Neovascularization evaluated by CD105 correlates well with prognostic factors in breast cancers

KUN-MING RAU[1,3], CHAO-CHENG HUANG[2,3], TAI-JAN CHIU[1], YEN-YANG CHEN[1], CHIEN-CHANG LU[4], CHIEN-TING LIU[1], SUNG-NAN PEI[1] and YU-CHING WEI[2]

[1]Department of Internal Medicine, Division of Hematology-Oncology, and [2]Department of Anatomical Pathology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung; [3]Chang Gung University, College of Medicine, Tao-Yuan; [4]Department of Colon and Rectal Surgery, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, R.O.C.

Received March 18, 2012; Accepted May 21, 2012

DOI: 10.3892/etm.2012.594

Abstract. Angiogenesis is critical for the growth, invasion and metastasis of cancers. Extensive neovascularization and tumor thrombus also correlate with a poor prognosis in breast cancer (BC). Although anti-angiogenic agents have been the therapies of choice for BC, in particular for triple-negative BCs, predictive markers for anti-angiogenic agents are lacking. Microvascular density (MVD) is commonly used to assess the neovascularization in tumors. Compared with pan-endothelial markers such as CD31, CD34 and von Willebrand factor (vWF), CD105 has a higher specificity for MVD in tumor tissues. In this study, we aimed to determine the prognostic value of CD105 in BCs. Paraffin-embedded tissue blocks from 201 BC patients were formed into tissue microarrays. Evaluation of MVD revealed that a median of 11 microvessels determined by CD105 staining correlated significantly with the pathological characteristics of BCs and also with the survival of patients. The expression of CD105 correlated inversely with hormone receptor (HR) expression but positively with Her-2 expression. Univariate analysis indicated that CD105 is a superior predictor of disease-free survival (DFS) in stage I and II diseases; multivariate analysis indicated that only hormone receptors (HRs) are suitable for predicting overall survival (OS) in stage III disease. These findings reveal for the first time that MVD measured by CD105 staining correlates positively with Her-2 expression but negatively with HR expression. The significance of MVD on OS is more apparent in early stage BCs. CD105 has the potential to be used as a predictive marker for anti-angiogenic agents; the targeting of CD105 may also be a potential anticancer strategy.

Correspondence to: Dr Yu-Ching Wei, Department of Anatomical Pathology, Kaohsiung Chang Gung Memorial Hospital, 123 Ta Pei Rd., Kaohsiung 833, Taiwan, R.O.C.
E-mail: liu07822@ms57.hinet.net

Key words: breast cancer, CD105, endoglin, hormone receptor, angiogenesis, neovascularization, prognosis

Introduction

Breast cancer (BC) is the most common malignancy (28% of all cancers in females) and the second most common cause of cancer mortality in women (15% of female cancer mortality) in the United States (1). The incidence of BC is also increasing in Taiwan. The number of new cases per 100,000 individuals increased from 34.3 in 1994 to 53.1 in 2007. The median age at diagnosis was 51 years, and stage IV disease accounted for approximately 1-5% of new cases. Although there are numerous adjuvant therapies for early stage BC, the recurrence rate remains 20-30%. Treatments for BC rely on the availability of robust clinical and pathological prognostic and predictive factors to guide decision making and the selection of treatment options. Pathological characteristics, including the size of the primary tumor, regional lymph node (LN) metastasis, estrogen receptor (ER), progesterone receptor (PR), Her-2 expression, lymphovascular invasion (LVI) and Ki-67 expression, are all established prognostic markers. In addition to histological prognostic factors, there are increasing numbers of molecular classification systems for BC, including the 21-gene RT-PCR assay (Oncotype DX), which can also be used to predict the benefits of adjuvant chemotherapy (2).

Angiogenesis, the formation of new vessels, is an essential process in the progression of malignant tumors since solid tumors cannot grow beyond 1-2 mm in diameter without angiogenesis (3). As in other malignancies, angiogenesis is also critical for the growth, invasion and metastasis of BC. In BC, extensive neovascularization and tumor thrombus in vessels have been reported to be signs of poor prognosis (4). Also, the tumor microvasculature may constitute a target for anti-angiogenic therapy. Clinical studies have demonstrated that anti-angiogenic agents such as bevacizumab are able to improve the progression-free survival (PFS) of patients with advanced BC when combined with chemotherapy. However, to date there are few predictive markers for anti-angiogenic agents.

