of functional annotation terms from diverse digital public sources will undoubtedly improve the quality and completeness of GO repositories and tools.

When functional annotation clustering was based on pathway analysis, ECM-receptor interaction, cell-cell communication and focal adhesion were enriched (enrichment score 8.45) ((Figure 1E, F). Not surprisingly, based on structure, the genes were enriched for collagen helix repeats (enrichment score 7.02) and von Willebrand factor A domains (enrichment score 3.39), both present in collagens and integrins. Also enriched were EGF domains (enrichment score 3.32), present in proteoglycans, and peptidase domains (enrichment score 2.96), present in matrix metalloproteinases (data not shown).

The genes overexpressed in the EC of different tumor types (Supplementary Table 1) were relatively equally distributed throughout the genome ((Figure 2A, B). Several chromosomal regions harbor multiple tumor angiogenesis associated genes ((Figure 2), and eight loci (7p12.3, 13q34, 22q13.3, 11p13, 5q35.1, 13q12.3, 1p36.3, 21q22.3) demonstrated enrichment as determined by binomial testing with Benjamini-Hochberg correction (Supplementary Table 3). Four genes, VWA1, SDF4, ISG15 and CPSF3L, reside at 1p36, a region frequently deleted in different tumors (16, 17). Though the genomic status of the different tumors these genes were identified in is not known, it supports the notion that endothelial cells are genetically stable and further points to the importance of this genomic region. Chromosome 21q22.3 contains five TEC genes, of which three are collagens (COL18A1, COL6A1, COL6A2), and 13q34 contains 4 TEC genes, two of which are collagens (COL4A1, COL4A2). This probably reflects ancestral paralogy, as many collagens are found in close chromosomal proximity of each other. Intriguingly, COL18A1 is overexpressed in TEC, but proteolytic cleavage of this collagen gives rise to endostatin, an endogenous inhibitor of angiogenesis (18).

When combining the available gene expression dataof human tumor endothelium, it emerges that extracellular matrix organization plays a major role in tumor angiogenesis. The large proportion of genes coding for cell surface proteins, matrix components and secreted molecules that are overexpressed in the diverse tumor endothelia also offers therapeutic opportunities. Targeting drugs to eradicate tumor (endothelial) cells is greatly facilitated by the presence of specific molecules accessible via the blood stream. Furthermore, the importance of matrix remodeling during tumor angiogenesis may be exploited by the administration of drugs that for example prevent the assembly of collagen, or inhibit the activity of

