2007-06

The Potential of New Tumor Endothelium-Specific Markers for the Development of Antivascular Therapy

Li, J-L

10.1016/j.ccr.2007.05.004
Cancer Cell
Elsevier BV

REFERENCES

Bracken, A.P., Dietrich, N., Pasini, D., Hansen, K.H., and Helin, K. (2006). Genes Dev. *20*, 1123–1136.

Chen, H., Tu, S.W., and Hsieh, J.T. (2005). J. Biol. Chem. *280*, 22437–22444.

Di Croce, L. (2005). Hum. Mol. Genet. *14*, R77–R84.

Fiskus, W., Pranpat, M., Balasis, M., Herger, B., Rao, R., Chinnaiyan, A., Atadja, P., and Bhalla, K. (2006). Mol. Cancer Ther. *5*, 3096–3104.

Kirmizis, A., Bartley, S.M., Kuzmichev, A., Margueron, R., Reinberg, D., Green, R., and Farnham, P.J. (2004). Genes Dev. *18*, 1592–1605.

Kubicek, S., O'Sullivan, R.J., August, E.M., Hickey, E.R., Zhang, Q., Teodoro, M.L., Rea, S., Mechtler, K., Kowalski, J.A., Homon, C.A., et al. (2007). Mol. Cell *25*, 473–481.

Kuzmichev, A., Margueron, R., Vaquero, A., Preissner, T.S., Scher, M., Kirmizis, A., Ouyang, X., Brockdorff, N., Abate-Shen, C., Farnham, P., and Reinberg, D. (2005). Proc. Natl. Acad. Sci. USA *102*, 1859–1864.

Licht, J.D. (2006). Cancer Cell *9*, 73–74.

Quina, A.S., Buschbeck, M., and Di Croce, L. (2006). Biochem. Pharmacol. *72*, 1563–1569.

Sparmann, A., and van Lohuizen, M. (2006). Nat. Rev. Cancer *6*, 846–856.

Squazzo, S.L., O'Geen, H., Komashko, V.M., Krig, S.R., Jin, V.X., Jang, S.W., Margueron, R., Reinberg, D., Green, R., and Farnham, P.J. (2006). Genome Res. *16*, 890–900.

Tan, J., Yang, X., Zhuang, L., Jiang, X., Chen, W., Lee, P.L., Karuturi, R.K., Tan, P.B., Liu, E.T., and Yu, Q. (2007). Genes Dev. *21*, 1050–1063.

Viré, E., Brenner, C., Deplus, R., Blanchon, L., Fraga, M., Didelot, C., Morey, L., Van Eynde, A., Bernard, D., Vanderwinden, J.M., et al. (2006). Nature *439*, 871–874.

Villa, R., Pasini, D., Gutierrez, A., Morey, L., Occhionorelli, M., Viré, E., Nomdedeu, J.F., Jenuwein, T., Pelicci, P.G., Minucci, S., et al. (2007). Cancer Cell, this issue.

The Potential of New Tumor Endothelium-Specific Markers for the Development of Antivascular Therapy

Ji-Liang Li[1,*] and Adrian L. Harris[1,*]
[1]Cancer Research UK Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford, OX3 9DS, UK
*Correspondence: lij@cancer.org.uk (J.-L.L.); aharris.lab@cancer.org.uk (A.L.H.)
DOI 10.1016/j.ccr.2007.05.004

Angiogenesis is a hallmark of solid tumors, and disruption of tumor vasculature is an active anticancer therapy in some cases. Several proteins expressed on the surface of tumor endothelium have been identified during the last decade. However, due to the expression in both physiological and tumor angiogenesis, only a few targets have been developed for clinical therapeutics. By thorough SAGE analysis of mouse endothelial cells isolated from various normal resting tissues, regenerating liver, and liver-metastasized tumor, Seaman and colleagues in this issue of *Cancer Cell* have demonstrated organ-specific endothelial markers, physiological angiogenesis endothelial markers, and tumor endothelial markers and revealed striking differences between physiological and pathological angiogenesis.

Angiogenesis is a hallmark of solid tumors. Disruption of tumor angiogenesis by blocking proangiogenic growth factors or shutdown of the established tumor blood vessels by vascular targeting agents has demonstrated therapeutic effects in human cancer. The vascular-disrupting effect can be mediated directly by toxic agents or selectively delivered by antibody or peptide targeting (Neri and Bicknell, 2005). The recent successful blockade of the VEGF pathway in several major cancers prolonged survival in phase III clinical trials and has encouraged the identification of new tumor endothelial markers (TEMs).