Microvascular density (MVD) has become the morphological gold-standard for assessing the neovascularization in human tumors. Several studies have shown that the angiogenic potential of BC as assessed by MVD correlates with progression and metastasis; it therefore predicts clinical outcome (5).

The evaluation of MVD requires the use of specific markers for the vascular endothelium and immunohistochemical (IHC) procedures to visualize microvessels (6). Traditionally, histological assessments of MVD in tumors have used pan-endothelial markers, including CD31, CD34 and von Willebrand factor (vWF) (7-9). Although CD34 has been reported to be more sensitive than CD31 or vWF for MVD in BC (10), these markers are specific for all endothelia and do not target only the vascular endothelium from tumor-induced neovascularization. In non-small-cell lung cancer, CD105 (endoglin) has been shown to be superior to CD34 and CD31 in the evaluation of neovascularization since it has a greater affinity for the endothelial cells in tumor-related angiogenic tissue, whereas CD34 and CD31 react nonspecifically with normal and pathological vessels (11). In solid tumors, CD105 has been revealed to be upregulated in the endothelial cells of peri- and intra-tumoral blood vessels and in the stromal components of several types of cancer (12). Studies have shown that increased MVD, as assessed by a CD105 antibody, is associated with worse overall and disease-free survival (DFS) for various types of cancer (13-15).

CD105 is expressed on the cell surface as a 180-kDa homodimeric transmembrane protein. It is a co-receptor of TGF-β1 and TGF-β2. TGF-β has a complex role in carcinogenesis, as it has tumor-suppressor and oncogenic activities. In the early stages of epithelial tumorigenesis, TGF-β has strong anti-proliferative, pro-apoptotic and tumor growth-inhibiting effects; however, it also acts as a tumor-promoting factor by stimulating the epithelial-to-mesenchymal transition and the invasiveness of cancer cells, inhibiting immune surveillance and stimulating angiogenesis by inducing vascular endothelial growth factor (VEGF) expression (16,17). It is expressed almost exclusively in the endothelial cells of peri- and intra-tumoral blood vessels and in tumor stromal components. CD105 antagonizes the inhibitory effects of TGF-β on proliferation and migration, thus promoting the growth and migration of tumor cells (18).

The use of an antisense agent to inhibit CD105 protein translation in cultured human endothelial cells has been reported to markedly enhance the ability of TGF-β1 to inhibit *in vitro* angiogenesis, suggesting that CD105 is a pro-angiogenic component in the endothelial cells (18). Monoclonal antibodies and their immunoconjugates (immunotoxins and radioimmunoconjugates) against CD105 have been reported to have anticancer effects (19).

In our current study, we analyzed the correlation between CD105 expression and prognostic markers of BC. We sought to determine whether CD105 has the potential to be a therapeutic target for further anti-angiogenic therapies in the future.

Materials and methods

Breast cancer tissue. This study was approved by the Ethics Committee of Kaohsiung Chang Gung Memorial Hospital. With permission from the Institutional Review Board of Chang Gung Memorial Hospital, we collected clinical data and pathological specimens from patients with BC who were diagnosed between 2000 and 2002 and treated in our hospital. Patients provided written consent for their tissue samples to be used for research purposes. In addition, all data were analyzed anonymously. Clinical data, including age at diagnosis, clinical stage and pathological stage, date of recurrence and mortality, and pathological features, including ER, PR and Her-2 status, were obtained from a combination of clinical and pathological record reviews and reports of external medical records.

Tissue microarray. Tissue microarray (TMA) blocks were constructed using the Manual Tissue Arrayer (MTA-1; Beecher Instruments, Sun Prairie, WI, USA). Targets for arraying (areas with BC) were identified by marking the areas on hematoxylin and eosin-stained sections from each paraffin-embedded block. Three tissue cores with a diameter of 2 mm were transferred from each donor block to the recipient TMA block. Liver and skeletal muscle tissue was placed in the first lane core of the three upper left and two lower left cores of the TMA block to ensure correct orientation.