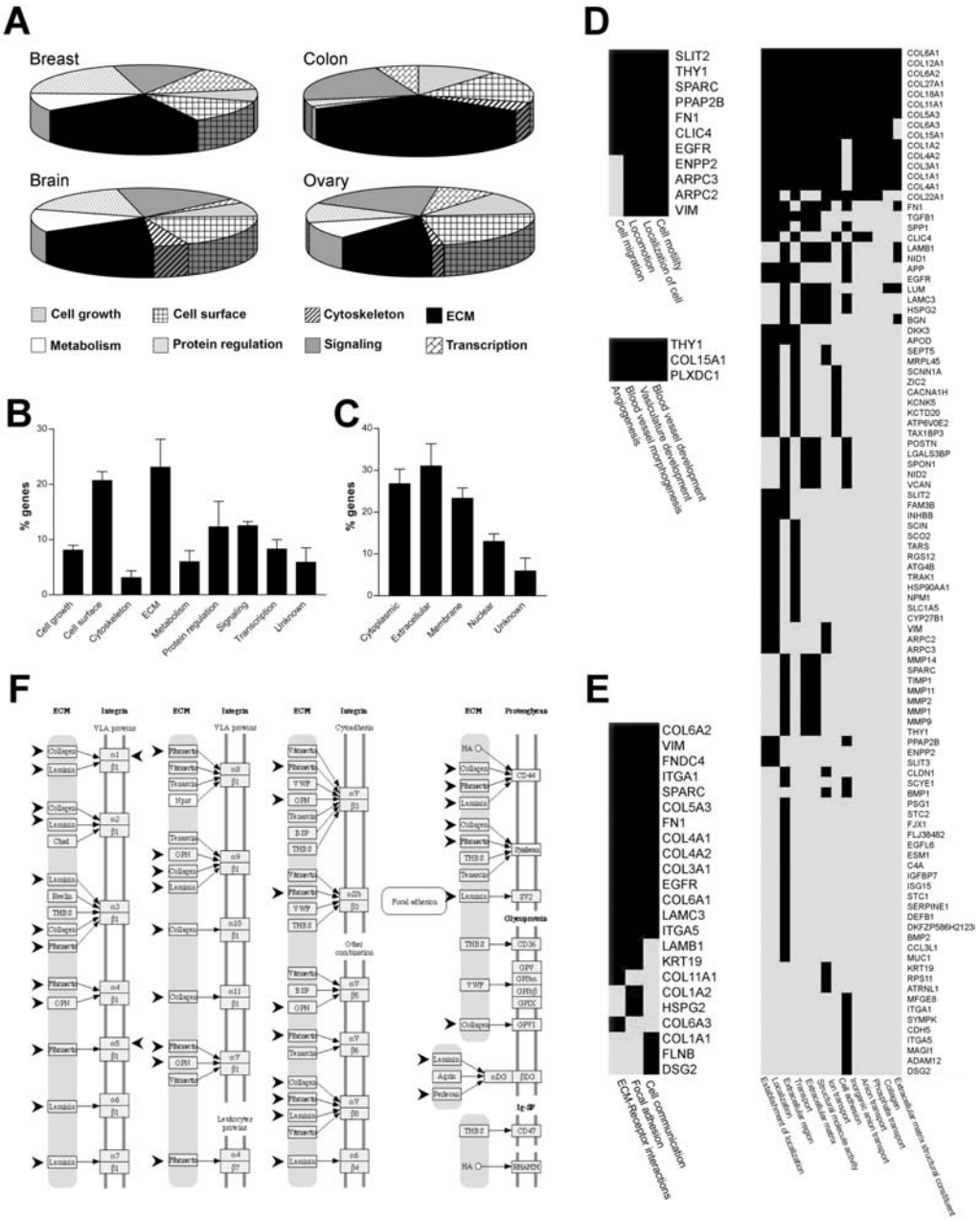
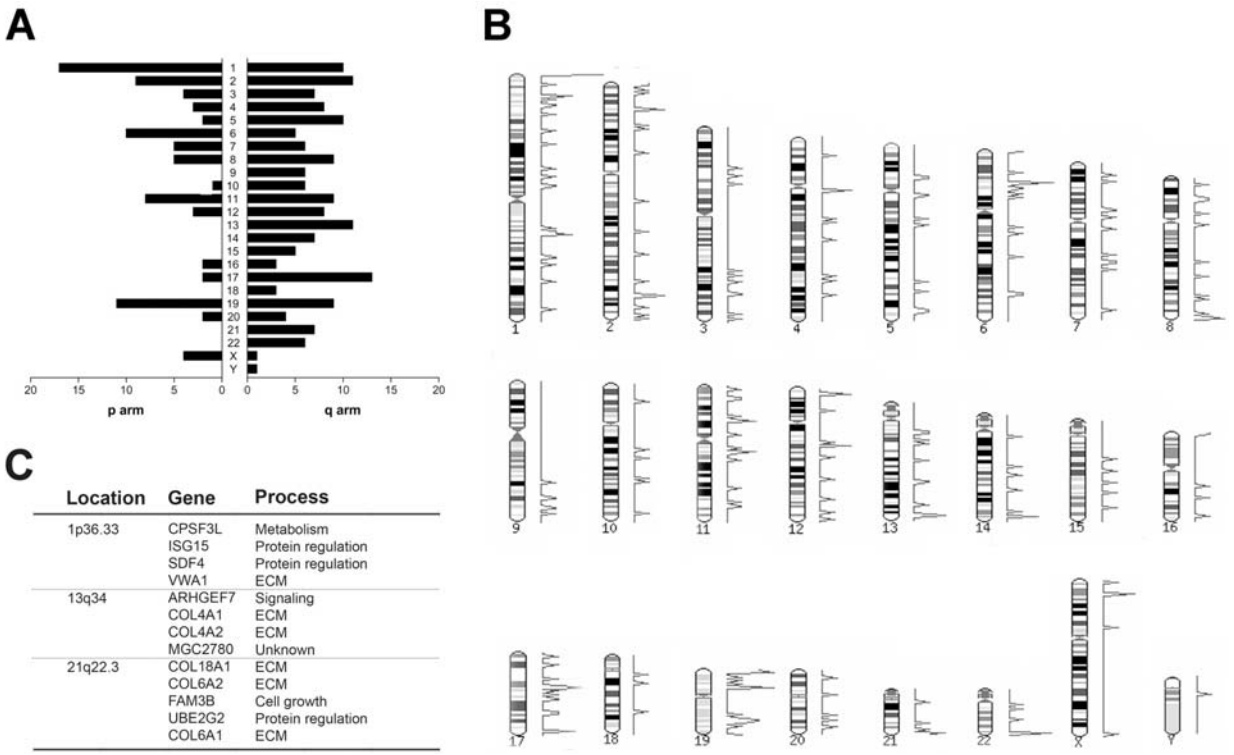


