Newer vascular targets: Endosialin (Review)

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Abstract. The identification of cell surface markers expressed selectively by tumor vasculature is challenging. To get as close to the human disease as possible, investigators have isolated endothelial cells from fresh human tumor specimens and subjected them to RNA-based gene expression analysis. The data indicate that there are few proteins that distinguish tumor vasculature from normal vasculature and re-enforce the notion that the endothelium is a tissue specialized cell-type. Endosialin was identified as a cell surface tumor endothelial marker. The selective expression of endosialin by tumor vasculature and stroma has been confirmed. Although the function of endosialin remains to be elucidated, the expression pattern for this protein may be favorable for cancer therapy.

Contents

1. Introduction

The field of antiangiogenic therapies has moved very quickly from laboratory discoveries into the clinic. As with other areas of science the rapidity of the development of the anti-angiogenic field was fueled by the availability of models and the identification of therapeutic targets. The field was also fueled by the early hypothesis which held that angiogenesis was the same no matter where it occurred. Therefore, angiogenesis during embryo development or wound healing was the same as angiogenesis during the growth of malignant disease (Fig. 1) (1-4). The corollary to this hypothesis was that models of normal embryo development and models working with mature well-differentiated endothelial cells in culture would be sufficient and satisfactory models for tumor endothelial cells. This hypothesis also held that because endothelial cells involved in malignant disease were normal, these cells would be less susceptible to developing drug resistance because they were genetically stable (5,6).

The current hypothesis is that angiogenesis occurring during malignant disease is abnormal and that therapeutic targets identified by studying endothelial cells isolated from fresh samples of human cancers will be most relevant for developing therapeutic agents to treat human malignant disease (7-10).

2. New target discovery

Early studies of gene expression were carried out primarily with cell lines. As the importance of the tissue micro-environment and the easy plasticity with which cells alter gene expression in response to the microenvironment became evident, the severe limitations, indeed, inaccuracies in disease representation by monolayer cell culture were recognized. ‘ Drug-target hunters’ realized the need to get as close to the human disease as possible to identify disease critical molecular targets. To accomplish this, fresh samples of human malignant tumors and corresponding normal tissues were used as starting materials (11-25). Gene expression profiling techniques such as microarray analysis (11-20) and serial analysis of gene expression (SAGE) (21-25) have provided global views of the levels of mRNAs in malignant tissues compared with normal tissues and allowed identification of genes and pathways involved in the malignant process. Specific diseases including ovarian cancer, breast cancer, gastric cancer, multiple myeloma, lung adenocarcinoma, Wilm's tumor and neuroblastoma have been analyzed for diagnostic and prognostic gene expression characteristics and for identification of potential drug targets (14-20). Chief among the issues being faced by these studies is developing data analysis methods that allow investigators to draw biologically meaningful conclusions from very large datasets (12,13).

The one of the challenges for gene expression studies is to translate research findings of multigene expression signature classifiers/genomic signatures of disease into applications in diagnostics and therapeutics (26-30). Integrative computational

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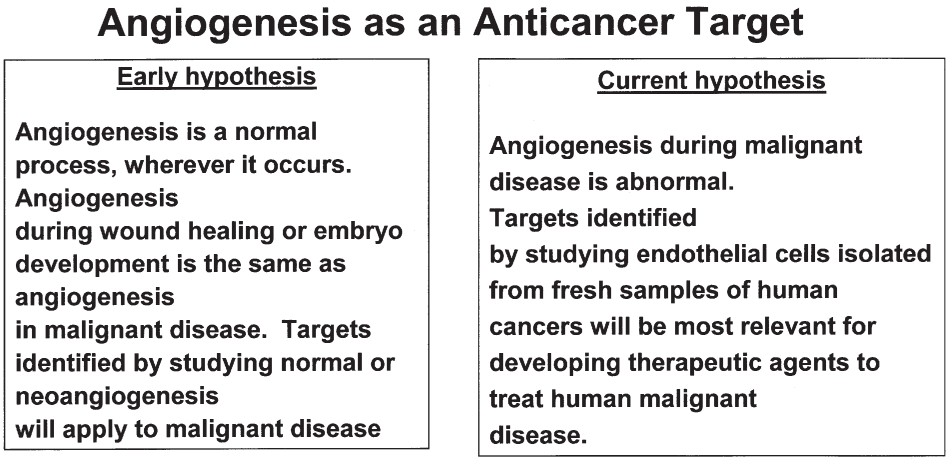

Figure 1. Hypotheses supporting angiogenesis as a target for cancer therapy is shown.

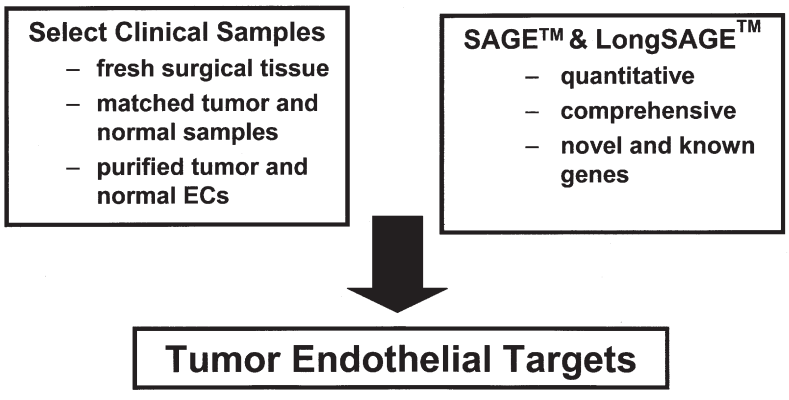

Figure 2. Schema for isolation of tumor endothelial cells from fresh samples of human tumors and normal tissues and subsequent preparation of tumor endothelial cell RNA for SAGE analysis is depicted. Because the tumor endothelial cells were never placed into culture, tissues samples of at least 2 gram were required to obtain sufficient numbers of cells for expression analysis.

and analytical data analysis approaches including meta-analysis, functional enrichment analysis, interactome analysis, transcriptional network analysis and integrative model system analysis are being applied to gene expression data. Some studies focus on the expression of mRNAs that code for enzymes as potential drug targets, some search for functional regulators driving large-scale transcriptional signatures and others focus on epigenetic alterations that regulate gene expression (27-32).