Immunohistochemical staining. TMA blocks constructed from formalin-fixed paraffin-embedded human BC tissue were sectioned at 3-μm thickness on adhesive-coated glass slides and dried overnight at 37˚C. The slides were deparaffinized in xylene and rehydrated through graded alcohols to water. For antigen retrieval, the slides were heated in 10 mM citrate buffer (pH 6.0) for 17 min using a pressure cooker. The slides were subsequently washed using TBS buffer with 0.1% Tween 20 for this and subsequent washes. Endogenous peroxidase activity was quenched by treatment with 3% H_2O_2. After washing, the slides were incubated with primary antibodies targeting CD105 (NCL-CD105; Novocastra, Leica, Newcastle, UK) for 3 h at room temperature (RT), ER (ER, clone SP1, #RM-9101, Neomarkers, Thermo Fisher Scientific, Suwanee, GA, USA) for 1 h at RT, PR (PR, NCL-L-PGR-312/2; Novocastra, Leica) for 1 h at RT and Her-2 (clone SP3, #RM-9103; Neomarkers, Thermo Fisher Scientific) for 1 h at RT. Following the incubation of the primary antibody and a wash step, the HRP polymer (87-8963; Invitrogen, Grand Island, NY, USA) was added. The slides were analyzed using the DAB substrate chromogen system K3468 (Dako, Glostrup, Denmark). The TMA slides were counterstained with hematoxylin and then coverslipped using Entellan® new (Merck KGaA, Darmstadt, Germany) mounting medium. Incubation without the primary antibody was used as a negative control.

The intensity of these markers was determined by two independent pathologists and classified into low and high. A score of low was given if fewer than 10% of the cells were stained either in the nucleus or cytosol; if more than 10% of the cells were stained, this was scored as high. The intensity of the protein expression was tested for correlation with the tumor stage and the prognosis of the disease. The Fisher's exact test was used to test the hypothesis of independence between categorical variables.

Evaluation of MVD. The MVD was evaluated by the immunohistochemical analysis of tumor vessels for CD105 expression in tissue microarrays. Any immune-positive single cell or cluster of cells clearly with lumen was considered to be an individual vessel, as previously recommended (20). Areas of fibrosis, necrosis and inflammation, as well as vessels with a muscle wall, were excluded from the count. The sections were

Table I. Patient and disease characteristics at baseline (n=201).

Characteristics	No. of patients	%
Age at diagnosis, years median (range)	66 (24-84)	
Histology		
Infiltrating ductal carcinoma	186	92.5
Infiltrating lobular carcinoma	6	3.0
Medullary carcinoma	5	2.5
Other	4	2.0
Stage at diagnosis		
I	34	16.9
II	106	52.7
III	52	25.9
IV	9	4.5
Estrogen receptor status		
Positive	132	65.7
Negative	63	31.3
Unknown	6	3.0
Progesterone receptor status		
Positive	117	58.2
Negative	66	32.8
Unknown	18	9.0
Her-2 status		
Negative	145	72.1
Positive[a]	31	15.4
Unknown	25	12.4
Triple-negative	29	14.4

[a]Her-2 positive indicates a immunohistochemical staining score of 3 or fluorescence *in situ* hybridization-positive.

scanned at a magnification level of x100 by two observers simultaneously to select the most vascularized (hot-spots) of the three tissue array spots for each patient. The microvessels in the hot-spots were counted at a magnification level of x200, and their density was expressed as the mean number of microvessels/mm^2. Mean values of CD105 staining were calculated for each individual tumor and used for further analysis.

Statistical analysis. Survival curves in relation to predictive markers were illustrated by Kaplan-Meier survival plots and the difference in time to progression was evaluated by the log-rank test. Spearman's rho correlation was used to test the correlation between the immunohistochemical findings and conventional clinical features, including pathological stage and expression of markers. P<0.05 was considered to indicate a statistically significant result. Statistics software (SPSS, version 10.0; SPSS, Inc., Chicago, IL, USA) was used for all calculations.

The cumulative probability of DFS and overall survival (OS) was estimated by the product-limit method. The log-rank test was used to compare the homogeneity of DFS and survival functions across strata defined by categories of prognostic variables.

Results

Characteristics of the patients and pathological findings. In total, 201 patients were enrolled into this study. The median age at diagnosis was 66 years. The major histological type was infiltrating ductal carcinoma. Approximately one-half of the patients were diagnosed at stage II disease. ER was positive in 132/201 (65.7%) patients, PR was positive in 117/201 (58.2%) patients and Her-2 was positive in only 31/201 (15.4%) patients (Table I).