Figure 1. Functional classification of tumor angiogenesis genes. A) Distribution of annotated genes reported to be overexpressed in endothelial cells isolated from breast, colon, brain and ovarian tumors in functional groups related to different biological processes. Gene ontology terms obtained from the Ensembl databases via Biomart were grouped in eight different categories (see Supplementary Tables 2A, B; Supplementary Table 4). Genes with functions in ECM remodeling and cell surface proteins, including signal transduction receptors and adhesion molecules, dominate. B) Average distribution of tumor angiogenesis genes categorized to different biological processes in all tumor types as reported in Supplementary Table 4. C) Average distribution of tumor angiogenesis genes to different cellular components based on gene ontology data (see Supplementary Table 4). D) Heatmaps of DAVID Functional Annotation Clustering analysis for gene ontology. Black squares indicate the presence of a given annotation for a gene; for gray squares the annotation is absent. Tumor angiogenesis genes were enriched for cell motility and extracellular matrix organization and biogenesis. Only very few (N=3) of the tumor angiogenesis genes analyzed by DAVID (N=247) have previously been annotated as involved in angiogenesis and blood vessel development. E) DAVID Functional Annotation Clustering analysis for pathways reveals an enrichment for genes involved in ECM-receptor interactions, focal adhesion and cell communication. F) ECM-receptor interactions within the set of tumor angiogenesis genes. Pathway information was generated by KEGG (http://www.genome.jp/kegg/pathway.html). Proteins highlighted with arrowheads are present in the list of tumor angiogenesis genes.



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Location	Gene	Process
1p36.33	CPSF3L	Metabolism
	ISG15	Protein regulation
	SDF4	Protein regulation
	VWA1	ECM
13q34	ARHGEF7	Signaling
	COL4A1	ECM
	COL4A2	ECM
	MGC2780	Unknown
21q22.3	COL18A1	ECM
	COL6A2	ECM
	FAM3B	Cell growth
	UBE2G2	Protein regulation
	COL6A1	ECM

Figure 2. Distribution of tumor angiogenesis genes. A) Number of different tumor angiogenesis genes (from Supplementary Table 1) present on the different chromosomes. B) The distribution of the tumor angiogenesis genes in the genome using KaryoView analysis. The histograms are indicative of the number of genes on each locus. C) Loci enriched for tumor angiogenesis genes.

matrix proteases that facilitate invasion and migration. However, though it is clear that resting endothelial cells differ considerably from activated tumor endothelial cells, we and others have observed a considerable overlap between genes expressed in tumor endothelium and during physiological angiogenesis e.g. during wound healing and in the female reproductive cycle (2, 3).Thus, for therapeutic applications, genes that distinguish between physiological and pathological angiogenesis need to be identified, to allow selective targeting of the tumor vasculature and to minimize potential side effects. Indeed, both physiological and pathological angiogenesis are dependent on the effect of angiogenic cytokines such as VEGF and bFGF; furthermore, tumors have been described as 'wounds that never heal' (19). On the other hand, in tumors, the presence of inflammatory cells that release cytokines, aberrant blood flow, hypoxia and supra-physiological concentrations of angiogenic cytokines may contribute to distinctive tumor endothelial gene expression profiles. Very recently, a large SAGE analysis was performed in which physiological angiogenesis after partial hepatectomy was compared with tumor angiogenesis in liver metastases in mice (20). Genes that were identified as overexpressed in EC from regenerating liver all demonstrated overexpression in tumor endothelial cells. Further analysis identified 13 genes that were 10-fold overexpressed in tumor EC as compared to normal EC and EC from regenerating liver (20). Interestingly, one of these markers is CD276, that has previously been identified in glioma EC (7). Thus, both qualitative and quantitative differences in gene expression between physiological and tumor angiogenesis exist that can be exploited therapeutically.