3. SAGE analysis of tumor sub-populations

SAGE is a gene expression profiling method that allows global unbiased, quantitative determination of the transcriptome in the sample at the time of RNA collection (21-26). SAGE expression profiling depends upon the notions that a short (10-27 base-pair sequence) fragment of mRNA cut by a restriction enzyme is sufficient to uniquely identify a transcript and that concatemerization of these fragments (tags) increases the efficiency of sequence-based transcriptome analyses (21). Approximately 90% of genes are represented by SAGE tags (Madden, unpublished data). Because SAGE does not depend upon *a priori* knowledge of the genes of interest; it can identify novel, un-named and unexpected transcripts. For these reasons SAGE methodology has been selected as the method of choice to examine gene expression from subpopulations of cells isolated from fresh clinical specimens (22-26).

Fresh specimens of colon carcinoma, normal colon mucosa, breast carcinoma, normal breast tissue, brain tumors and normal brain were obtained for analysis of cellular subpopulations by SAGE analysis (Fig. 2) (25,26,33,34). The tissues were disaggregated and the endothelial cells were isolated using selection with an antibody to P1H12 linked to a magnetic bead (33-36). The RNA from the endothelial cells isolated from tumor and normal tissues was collected and subjected to SAGE analysis. This methodology allows elucidation of the RNA transcripts in the cells at the time of RNA isolation providing the identity of the transcript and the relative abundance of each transcript. Thus far, SAGE-derived transcript libraries have been generated for endothelial cells isolated from 7 fresh human tumor specimens and 5 specimens of corresponding normal tissues.

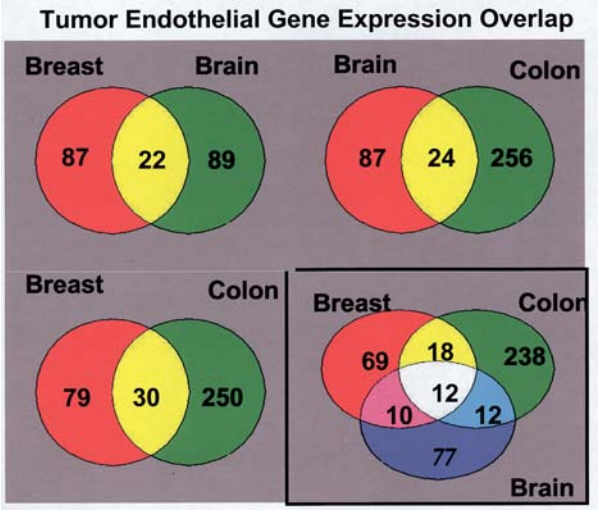

Figure 3. Venn diagrams depicting the overlap in the number of genes expressed at higher levels in tumor endothelial cells derived from breast, brain and colon tumors compared with endothelial cells from the corresponding normal tissues. The selected genes were over-expressed in tumor endothelial cells with >98% confidence by χ^2 analysis. The data include known and unnamed genes.

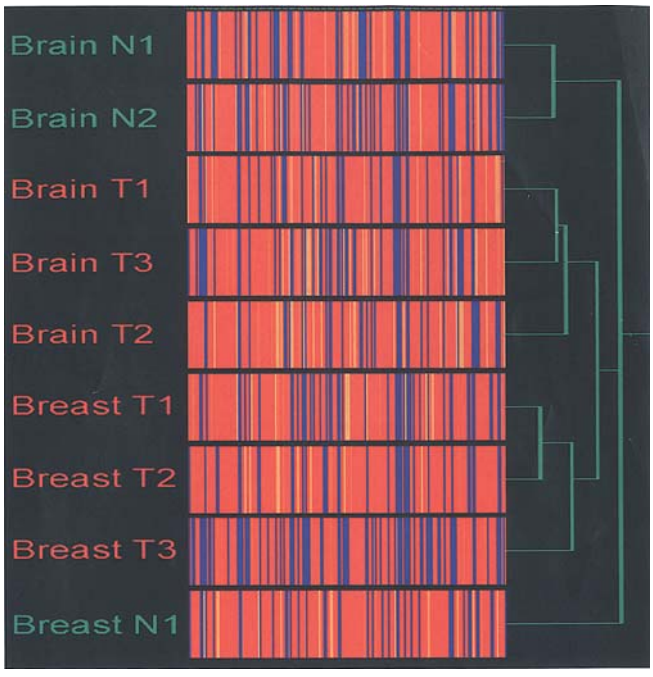

Figure 4. Hierarchical clustering of tumor endothelial cell and normal tissue endothelial cells SAGE libraries by GeneSpring™ is shown for breast tumor and normal breast and brain tumors and normal brain. SAGE tags from statistical confidence filtering (90% confidence) were used. At the 90% confidence level shown, the tumor endothelial cells libraries formed distinctive sub-clusters from the normal endothelial cell libraries indicating that there is a group of genes expressed by endothelial cell involved in the switch from normal to tumor independent of tissue of origin.

The first bioinformatics analysis was to compare the genes/mRNA expressed in each of the three tumor types with the genes/mRNA expressed in each corresponding normal tissue. In each case, a similar pattern emerged. The vast majority of the genes/mRNA expressed by the tumor endothelial cells was very similar to the genes/mRNA expressed by the endothelial cells from the corresponding normal tissue. However, there was a small subpopulation of genes/mRNA that was expressed at much higher levels by the tumor endothelial cells than by normal endothelial cells and a different small subpopulation of genes/mRNA that was expressed at much higher levels by the normal endothelial cells than by the tumor endothelial cells. Generally, the tumor endothelial cells appeared to be expressing at least a partial 'malignant phenotype'. The tumor endothelial cells appeared to be relatively de-differentiated or immature relative to the corresponding normal endothelial cells.

The second bioinformatics analysis was to compare the genes/mRNA that were expressed at high levels by the tumor endothelial cells from the colon carcinoma, breast cancer and brain tumors with each other. Venn diagrams were developed for the subpopulations of genes that by the χ^2 test had >99% confidence of being over-expressed in the tumor endothelial cells compared with the corresponding normal endothelial cells (Fig. 3). The genes/mRNA that fulfilled these criteria included 280 genes from the colon carcinoma, 109 genes from the breast carcinomas and 111 genes from the brain tumors. The number of genes that were over-expressed in endothelial cells from both breast cancer and brain cancers was 22, from brain cancers and colon cancer was 24 and from breast cancer and colon cancer was 30. Thus, there is a high degree of organ/tissue specificity in the endothelium and there is a high degree of heterogeneity among tumor endothelium. When the highly over-expressed genes from the endothelial cell libraries for each of the three tumor types were compared, there were only 12 genes that were highly over-expressed in all three tumor types. Based on these findings, it may be less likely that therapeutic antiangiogenesis targets can be identified that are universally applicable. It may be more likely that antiangiogenic therapeutic targets can be found that will apply to major tumor categories.