Early attempts to identify tumor vascular targets focused on the study of in vitro endothelial cell (EC)-isolates using a range of molecular, biochemical, and immunological techniques. These efforts have led to the identification of a limited number of molecular markers predominantly expressed on angiogenic vessels, but in both tumor and physiological angiogenesis. With the advent of new techniques, a great number of tumor endothelial molecules have been identified during the last decade. In silico methods have been used to define new angiogenesis genes such as Robo 4 (Huminiecki and Bicknell, 2000). In vivo phage display has been used to deliver peptides that selectively recognize organ-specific and tumor endothelium, leading to the

identification of aminopeptidase-N as a potential marker of tumor vasculature (Ruoslahti, 2002).

In an earlier study from St. Croix et al. (2000), global screening of gene expression by serial analysis of gene expression (SAGE) revealed 79 transcripts differentially expressed between ECs isolated from a human colorectal tumor and those isolated from its adjacent normal tissue, including 46 specifically elevated more than 10-fold in tumor-associated ECs. In situ hybridization showed that all nine chosen for the validation were prominently expressed in tumor ECs but were absent or barely detectable in normal ECs. Mouse orthologs were also identified, and some were strongly expressed in tumor vessels and in the developing embryonic vasculature, but essentially absent from adult normal tissues

Others developed and applied this approach further for several tumor types, usually comparing tumor endothelium with adjacent normal tissue endothelium. SAGE analysis of human brain ECs revealed 14 glioma endothelial markers upregulated in ECs isolated from three grade-III/IV gliomas compared to ECs from two nonneoplastic temporal lobe tissues. Of these, 12 are known to be present on the cell surface or secreted. In situ hybridization demonstrated the overexpression of the G protein-coupled receptor RDC1 in both brain and colon tumor ECs (Madden et al., 2004). SAGE-analysis of purified ECs from freshly resected specimens of two invasive breast cancers and one normal reduction mammoplasty revealed 29 genes that were expressed at least 6-fold higher in breast tumor ECs than in normal breast ECs; five of these were 5-fold higher in breast tumor than in both colon and brain tumors. HEYL, one of the TEMs, was restricted to invasive breast tumor ECs and capable of increasing proliferation and reducing apoptosis of primary ECs in vitro (Parker et al., 2004).

van Beijnum et al. (2006) used suppression subtractive hybridization to compare gene-expression profiles of the ECs isolated from five human colon carcinoma, five normal colon tissues from the same patients, and five fresh placental tissues as well as colorectal tumor-conditioned HUVEC and quiescent HUVEC cells. Forty-six general angiogenesis genes were upregulated in tumor and placental ECs compared with patient-matched normal ECs; 17 tumor angiogenesis genes were overexpressed in tumor ECs compared with angiogenic (placental) and nonangiogenic ECs. Four of these markers (vimentin, CD59, IGFBP7, and HMGB1) were overexpressed on tumor vasculature at the protein level. Antibodies targeting these markers inhibited angiogenesis in vitro and in vivo and targeting endothelial vimentin in a xenograft mouse model markedly inhibited tumor growth and tumor angiogenesis.

Following the isolation of ECs from ten invasive epithelial ovarian cancers and five normal ovaries, Lu et al. (2007) examined gene-expression differences between ovarian tumor ECs and normal ECs by microarrays and revealed more than 400 differentially expressed genes in ovarian tumor ECs. Among them, all six validated genes were overexpressed in tumor ECs at the protein level. Reducing the expression of EZH2, Jagged1, or PTK2 with siRNA blocked EC migration and tube formation in vitro.

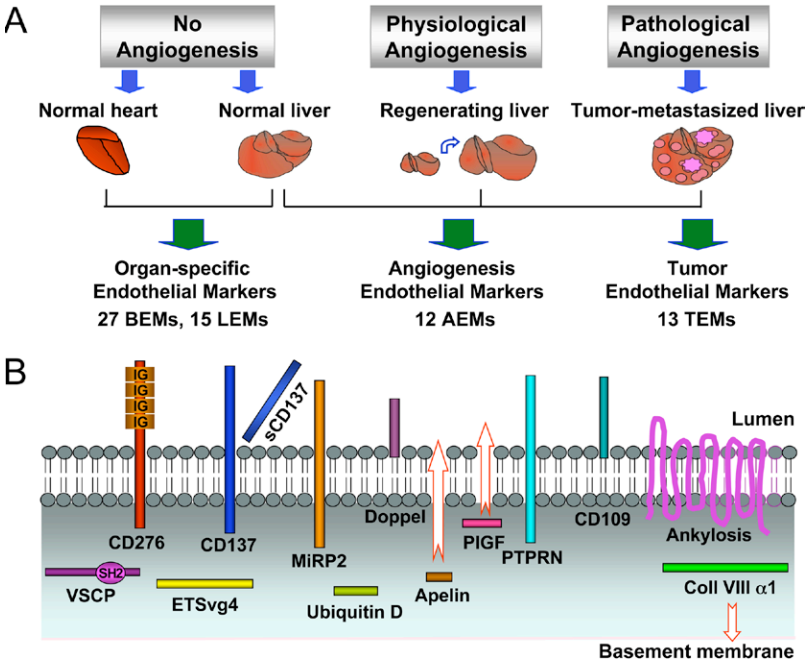

Figure 1. Schematic Diagram Depicting the Identification of Organ-Specific, Physiological, and Tumor Angiogenesis Markers