Evaluation of MVD from the intensity of CD105 staining. Positive CD105 was observed as thin, linear deposits in the membranes and cytoplasm of the endothelial cells within microvessels. Evaluation of MVD from the intensity of the CD105 staining revealed a median of 11 microvessels. The median microvessel count of 11 was selected as the cut-off value to define low and high groups (Fig. 1).

CD105 correlated inversely with hormone receptor (HR) expression. In a bivariant correlation analysis between clinical-pathological variables and CD105, no correlation was found among age of diagnosis, stage and tumor grade. However, we found that CD105 correlated inversely with ER (p<0.001) and PR (p<0.001) expression. On the other hand, it correlated positively with Her-2 (p=0.005) expression (Fig. 2). No correlation between CD105 expression and triple-negative BCs was identified.

CD105 is a poor prognostic factor for early stage BCs. Since angiogenesis is a predictive marker for cancer prognosis, we also analyzed the correlation between CD105 expression and survival for different stages of BC. We found that although the number of microvessels (cut-off, 11 microvessels) did not correlate with the DFS of stage I to III BC patients, it had a stronger correlation with OS in patients with early stage BC (stages I and II) than in patients with late stage BC (stages III and IV; Fig. 3).

Hormone receptors correlate with the OS of BC patients of all stages. We also analyzed a combination of CD105 expression and the expression of HRs and Her-2, using a microvessel count of 11 as a cut-off point. We found that at the early stages (stages I and II), both HRs and MVD influenced OS; we found no difference between CD105 and HRs in the prediction of OS. However, at stage III, only HRs had a predictive value for OS, either in patients with tumors expressing lower levels of CD105 (p=0.018) or in patients with tumors expressing higher levels of CD105 (p=0.034; Fig. 4).

Discussion

Breast cancer is at present the most common cancer in women in Taiwan and its incidence is growing. Although TNM status is the first consideration when selecting treatment for BC, in this era of high-throughput methods, there are numerous novel biomarkers which have been reported for prognostic and predictive purposes. Among these, the most commonly used biomarkers are HRs and Her-2, which categorize BCs into luminal A, luminal B, Her-2 type and basal-type classifica-