Hierarchical clustering analysis using GeneSpring™ software was applied to the SAGE data from the normal and tumor brain and breast endothelial cell libraries. Each SAGE library included 30-40,000 SAGE tags. When the complete gene expression libraries were analyzed the libraries formed two sub-clusters based upon the tissue of origin of the endothelial cells, i.e. the normal and tumor breast endothelial cells clustered apart from the normal and tumor brain endothelial cells. Therefore, genes that distinguish tumor from normal endothelial cells did not dictate the general gene expression profiles. Statistical confidence filtering was then applied to all the libraries to isolate genes upregulated with 90%, 95% and 99% confidence. When hierarchical clustering was applied to the gene population upregulated in these libraries with 90% and 95% confidence, tumor endothelial cell libraries formed a distinctive sub-cluster from the normal endothelial cell libraries. Thus, a group of genes could be identified that were involved in the switch from normal tissue endothelium to malignant disease tissue endothelium without tissue type distinction (Fig. 4). Interestingly, when hierarchical clustering analysis was performed with genes upregulated at the 99% confidence level, the libraries from different tissues formed distinctive sub-clusters. Thus, at this high level of

statistical stringency, genes expressed by the endothelial cells were dominated by the tissue of origin of the cells and not by the normalcy or malignancy of tissue.

The final bioinformatics analysis was to examine the expression of the genes/mRNA that were highly up-regulated in tumor endothelial cells with genes/mRNA expressed in cells commonly used as a model systems in the angiogenesis and antiangiogenesis fields. The cells whose gene/mRNA expression was examined included HUVEC (human umbilical vein endothelial cells), HMVEC (human microvascular endothelial cells) and EPC (human endothelial precursor cells) (37). SAGE libraries were available for several cell culture conditions including stimulated with VEGF and unstimulated cells. Many of the genes/mRNA expressed at high levels in the tumor endothelial cells isolated from fresh human tumor specimens were either not expressed or were expressed at very low levels in HUVEC and HMVEC under stimulated and unstimulated conditions.

4. Endothelial precursor cells, pericytes and mesenchymal stem cells

Growth of blood vessels normally occurs during embryo development and wound-healing and abnormally as a component of tumor and inflammatory disease processes (38). The abnormality of tumor vasculature and the value of working with fresh endothelial cells isolated from solid tumors were recognized by cancer researchers and the role of endothelial precursor cells from bone marrow was recognized by developmental biologists (39,40). Asahara *et al* isolated putative endothelial precursor cells (angioblasts) from human peripheral blood by magnetic bead selection and described a role for these cells in postnatal vasculogenesis and pathological neovascularization (41-43). Studies in allogeneic bone marrow transplant recipients confirmed that circulating endothelial precursor cells in peripheral blood originated from the bone marrow (44). $CD34^+/AC133^+$ progenitor cells from bone marrow can differentiate into endothelial cells in culture (37,38). Several studies have tied circulating endothelial precursor cells to the development of tumor vasculature (45-49).

In culture with VEGF, $AC133^+$ multipotent human bone marrow progenitor cells differentiate into $CD34^+$/VE-cadherin$^+$/$VEGFR2^+$ cells (37). Upon maintenance in cell culture these cells continue to differentiate toward a more mature endothelial phenotype.

Most research directed toward the development of antiangiogenic anticancer agents has utilized HUVEC and HMVEC, as the cell-based models of the tumor endothelium (50). As determined by gene expression profiling using SAGE, the endothelial precursor cell developed by driving $AC133^+/CD34^+$ human bone marrow progenitor cells toward endothelial cell differentiation in cell culture was a better model for tumor endothelial cells than were HUVEC and HMVEC (37). Analysis of several cell surface markers by flow cytometry showed that endothelial precursor cells, HUVEC and HMVEC have similar expression of P1H12, VEGFR2 and endoglin but that endothelial precursor cells have much lower expression of ICAM1, ICAM2, VCAM1 and thrombomodulin than do HUVEC and HMVEC. The endothelial precursor cells generated can form tubes/networks on Matrigel™, migrate through porous membranes and invade through thin layers of Matrigel similarly to HUVEC and HMVEC. However, in a co-culture assay using human SKOV3 ovarian cancer cell clusters in collagen as a stimulus for invasion through Matrigel, endothelial precursor cells were able to invade into the malignant cell cluster while HMVEC were not able to invade the malignant cell cluster. *In vivo* a Matrigel plug assay where human endothelial precursor cells were suspended in the Matrigel allowed tube/network formation by human endothelial precursor cells to be carried out in a murine host.

Endothelial precursor cells appear to represent a more immature endothelial cell or a more de-differentiated endothelial cell than do HUVEC and HMVEC and thus provide a more accurate mimic of tumor endothelial cells. These cells function well in cell-based assays including proliferation, tube formation, migration and invasion. Endothelial precursor cells from several donors express targets identified by studying tumor endothelial cells and thus may represent an improved or second generation model cell system that can be used to study and screen potential antiangiogenic therapeutics (37).