Various mouse ECs were isolated from eight normal resting tissues (including brain, heart, kidney, liver, lung, muscle, spleen, and intestine), regenerating liver (24, 48, and 72 hr posthepatectomy), two types of liver-metastasized, and three types of subcutaneously-implanted tumors by using CD105 (endoglin) and/or VE-cadherin markers. Numerous SAGE libraries were then constructed and sequenced. Thorough analysis of the SAGE Tags revealed 27 brain endothelial markers (BEMs) with 20-fold or higher expression in brain ECs compared to other normal tissue ECs, 15 liver endothelial markers (LEMs) with 20-fold or higher expression in normal resting liver ECs compared to normal ECs from other tissues, 12 angiogenesis endothelial markers (AEMs) overexpressed with 10-fold or higher expression in regenerating liver ECs and tumor ECs compared to nonangiogenic ECs of all resting tissues, and 13 tumor endothelial markers (TEMs) overexpressed at least 10-fold or higher in tumor ECs compared to normal resting ECs and regenerating liver ECs (A). Of the 13 TEMs identified (B), seven of them including CD276 (B7-H3), CD137 (4-1BB), MiRP2, Doppel (Prion-PLP), PTPRN (IA-2), CD109, and ankylosis have been found to be expressed on the cell surface; PlGF and apelin are secreted angiogenic factors. The most differentially expressed of the TEMs was vascular SH2-containing protein (VSCP), followed by CD276, ETSvg4 (Pea3), CD137 (including its soluble form, sCD137), and MiRP2. The sizes of TEMs are not to scale. Part of panel A is adapted from Figure 1D of Seaman et al. (2007) in this issue of *Cancer Cell*.

Buckanovich et al. (2007) used immunohistochemistry-guided laser-capture microdissection and transcriptional profiling to characterize tumor vascular cells from 21 epithelial ovarian cancers and four normal ovaries and identified 12 ovarian tumor vascular markers that were highly expressed in purified tumor ECs and localized to tumor vasculature. Some of these markers were found to be specifically expressed in ovarian cancer but absent in various normal tissues, including placental and female reproductive tissues with physiological angiogenesis. The expression of STC2, EGFL6, and FZD10 in ovarian vascular cells was significantly associated with decreased disease-free interval.

One major concern arising from the above studies is the relative lack of data on multiple normal vascular beds besides that of tissue adjacent to the tumor, which was available for obvious surgical reasons. An ideal TEM would discriminate between tumor angiogenesis and physiological or regenerative angiogenesis, such as those occurring in female reproductive and wound healing tissues, and does not express in any type of normal cells. Many of the TEMs described so far were also expressed, more or less, in physiological angiogenesis of corpus luteum formation and wound healing, or even in normal ECs from various organs, though at a low level (Bonuccelli et al., 2005; Buckanovich et al., 2007; St. Croix et al., 2000; van Beijnum et al., 2006). Therefore, a major challenge in identifying tumor endothelium-specific markers seems to be obtaining sufficiently pure EC populations from natural tumors, organ-matched normal and regenerative tissues, and other resting tissues.

Seaman and colleagues extend their previous studies and now provide new information to answer these issues on organ-specific endothelial markers, angiogenesis endothelial markers (AEMs), and TEMs (Seaman et al., 2007, this issue of *Cancer Cell*) (Figure 1). This time they used mouse models and comprehensively SAGE-analyzed gene-expression profiles in mouse ECs that were isolated from various normal tissues, regenerating liver, and liver-metastasized tumors as well as subcutaneously implanted tumors. Twenty-seven brain endothelial markers and 15 liver endothelial markers were identified to be highly expressed in resting brain or liver ECs compared to normal ECs from other tissues. In addition, 12 AEMs were overexpressed with 15- to 100-fold higher in regenerating liver ECs compared to nonangiogenic ECs, and most of AEMs have expected roles in cell-cycle control. AEMs were also upregulated in tumor ECs, highlighting the overlapping processes in physiological and tumor angiogenesis.