4. COMMON TUMOR ANGIOGENESIS GENES

Though all different tumor EC data sets described here are enriched for genes with functions in extracellular matrix organization, rather few individual genes are present in multiple data sets. Only 28 of the 261 reported tumor angiogenesis genes (11%) are reported overexpressed in more than one study. Moreover, only 18 genes (7%) are overexpressed in the endothelium of more than one tumor type (Table 2). The different gene expression analysis platforms that were used to generate the described data sets are likely to contribute to limited consensus. Furthermore, the authors applied various criteria to select those genes that they considered most relevant. It would therefore be valuable to have the raw data sets available to mine for possibly more genes that are differentially expressed between tumor and normal endothelial cells.

From Table 2 it emerges that the gene expression profiling method is probably more influential to the data retrieved than the method used for isolation of the endothelial cells. Nine different genes were reported to be overexpressed in ovarian TEC by two independent groups (6, 9), both using oligonucleotide arrays, but using different methods for EC isolation. Furthermore, several of the genes

listed in Table 2 were identified by a single gene expression profiling platform though from different tumors, e.g. COL4A2, suggesting these different technologies may be biased for certain transcripts.

Eight different genes were reported in three or four different studies, representing two or more different tumor types. Collagens 1A1, 4A1 and 4A2 are reported overexpressed in the TEC of glioma, colon and breast (2, 3, 7, 8). Insulin-like growth factor binding protein 7 (IGFBP7) was identified as overexpressed in the endothelium of colon tumors and gliomas (2, 3, 10). IGFBP7 is a cell-adhesive glycoprotein and has structural homology to insulin-like growth factor binding proteins. It has lower affinity for IGF than other IGFBPs (21). Rather than functioning in modulation of IGF function, IGFBP7 interacts with diverse ECM proteins including type IV collagens and heparan sulfate proteoglycans (22, 23). The precise biological role of IGFBP7 remains poorly defined, but it is likely to contribute to vascular remodeling (10). However, its general role in cancer is ambiguous, as the protein has been reported both as a poor prognostic indicator when overexpressed (24, 25), as well as a tumor suppressor role (26, 27).

Matrix metalloproteinase 9 (MMP9) is involved in the breakdown of extracellular matrix components including type IV collagens, both in normal physiological processes as well as in disease processes. Interestingly, proteolytic cleavage of COL4A3 by MMP9 was reported to generate tumstatin, a putative angiogenesis suppressor (28). MMP9 has extensively been associated with tumor invasion and angiogenesis (29), and inhibition of MMP9 production or activity reduces tumor invasion and angiogenesis (30-33).

Thy-1 cell surface antigen (THY1), also known as CD90, was originally identified as a lymphocyte marker (34). It is a heavily glycosylated GPI-anchored cell surface protein containing IgG domains (35). Thy-1 is not only expressed on endothelial cells and lymphocytes, but also different types of neuronal and glial cells. Thy-1 expression is upregulated on the surface of newly formed blood vessels after injury, during pregnancy and in transplanted tumors *in vivo*, but not during embryogenesis (36). Furthermore, IL1β and TNFα upregulated Thy-1 in EC *in vitro*, confirming its role in (tumor) angiogenesis (36). Thy-1 can interact with integrin αVβ3, and the transmigration of melanoma cells expressing this integrin over a layer of EC was dependent on the interaction with endothelial Thy-1 (37). Furthermore, endothelial Thy-1 can interact with leukocyte integrin Mac-1 (αMβ2), and this interaction mediates the adhesion and transmigration of leukocytes (38). Thus, Thy-1 is an important endothelial adhesion molecule that can mediate both metastasis formation and leukocyte infiltration. Interestingly, Thy-1 was also expressed on vascular leukocytes, tumor-infiltrating cells that express both CD45 and VE-cadherin, and that contribute to tumor vascularization (39). Given its surface localization and accessibility, as well as its role in diverse cell-cell communication processes, Thy-1 may be a worthwhile target for therapeutic interference in tumor angiogenesis.