In the search for tumor vascular targets, it became evident that some potential therapeutic target proteins were expressed by tumor endothelial cells and by tumor-associated pericytes (34,51). Pericytes are key cells in vascular development, stabilization, maturation and remodeling and are intimately associated with endothelial cells (52-55). In normal tissue the pericyte/endothelial interface reflects the vessel function. In tumors, however, endothelial cells of tumor vessels do not form a tight barrier and pericytes are loosely attached (52). Pericytes express several cell surface markers including smooth muscle α-actin (αSMA), desmin, NG-2, platelet-derived growth factor receptor (PDGFR)-ß, aminopeptidase A and N, and RGS5; however, none of these cell surface proteins are exclusive to pericytes. Several secreted factors including transforming growth factor ß, angiopoietins 1 and 2, platelet-derived growth factors, sphingosine-1-phosphate and Notch ligands are involved in the intercellular communication between endothelial cells and pericytes (55). Pericytes are likely of mesenchymal origin, although other possibilities include trans-differentiation of endothelial cells into pericytes and derivation for bone marrow progenitor cells (56). Pericyte progenitor cells may move from the bone marrow to differentiate into fibroblast-like cells and contribute to extracellular matrix formation during wound healing, chronic inflammation and to tumor stroma. Tumor vessels are heterogeneous in their pericyte coverage. It appears that antiangiogenic therapies directed toward endothelial targets can produce an ablation of naked endothelial tubes and that pericyte covered endothelium is less susceptible to damage. Thus, pericytes may be a valid target for anticancer therapeutics. Using the RIP/Tag2 mouse, a single transgenic that is a model of islet cell carcinogenesis, Bergers *et al* found that a combination of tyrosine kinase inhibitors directed toward endothelial and pericyte targets was a superior therapy compared with each molecule administered alone (57). As primary cells in culture, pericytes and endothelial precursor cells share many properties such as tube/network formation and response to kinase inhibitors selective for angiogenic pathways. Expression of cell surface proteins including PDGFR, VCAM, ICAM, endoglin, desmin and NG2 were

similar between pericytes and endothelial precursor cells while expression of P1H12 and LFA-1 clearly differentiate the cell types (51).

Mesenchymal stem cells are multipotent bone-marrow-derived cells (58). All mesenchymal tissues develop from mesenchymal stem cells. The remarkable plasticity of mesenchymal stem cells allows them under different conditions to differentiate into the tissues and organs which they form in the body including bone, cartilage, muscle, ligament, tendon, adipose and stroma (58). Mesenchymal stem cells can readily be isolated from bone marrow and grown in culture (59). These cells have been shown to be immunologically neutral and to home to sites of tissue injury. Therefore mesenchymal stem cells have been proposed for use in several cellular therapy applications including induction of vascular network formation following ischemic injury, and targeting tumors with gene therapy to activate prodrugs or deliver anticancer protein therapeutics and/or imaging agents (60,61).

5. Newer vascular targets: endosialin

In 2000 St. Croix *et al* reported results of a SAGE (serial analysis of gene expression) study using RNA prepared from endothelial cell samples from the colon carcinoma and normal colon mucosa of a human patient (33). The SAGE tag for TEM1/endosialin was found at a level of 0 tags in the normal endothelial cells and 28 tags in the tumor endothelial cells per 100,000 tags sequenced. Earlier, Rettig *et al* recognized that cells of the reactive tumor stroma differed from corresponding cells in normal tissues in proliferative and invasive behavior and raised an antibody against cultured fetal fibroblasts and identified the tumor vascular endothelial antigen, endosialin (63). In cell culture, several human fibroblast cell lines and human neuroblastoma cell lines (some of these are now classified as Ewing's sarcoma) were positive for endosialin protein using the FB5 antibody; while melanoma, glioma, sarcoma, carcinoma and leukemia cell lines and both growth factor stimulated- and unstimulated-HUVEC were negative for endosialin. Most normal human tissues were immunohistochemically negative for endosialin while 41/61 sarcomas, 26/37 carcinomas, 18/25 neuroectodermal tumors and 0/5 lymphomas were endosialin positive by FB5 staining. Later, the full-length cDNA for endosialin was cloned and found to encode a type I membrane protein (757 aa) that corresponds to TEM 1 described by St. Croix *et al* (64). Endosialin is a C-type lectin-like protein with a signal leader peptide, five globular extracellular domains (a C-lectin domain, one domain similar to the Sushi/ccp/scr pattern and three EGF repeats), followed by a mucin-like region, a transmembrane segment and a short cytoplasmic tail (64). The core protein is abundantly sialylated with O-linked oligosaccharides and is sensitive to O-sialoglycoprotein endopeptidase and is therefore in the group of sialomucin-like molecules. The N-terminal (360 aa) shows homology to thrombomodulin, a receptor involved in regulating blood coagulation and to complement receptor C1qRp (65-67). This overall protein structure indicates that endosialin may be a receptor (64).

The murine homolog of TEM 1 was found to be expressed abundantly in the vasculature of the developing embryo but only in very limited adult vasculature by Carson-Walter *et al* (36). Opavsky *et al* found 77.5% identity between human and murine endosialin and 100% identity between the transmembrane portion of the human and murine proteins (68). Using Rapid-Scan panel (Ori-Gene) for mouse mRNA, Opavsky *et al* were able to detect endosialin message in all tissues with highest expression in heart, kidney, stomach, skin, pancreas, uterus embryo and virgin breast. In the human gene panel, the highest expression of endosialin message was found in placenta, ovary, heart, skeletal muscle, small intestine and the cardiovascular system. Mouse cell lines from embryonic fibroblasts, pre-adipocytes and immortalized endothelial cells expressed endosialin; however, human HUVEC cells were negative for endosialin message.

An endosialin knockout (KO) mouse was fertile and appeared to develop normally in body weight, vasculature and wound healing (69). However, when human HCT116 colon carcinoma was implanted orthotopically on the cecum of nude endosialin KO mice, the take rate was about 33% compared with 90% take rate in normal nude mice. The HCT116 tumors that grew in the endosialin KO animals were slower growing than the tumors in the normal nude mice. There were significantly fewer HCT116 liver metastases in the endosialin KO mice. The tumors in the endosialin KO mice had a larger number of very small vessels than did the tumors in the normal nude mice.

Several recent reports have detected endosialin mRNA and/or endosialin protein in various tumor settings. Davies *et al* examined the levels of expression for tumor endothelial markers in human breast cancer (70). Breast cancer tissues (n=120) and normal breast tissues (n=33) were obtained after surgery. RNA was extracted from frozen sections for gene amplification. The expression of tumor endothelial markers was assessed using RT-PCR and the quantity of the transcripts was determined using real-time quantitative PCR (Q-RT PCR). After a median follow-up of 72.2 months it was found that patients who had recurrent disease and/or who had died from breast cancer had a significantly ($P<0.05$) elevated levels of endosialin compared to those patients who were disease-free. Patients who had developed nodal involvement exhibited significantly ($P<0.05$) higher levels of endosialin compared to patients who were node negative. The data indicated that elevated levels of endosialin associated with either nodal involvement, and/or disease progression, and may have a prognostic value in breast cancer.