However, 13 TEMs were expressed at least 10-fold higher in tumor ECs than in normal ECs and regenerating liver ECs, of which seven TEMs were found to be cell surface receptors. Validation of the top nine TEMs by in situ hybridization showed that each of them was expressed in the ECs of various tumor types. Further investigation of CD276, the most differentially expressed TEM, with multiple human tumor samples revealed that CD276 was highly expressed in tumor ECs of various tumor types and also in tumor cells in some cases. Thus, this study revealed not only organ-specific and angiogenesis-specific EC markers but also striking differences between physiological and pathological angiogenesis at the molecular level, which are potentially important for the development of tumor-specific vascular targeting therapy.

Nevertheless, several issues remain to be further investigated. The gene-expression profiling of the ECs from xenograft or allograft tumors could be very different from those in endogenously formed primary and metastatic tumors. Moreover, transcriptional profiling at a later stage of regenerating liver or from chronically inflamed tissue or inflammatory responses is likely to be more informative when compared to tumor angiogenesis since tumors represent "unhealed wounds" and are chronic. The threshold for analysis of these genes was set at 10-fold, but analysis of genes with lower differential may be just as important. Splice variants, which have been shown to be important for other vascular targets such as fibronectin and CD44, will need to be analyzed. The extent of heterogeneity of the TEM profile, such as expression at different sites of metastasis, interindividual variation, and variation from tumor to tumor, will be important to evaluate. Clearly identifying organ-specific EC markers in addition to those of brain and liver is also important. In vivo imaging studies should help resolve some of these issues.

The most important point perhaps is still the specificity of these TEMs. Four of them (CD276, CD137, PTPRN, and CD109) had been shown to be involved in regulating inflammatory or autoimmune responses. CD276 can be induced on T cells, B cells, and dendritic cells by various cytokines. Therefore, more cautious evaluation of these TEMs for their tissue/cell specificity and biological function should be warranted prior to developing these TEMS as therapeutic targets clinically.

Could some of these markers be mechanistic? What is their role in angiogenesis and cell trafficking, e.g., attracting endothelial progenitors and immune cells? What are the mechanisms for their upregulation? Could they be regulated by cytokines secreted by tumor, which activate distant sites of metastasis to prepare the "soil"? If so, early therapy could be used to prevent establishing growth of micrometastasis. The findings of Seaman et al. (2007) provide a great resource for further investigation of these issues.

REFERENCES

Bonuccelli, G., Sotgia, F., Frank, P.G., Williams, T.M., de Almeida, C.J., Tanowitz, H.B., Scherer, P.E., Hotchkiss, K.A., Terman, B.I., Rollman, B., et al. (2005). Am. J. Physiol. Cell Physiol. *288*, C1402–C1410.

Buckanovich, R.J., Sasaroli, D., O'Brien-Jenkins, A., Botbyl, J., Hammond, R., Katsaros, D., Sandaltzopoulos, R., Liotta, L.A., Gimotty, P.A., and Coukos, G. (2007). J. Clin. Oncol. *25*, 852–861.

Huminiecki, L., and Bicknell, R. (2000). Genome Res. *10*, 1796–1806.

Lu, C., Bonome, T., Li, Y., Kamat, A.A., Han, L.Y., Schmandt, R., Coleman, R.L., Gershenson, D.M., Jaffe, R.B., Birrer, M.J., and Sood, A.K. (2007). Cancer Res. *67*, 1757–1768.

Madden, S.L., Cook, B.P., Nacht, M., Weber, W.D., Callahan, M.R., Jiang, Y., Dufault, M.R., Zhang, X., Zhang, W., Walter-Yohrling, J., et al. (2004). Am. J. Pathol. *165*, 601–608.

Neri, D., and Bicknell, R. (2005). Nat. Rev. Cancer *5*, 436–446.

Parker, B.S., Argani, P., Cook, B.P., Liangfeng, H., Chartrand, S.D., Zhang, M., Saha, S., Bardelli, A., Jiang, Y., St Martin, T.B., et al. (2004). Cancer Res. *64*, 7857–7866.

Ruoslahti, E. (2002). Nat. Rev. Cancer *2*, 83–90.

Seaman, S., Stevens, J., Yang, M.Y., Logsdon, D., Graff-Cherry, C., and St. Croix, B. (2007). Cancer Cell, this issue.

St. Croix, B., Rago, C., Velculescu, V., Traverso, G., Romans, K.E., Montgomery, E., Lal, A., Riggins, G.J., Lengauer, C., Vogelstein, B., and Kinzler, K.W. (2000). Science *289*, 1197–1202.

van Beijnum, J.R., Dings, R.P., van der Linden, E., Zwaans, B.M., Ramaekers, F.C., Mayo, K.H., and Griffioen, A.W. (2006). Blood *108*, 2339–2348.