SPARC, demonstrated overexpression in five different studies representing 3 different tumor types. SPARC has been recognized for years to be involved in matrix remodeling and angiogenesis (14). Proteolysis of matrix components by SPARC results in the production of peptides that may have pro-angiogenic activity (40, 41). Contrasting its pro-angiogenic and pro-invasive functions (42-44) however, are reports that suggest SPARC to have tumor suppressor activities (45-47).

4.1. Plexin-domain containing 1

Plexin-domain containing 1 (PLXDC1) was first identified as TEM7, and shown to be expressed on the cell surface (2, 12). The gene encodes a type I transmembrane protein containing a large extracellular domain, and a short cytoplasmic tail. The extracellular domain contains a plexin-semaphorin-integrin (PSI) domain and has weak homology to nidogen (12). A second related transcript, TEM7R (PLXDC2) was also identified and differs from PLXDC1 by the use of alternative polyadenylation sites. Both PLXDC1 and PLXDC2 were expressed in the endothelium of colorectal tumors but not in normal endothelium (12). Furthermore, PLXDC1 was predominantly localized to the tight junctions between endothelial cells (48). Unfortunately, the mouse homologs of PLXDC1 were not detected in the tumor endothelium of mouse tumors (12). PLXDC1 appears to be expressed predominantly in the nervous system in rodents, most notably the Purkinje cells in the cerebellum, various layers in the cerebral cortex, hippocampus and hypothalamic nucleus (12, 49, 50). However, PLXDC1 expression was hardly detectable during embryonic development (49-51). The absence of the transcript in mouse tumor endothelium seriously hampers the use of preclinical mouse studies to evaluate the contribution of PLXDC1 to tumor angiogenesis and the possibility of using PLXDC1 as a target molecule for therapy.

Interestingly, though PLXDC1 was absent in the endothelium of mouse tumors, *in vitro* in fibrin/fibrinogen-based three-dimensional culture systems it was present in mouse endothelial cells and upregulated during capillary morphogenesis (52). PLXDC1 was downregulated in proliferating EC by serum supplementation to the culture, and upregulated by PMA (52). The discrepancy with the absence of PLXDC1 in TEC of mouse models may be explained by the generally high growth rate of mouse tumors with a concomitant high proliferative rate of the tumor endothelium, resulting in downregulation of the protein. In contrast, human tumors tend to grow more slowly resulting in a higher ratio of differentiating vs proliferating endothelial cells and hence a high expression of PLXDC1.

Different possible binding partners for PLXDC1 have so far been identified. Cortactin, an intracellular actin binding protein, coprecipitates with PLXDC1. A plexin-like domain in cortactin was minimally required for its binding to PLXDC1 (48). However, the functional relevance of this interaction is as yet unknown. More recently, the basement membrane protein nidogen was identified as a binding partner for PLXDC1 (53). PLXDC1-

nidogen interactions stimulate cell spreading, though do not influence cell adhesion (53), confirming a presumed role of PLXDC1 in vessel morphogenesis. Interestingly, nidogen-1 and nidogen-2 are both overexpressed in TEC (2, 9).

The possible therapeutic applications of PLXDC1 remain largely uninvestigated. Its cell surface localization on the endothelium of diverse human tumors promises wide applicability and highly efficient targeting. Interestingly, administration of PLXDC1 extracellular domain inhibits capillary morphogenesis *in vitro* (52), possibly by interfering with cell spreading. Furthermore, PLXDC1 was highly expressed in osteosarcoma cells of mesenchymal origin, and downregulation by RNA interference inhibited migration and invasion *in vitro* (52). Moreover, high expression of PLXDC1 in osteosarcomas was correlated with high-grade tumors and poor prognosis (52). Taken together, PLXDC1 may be a promising target for anti-cancer and anti-angiogenic treatment strategies.

5. CONCLUSIONS

Tumor endothelial cells exhibit gene expression profiles that distinguish them from normal endothelial cells and these differences may be exploited for therapeutic use. Several studies have attempted to characterize the gene expression profile of endothelial cells isolated from human tumors and corresponding normal tissues, resulting in the identification of a few hundred genes with putative therapeutic applications. Though only few genes are commonly overexpressed in the tumor endothelium of different tumor types, a gene expression signature involved in extracellular matrix organization is a general hallmark of tumor endothelium.

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Abbreviations: EC: endothelial cell; TEC: tumor endothelial cell; NEC: normal endothelial cell; ECM: extracellular matrix; PLXDC1: plexin domain containing 1

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