Kaposi's sarcoma is a multifocal, vascular, proliferative disease made up of clusters of spindle-shaped cells, slit-like vessels and a variable inflammatory infiltrate (71). Wang *et al* (72) showed by gene expression microarrays that neoplastic cells of Kaposi's sarcoma are closely related to lymphatic endothelial cells and that Kaposi sarcoma herpesvirus infects both lymphatic endothelial cells and blood vascular endothelial cells *in vitro*. Oligonucleotide microarrays were used to compare the gene expression profiles of Kaposi sarcoma and normal skin. Nodular Kaposi sarcoma biopsy samples with >80% spindle cells were used to minimize the dermal and epidermal components. A subset of the global expression profile provided a 'Kaposi sarcoma expression signature'. The signature contained 1,482 genes that differentiate Kaposi sarcoma from normal skin ($P\leq0.05$). Endosialin was among

the genes highly upregulated in Kaposi's sarcoma. Recent studies using standardized high-throughput RNA detection with microarray chips allowing for electronic Northern blot analysis of marker genes and laser capture microdissection on antibody-stained tissue sections for collection of RNA, confirmed that endosialin was a tumor stromal marker and a tumor vascular marker (73-76). These studies also showed that endosialin was expressed on malignant cells of mesenchymal origin including malignant fibrous histiocytoma, liposarcoma and other sarcomas.

Conejo-Garcia *et al* analyzed tumor-infiltrating host cells from ten consecutive stage III ovarian carcinomas for their expression of leukocyte marker CD45 or endothelial marker VE-cadherin by flow cytometry (77). A sub-population of cells that co-express CD45 and VE-cadherin were identified and termed vascular leukocytes. Real-time RT-PCR of sorted human tumor endothelial cells, vascular leukocytes, HUVEC and normal human spleen indicated similar levels of CD31 mRNA in the vascular leukocytes and HUVEC and higher levels in tumor endothelial cells. The CD45 mRNA expression was high in vascular leukocytes and spleen cells and very low in tumor endothelial cells and HUVEC. Endosialin was expressed by both tumor endothelial cells and vascular leukocytes. Thus, a new cell type was defined as $CD45^+$VE-cadherin$^+$ $P1H12^+CD34^+CD31^+TEM\ 1^+TEM\ 7^+$ that can form functional blood vessels (77).

When Madden *et al* performed SAGE analysis on endothelial cells derived from fresh surgical samples of normal temporal lobe cortex (2 patients) or glioma (3 patients), sixteen genes were highly upregulated in tumor endothelial cells compared with normal endothelial cells and endosialin was amongst these genes (25). Brady *et al* investigated the expression pattern of endosialin in human brain tumors and brain metastasis (78). A rabbit polyclonal antibody to endosialin was generated and used to study 30 human brain tumor specimens by immunoblotting and immunohistochemistry. Twenty of 30 tumors expressed endosialin protein. The largest proportion of endosialin-expressing tumors was highly invasive glioblastoma multiforme (6/6), anaplastic astrocytomas (2/3), and carcinoma brain metastasis (4/7). Endosialin was expressed by melanoma (1/1), oligodendroglioma (2/2), astrocytomas (2/5), menigioma (2/5) and ependymoma (2/7). Endosialin localized to small and large vessels and was also expressed by Thy-1^+ fibroblast-like cells in some vessels. Endosialin expression was associated with high-grade primary and metastatic tumors and was absent in normal blood vessels (78). Rettig *et al* reported that radiolabeled FB5:endosialin complex was rapidly internalized into endothelial cells (63).

In a recent study, MacFayden *et al* immunized Balb/C mice with human AG1523 foreskin diploid fibroblasts and isolated 4 mouse monoclonal antibodies that bind to endosialin/CD248 (79). In several tissues, endosialin was a cell surface glycoprotein expressed predominately by fibroblasts and pericytes associated with tumor vasculature. Using phage display technology, Marty *et al* isolated a single chain antibody fragment directed toward the endosialin extracellular domain (80). The single chain antibody fragment was used to guide a liposome encapsulated cytotoxic agent to tumor vasculature.

The function of endosialin remains undetermined. Endosialin belongs to the superfamily of proteins containing C-type lectin domains and specifically to Group XIV along with CETM, thrombomodulin and C1qRP (81,82). These proteins are involved several biological processes including coagulation, inflammation and recognition of self versus non-self. Lectin carbohydrate recognition domains play a major role in pathogen detection (83-86). Pathogens can use carbohydrates specifically targeting C-type lectin domains to escape immune surveillance (84). Autoimmune peripheral vascular in antiphospholipid syndrome may in part represent a breakdown in this system (85). By analogy to other Group XIV members, endosialin may have roles in cell:cell interaction and maintenance of immune system recognition of the proliferating tumor vasculature as self (87).

6. Conclusion

The message for endosialin was amongst those identified as tumor endothelial markers by SAGE. Endosialin is expressed by endothelial precursor cells, pericytes, mesenchymal stem cells, a subset of T-cells (vascular leukocytes) and some malignant cells of messenchymal origin (sarcomas). The protein structure of endosialin places it in the Group XIV thrombomodulin-like family of C-lectin domain proteins. However, the absence of expression of endosialin in mature endothelium differentiates it from thrombomodulin. While the function of endosialin has yet to be elucidated, the expression pattern of this protein may make it a favorable target for cancer therapy with an antibody or antibody-toxin conjugate.

References

1. Folkman J: Angiogenesis. Annu Rev Med 57: 1-18, 2006.
2. Folkman J: Antiangiogenesis in cancer therapy - endostatin and its mechanisms of action. Exp Cell Res 312: 594-607, 2006.
3. Folkman J: Angiogenesis and apoptosis. Semin Cancer Biol 13: 159-167, 2003.
4. Kerbel R and Folkman J: Clinical translation of angiogenesis inhibitors. Nat Rev Cancer 2: 727-739, 2002.
5. Herbst RS, Onn A and Sandler A: Angiogenesis and lung cancer: prognostic and therapeutic implications. J Clin Oncol 23: 3243-3256, 2005.
6. Riesterer O, Honer M, Jochum W, Oehler C, Ametamey S and Pruschy M: Ionizing radiation antagonizes tumor hypoxia induced by antiangiogenic treatment. Clin Cancer Res 12: 3518-3524, 2006.
7. Hida K, Hida Y, Amin DN, Flint AF, Panigrahy D, Morton CC and Klagsbrun M: Tumor-associated endothelial cells with cytogenetic abnormalities. Cancer Res 64: 8249-8255, 2004.
8. Gasparini G, Longo R, Fanelli M and Teicher BA: Combination of antiangiogenic therapy with other anticancer therapies: results, challenges and open questions. J Clin Oncol 23: 1295-1311, 2005.
9. Teicher BA: Hypoxia, tumor endothelium and targets for therapy. In: Advances in Experimental Medicine & Biology. Okunieff P, Williams J and Chen Y (eds). Springer, New York NY, pp31-38, 2005.
10. Fiegl H, Millinger S, Goebel G, Muller-Holzner E, Marth C, Laird PW and Widschwendter M: Breast cancer DNA methylation profiles in cancer ccells and tumor stroma: association with HER-2/neu status in primary breast cancer. Cancer Res 66: 29-33, 2006.
11. Weeraratna AT: Discovering causes and cures for cancer from gene expression analysis. Ageing Res Rev 4: 548-563, 2005.
12. Brentani RR, Carrari DM, Verjovski-Almeida S, Reis EM, Neves EJ, De Souza SJ, Carvalho AF, Brentani H and Reis LFL: Gene expression arrays in cancer research: methods and applications. Crit Rev Oncol/Hematol 54: 95-105, 2005.

13. Segal E, Friedman N, Kaminski N, Regev A and Koller D: From signatures to models: understanding cancer microarrays. Nat Genet 37: S38-S45, 2005.
14. Sieben NL, Oosting J, Flanagan AM, Prat J, Roemen GMJM, Kolkman-Uljee SM, van Eijk R, Cornelisse CJ, Fleuren GJ and van Engeland M: Differential gene expression in ovarian tumors reveals Dusp 4 and Serpina 5 as key regulators for benign behavior of serous borderline tumors. J Clin Oncol 23: 7257-7264, 2005.
15. Espinosa E, Vara JAF, Redondo A, Sanchez JJ, Hardisson D, Zamora P, Pastrana F, Gomez CP, Martinez B, Suarez A, Calero F and Baron MG: Breast cancer prognosis determined by gene expression profiling: a quantitative reverse transcriptase polymerase chain reaction study. J Clin Oncol 23: 7278-7285, 2005.
16. Chen C-N, Lin J-J, Chen J JW, Lee P-H, Yang C-Y, Kuo M-L, Chang K-J and Hsieh F-J: Gene expression profile predicts patient survival of gastric cancer after surgical resection. J Clin Oncol 23: 7286-7295, 2005.
17. Agnelli L, Bicciato S, Mattioli M, Fabri S, Intini D, Verdelli D, Baldini L, Morabito F, Callea V, Lombardi L and Neri A: Molecular classification of multiple myeloma: a distinct transcriptional profile characterizes patients expressing CCND1 and negative for 14q32 translocations. J Clin Oncol 23: 7296-7306, 2005.
18. Spinola M, Leoni V, Pignatiello C, Conti B, Ravagnani F, Pastorino U and Dragani TA: Functional FGFR4 Gly388Arg polymorphism predicts prognosis in lung adenocarcinoma patients. J Clin Oncol 23: 7307-7311, 2005.
19. Grundy PE, Breslow NE, Li S, Perlman E, Beckwith JB, Ritchey ML, Shamberger RC, Haase GM, D'Angio GJ, Donaldson M, Coppes MJ, Malogolowkin M, Shearer P, Thomas PRM, Macklis R, Tomlinson G, Huff V and Green DM: Loss of heterozygosity for chromosomes 1p and 16q Is an adverse prognostic factor in favorable-histology Wilms tumor: a report from the National Wilms Tumor Study Group. J Clin Oncol 23: 7312-7321, 2005.
20. Bilke S, Chen Q-R, Westerman F, Schwab M, Catchpoole D and Khan J: Inferring a tumor progression model for neuroblastoma from genomic data. J Clin Oncol 23: 7322-7331, 2005.
21. Velculescu VE, Zhang L, Vogelstein B and Kinzler KW: Serial analysis of gene expression. Science 270: 484-487, 1995.
22. Porter D, Yao J and Polyak K: SAGE and related approaches for cancer target identification. Drug Discov Today 11: 110-118, 2006.
23. Beauchamp NJ, van Achterberg TAE, Engelse MA, Pannekoek H and De Vries CJM: Gene expression profiling of resting and activated vascular smooth muscle cells by serial analysis of gene expression and clustering analysis. Genomics 82: 288-299, 2003.
24. Polyak K and Riggins GJ: Gene discovery using the serial analysis of gene expression technique: implications for cancer research. J Clin Oncol 19: 2948-2958, 2001.
25. Madden SL, Cook BP, Nacht M, Weber WD, Callahan MR, Jiang Y, Dufault MR, Zhang X, Zhang W, Walter-Yohrling J, Rouleau C, Akmaev VR, Wang CJ, Cao X, St. Martin TB, Roberts BL, Teicher BA, Klinger KW, Stan R-V, Lucey B, Carson-Walter EB, Laterra J and Walter KA: Vascular gene expression in non-neoplastic and malignant brain. Am J Pathol 165: 601-608, 2004.
26. Parker BS, Argani P, Cook BP, Liangfen H, Chartrand SD, Zhang M, Saha S, Bardelli A, Jiang Y, St. Martin TB, Nacht M, Teicher BA, Klinger KW, Sukumar S and Madden SL: Alerations in vascular gene expression in invasive breast carcinoma. Cancer Res 64: 7857-7866, 2004.
27. Simon R: Roadmap for developing and validating therapeutically relevant genomic classifiers. J Clin Oncol 23: 7332-7341, 2005.
28. Bono H and Okazaki Y: The study of metabolic pathways in tumors based on the transcriptome. Semin Cancer Biol 15: 290-299, 2005.
29. Adler AS, Lin M, Horlings H, Nuyten DSA, van de Vijver MJ and Chang HY: Genetic regulators of large-scale transcritptional signatures in cancer. Nat Genet 38: 421-430, 2006.
30. Rhodes DR and Chinnaiyan AM: Integrative analysis of the cancer transcriptome. Nat Genet 37: S31-S37, 2005.
31. Yoo CB and Jones PA: Epigenetic therapy of cancer: past, present and future. Nat Rev Drug Discov 5: 37-50, 2006.
32. Teicher BA, Ara G, Keyes SR, Herbst RS and Frei E III: Acute *in vivo* resistance in high-dose therapy. Clin Cancer Res 4: 483-491, 1998.
33. St. Croix B, Rago C, Velculescu V, Traverso G, Romans KE, Montgomery E, Lal A, Riggins GJ, Lengauer C, Vogelstein B and Kinzler KW: Genes expressed in human tumor endothelium. Science 289: 1197-1202, 2000.
34. Bagley RG, Rouleau C, Morgenbesser SD, Weber W, Cook BP, Shankara S, Madden SL and Teicher BA: Pericytes from human non-small cell lung carcinomas: an attractive target for anti-angiogenic therapy. Vasc Res 71: 163-174, 2006.
35. Nanda A and St. Croix B: Tumor endothelial markers: new targets for cancer therapy. Curr Opin Oncol 16: 44-49, 2004.
36. Carson-Walter EB, Watkins DN, Nanda A, Vogelstein B, Kinzler K and St. Croix B: Cell surface tumor endothelial markers are conserved in mice and humans. Cancer Res 61: 6649-6655, 2001.
37. Bagley R, Walter-Yohrling J, Cao X, Weber W, Simons B, Cook BP, Chartrand SD, Wang C, Madden SL and Teicher BA: Endothelial precursor cells as a model of tumor endothelium: characterization and comparison to mature endothelial cells. Cancer Res 63: 5866-5873, 2003.
38. Garmy-Susini B and Varner JA: Circulating endothelial progenitor cells. Br J Cancer 93: 855-858, 2005.
39. Modzelewski RA, Davies P, Watkins SC, Auerbach R, Chang M-J and Johnson CS: Isolation and identification of fresh tumor-derived endothelial cells from a murine RIF-1 fibrosarcoma. Cancer Res 54: 336-339, 1994.
40. Kaufman D, Hanson E, Lewis R, Auerbach R and Thomson J: Hematopoietic colony-forming cells derived from human embryonic stem cells. Proc Natl Acad Sci USA 98: 10716-10721, 2001.
41. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G and Isner J: Isolation of putative progenitor endothelial cells for angiogenesis. Science 275: 964-967, 1997.
42. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M and Isner J: Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 85: 221-228, 1999.
43. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M and Isner J: VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J 18: 3964-3972, 1999.
44. Lin Y, Weisdorf DJ, Solovey A and Hebbel RP: Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest 105: 71-77, 2000.
45. Rafii S: Circulating endothelial precursors: mystery, reality, and promise. J Clin Invest 105: 17-19, 2000.
46. Peichev M, Naiyer A, Pereira D, Zhu Z, Lane W, Williams M, Oz M, Hicklin D, Witte L, Moore M and Rafii S: Expression of VEGFR-2 and AC133 by circulating human $CD34^+$ cells identifies a population of functional endothelial precursors. Blood 95: 952-958, 2000.
47. Gill M, Dias S, Hattori K, Rivera, M, Hicklin D, Witte L, Girardi L, Yurt R, Himiel H and Rafii S: Vascular trauma induces rapid but transient mobilization of $VEGFR2^+AC133^+$ endothelial precursor cells. Circ Res 88: 167-174, 2001.
48. Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett N, Crystal R, Moore M, Hajjar K, Manova K, Benezra R and Rafii S: Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat Med 7: 1194-1201, 2001.
49. De Bont E, Guikema J, Scherpeh F, Meeuwsen T, Kamps W, Vellenga E and Bos N: Mobilized human $CD34^+$ hematopoietic stem cells enhance tumor growth in a non-obese diabetic/severe combined immunodeficient mouse. Cancer Res 61: 7654-7659, 2001.
50. Auerbach R, Akhtar N, Lewis L and Shinners BL: Angiogenesis assays: problems and pitfalls. Cancer Metastasis Rev 19: 167-172, 2000.
51. Bagley RG, Weber W, Rouleau C and Teicher BA: Pericytes and endothelial precursor cells: cellular interactions and contributions to malignancy. Cancer Res 65: 9741-9750, 2005.
52. Baluk P, Hashizume H and McDonald DM: Cellular abnormalities of blood vessels as targets in cancer. Curr Opin Genet Dev 15: 102-111, 2005.
53. Von Tell D, Armulik A and Betsholtz C: Pericytes and vascular stability. Exp Cell Res 312: 623-629, 2006.
54. Armulik A, Abramsson A and Betsholtz C: Endothelial/pericyte interactions. Circ Res 97: 512-523, 2005.

55. Chantrain CF, Henriet P, Jodele S, Emonard H, Feron O, Courtoy PJ, De Clerck YA and Marbaix E: Mechanisms of pericyte recruitment in tumor angiogenesis: a new role for metalloproteinases. Eur J Cancer 42: 310-318, 2006.
56. Rajantie I, Ilmonen M, Alminaite A, Ozerdem U, Alitalo K and Salven P: Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. Blood 104: 2084-2086, 2004.
57. Bergers G, Song S, Meyer-Morse N, Bergsland E and Hanahan D: Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 111: 1287-1295, 2003.
58. Kemp KC, Hows J and Donaldson C: Bone marrow-derived mesenchymal stem cells. Leuk Lymphoma 46: 1531-1544, 2005.
59. Martens TP, See F, Schuster MD, Sondermeijer HP, Hefti MM, Zannettino A, Gronthos S, Seki T and Itescu S: Mesenchymal lineage precursor cells induce vascular network formation in ischemic myocardium. Nat Clin Prac 3 (Suppl 1): S18-S23, 2006.
60. Hung S-C, Deng W-P, Yang WK, Liu R-S, Lee C-C, Su T-C, Lin R-J, Yang D-M, Chang C-W, Chen W-H, Wei H-J and Gelovani JG: Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by non-invasive *in vivo* positron emission tomography imaging. Clin Cancer Res 11: 7749-7756, 2005.
61. Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J, Chen J, Hentschel S, Vecil G, Dembinski J, Andreeff M and Lang FF: Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. Cancer Res 65: 3307-3318, 2005.
62. Hofmeister V, Vetter C, Schrama D, Brocker E-B and Becker JC: Tumor stroma-associated antigens for anti-cancer immunotherapy. Cancer Immunol Immunother 55: 481-494, 2006.
63. Rettig WJ, Garin-Chesa P, Healey JH, Su SL, Jaffe EA and Old LJ: Identification of endosialin, a cell surface glycoprotein of vascular endothelial cells in human cancer. Proc Natl Acad Sci USA 89: 10832-10836, 1992.
64. Christian S, Ahorn H, Koehler A, Eisenhaber F, Rodi H-P, Garin-Chesa P, Park JE, Rettig WJ and Lenter MC: Molecular cloning and characterization of endosialin, a C-type lectin-like cell surface receptor of tumor endothelium. J Biol Chem 276: 7408-7414, 2001.
65. Peerschke EIB and Gherbrehiwet B: Platelet C1Q receptor interactions with collagen and C1Q coated surfaces. J Immunol 145: 2984-2988, 1990.
66. Webster SD, Park M, Fonseca MI and Tenner AJ: Structural and functional evidence for microglial expression of C1qRp, the C1q receptor that enhances phagocytosis. J Leukoc Biol 67: 109-116, 2000.
67. Dean YD, McGreal EP, Akatsu H and Gasque P: Molecular and cellular properties of the rat AA4 antigen, a C-type lectin-like receptor with structural homology to thrombomodulin. J Biol Chem 275: 34382-34392, 2000.
68. Opavsky R, Haviernik P, Jurkovicova D, Garin MT, Copeland NG, Gilbert DJ, Jenkins NA, Bies J, Garfield S, Pastorekova S, Oue A and Wolff L: Molecular characterization of the mouse TEM 1/ endosialin gene regulated by cell density *in vitro* and expressed in normal tissues *in vivo*. J Biol Chem 276: 38795-38807, 2001.
69. Nanda A, Karim B, Peng Z, Liu G, Qiu W, Gan C, Vogelstein B, St. Croix B, Kinzler KW and Huso DL: Tumor endothelial marker 1 (TEM1) functions in the growth and progression of abdominal tumors. Proc Natl Acad Sci USA 103: 3351-3356, 2006.
70. Davies G, Cunnick GH, Mansel RE, Mason MD and Jiang WG: Levels of expression of endothelial markers specific to tumor-associated endothelial cells and their correlation with prognosis in patients with breast cancer. Clin Exp Metastasis 21: 31-37, 2004.
71. Koon HB, Bubley GJ, Pantanowitz L, Masiello D, Smith B, Crosby K, Proper J, Weeden W, Miller TE, Chatis P, Egorin MJ, Tahan SR and Dezube BJ: Imatinib-induced regression of AIDS-related Kaposi's sarcoma. J Clin Oncol 23: 982-989, 2005.
72. Wang H-W, Trotter MWB, Lagos D, Bourboulia D, Henderson S, Makinen T, Elliman S, Flanagan AM, Alitalo K and Boshoff C: Kaposi sarcoma herpesvirus-induced cellular reprogramming contributes to the lymphatic endothelial gene expression in Kaposi sarcoma. Nat Genet 36: 687-693, 2004.
73. Dolznig H, Schweifer N, Puri C, Kraut N, Rettig WJ, Kerjaschki D and Garin-Chesa P: Characterization of cancer stroma markers: *in silico* analysis of an mRNA expression database for fibroblast activation protein and endosialin. Cancer Immunity 5: 10-19, 2005.
74. Rupp C, Dolznig H, Puri C, Schweifer N, Sommergruber W, Kraut N, Rettig WJ, Kerjaschki D and Garin-Chesa P: Laser capture microdisection of epithelial cancers guided by antibodies against fibroblast activation protein and endosialin. Diagn Mol Pathol 15: 35-42, 2006.
75. Tentori L, Vergati M, Muzi A, Levati L, Ruffini F, Forini O, Vernole P, Lacal PM and Graziani G: Generation of an immortalized human endothelial cell line as a model of neo-vascular proliferating endothelial cells to assess chemosensitivity to anti cancer drugs. Int J Oncol 27: 525-535, 2005.
76. Huber MA, Kraut N, Schweifer N, Dolznig H, Peter RU, Schibert RD, Scharffetter-Kochanek K, Pehamberger H and Garin-Chesa P: Expression of stromal cell markers in distinct compartments of human skin. J Cutan Pathol 33: 145-155, 2006.
77. Conejo-Garcia JR, Buckanovich RJ, Benencia F, Courreges MC, Rubin SC, Carroll RG and Coukos G: Vascular leukocytes contribute to tumor vascularization. Blood 105: 679-681, 2005.
78. Brady J, Neal J, Sadakar N and Gasque P: Human endosialin (tumor endothelial marker 1) is abundantly expressed in highly malignant and invasive brain tumors. J Neuropathol Exp Neurol 63: 1274-1283, 2004.
79. MacFayden JR, Haworth O, Roberston D, Hardie D, Webster M-T, Morris HR, Panico M, Sutton-Smith M, Dell A, van der Greer P, Wienke D, Buckley CD and Isacke CM: Endosialin (TEM1, CD248) is a marker of stromal fibroblasts and is not selectively expressed on tumor endothelium. FEBS Lett 579: 2569-2575, 2005.
80. Marty C, Langer-Machova Z, Sigrist S, Schott, Schwendener RA and Ballmer-Hofer K: Isolation and characterization of a scFv antibody specific for tumor endothelial marker 1 (TEM1), a new reagent for targeted tumor therapy. Cancer Lett 235: 1-11, 2006.
81. Zelensky AN and Gready JE: The C-type lectin-like domain superfamily. FEBS J 272: 6179-6217, 2005.
82. Zelensky AN and Gready JE: C-type lectin-like domains in Fugu rubripes. BMC Genom 5: 51-73, 2004.
83. Cambi A, Koopman M and Figdor CG: How C-type lectins detect pathogens. Cell Miobiol 7: 481-488, 2005.
84. Van Kooyk Y, Engering A, Lekkerkerker AN, Ludwig IS and Geijtenbeek TBH: Pathogens use carbohydrates to escape immunity induced by dendritic cells. Curr Opin Immunol 16: 488-493, 2004.
85. Vlachoyiannopoulos PG and Samarkos M: Peripheral vascular disease in antiphospholipid syndrome. Thrombosis Res 114: 509-519, 2004.
86. Van de Wouwer M, Collen D and Conway EM: Thrombomodulin-protein C-EPCR system: integrated to regulate coagulation and inflammation. Arterioscler Thromb Vasc Biol 24: 1374-1383, 2004.
87. Hanly AM, Hayanga A, Winter DC and Bouchier-Hayes DJ: Thrombomodulin: tumor biology and prognostic implications. J Cancer Surg 31: 217-220, 2005.