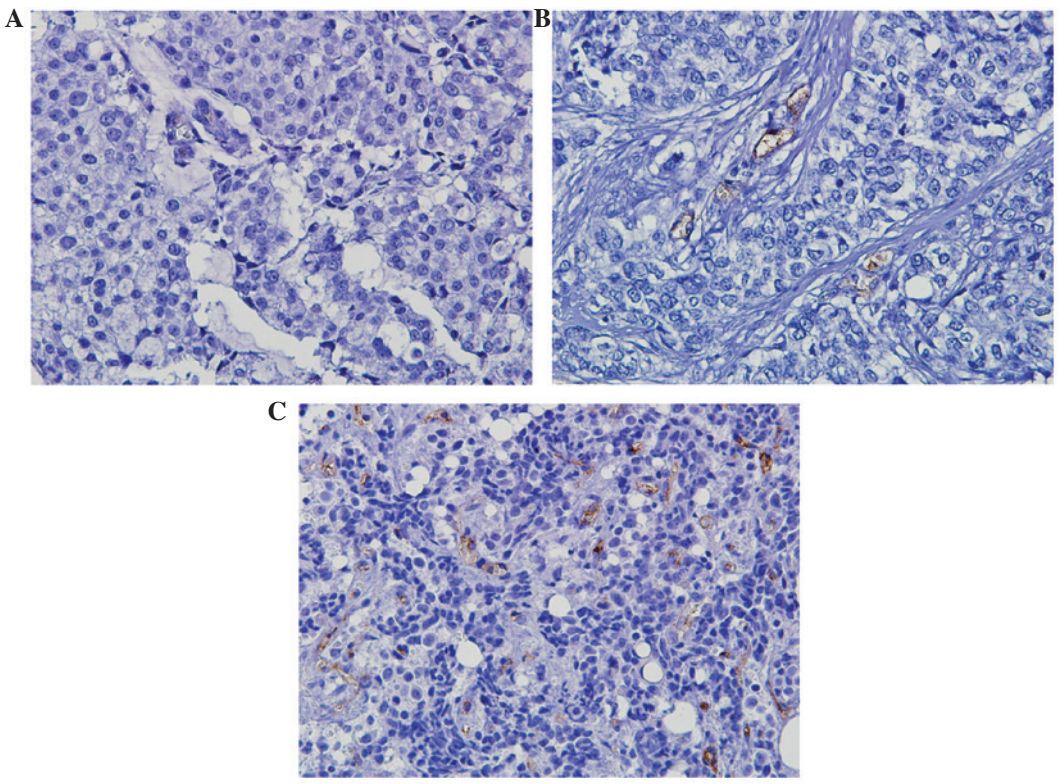

Figure 1. Scoring and illustrations of the expression of CD105. Positive CD105 was observed as thin, linear deposits in the membrane and cytoplasm of endothelial cells within the microvessels. The mean values of CD105 staining in (A-C) are 0, 13 and 43, respectively (x400).

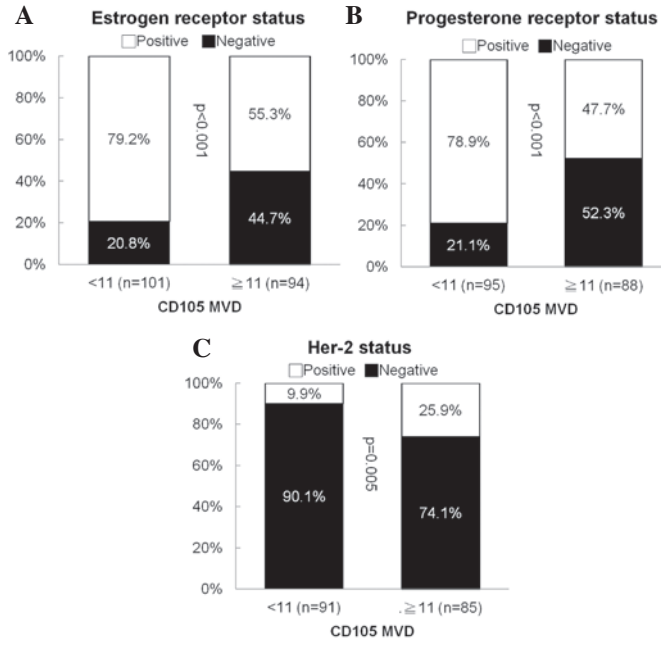

Figure 2. Correlation analysis between pathological variables and CD105. CD105 correlated (A) inversely with estrogen receptor expression ($p<0.001$), (B) inversely with progesterone receptor expression ($p<0.001$) and (C) positively with Her-2 expression ($p=0.005$). MVD, microvascular density.

tions (21); such classifications are also relevant in prognosis. The identification of new markers has led to a more definitive insight into tumor biology and substantiates the importance of existing biomarkers.

There are several drugs that target molecules in various oncogenic pathways. These include trastuzumab and lapatinib which target Her-2, RAD-001 which targets mTOR and bevacizumab which targets VEGF. The development of anti-Her-2 agents has improved the poor prognosis for Her-2-positive BC. In order to maximize the therapeutic effects of the anti-Her-2 agents, only Her-2-positive BCs are indicated for these drugs. In contrast, anti-angiogenic agents have been reported to be more effective in the treatment of triple-negative BC (22).

Angiogenesis is critical for the proliferation, growth and metastasis of cancer cells. Therefore, by inhibiting angiogenesis, these processes are also inhibited. Currently there are several agents that target the neovascularization pathway, but none of them has a predictive marker. Even elevated VEGF levels in the circulations of cancer patients may not be a relevant biomarker (23). Although several types of malignancies are hypervascular (24,25), it is not known how significant the angiogenesis pathway is to these tumors. High MVD alone is not a predictive marker for anti-angiogenic agents since anti-angiogenic agents target new vessels in tumors only. As CD105 expression is more specific for areas of neovascularization, CD105 has the potential to be a predictive marker for anti-angiogenic agents.

HR-positive BCs usually have a more favorable prognosis than HR-negative cases of BC. A primary reason for this is the development of antihormonal agents which can prevent the recurrence and delay the progression of HR-positive BC (26). This reverse correlation also explains the favorable prognosis and slow progression of HR-positive BC. However, the overexpression of Her-2 has been reported to upregulate

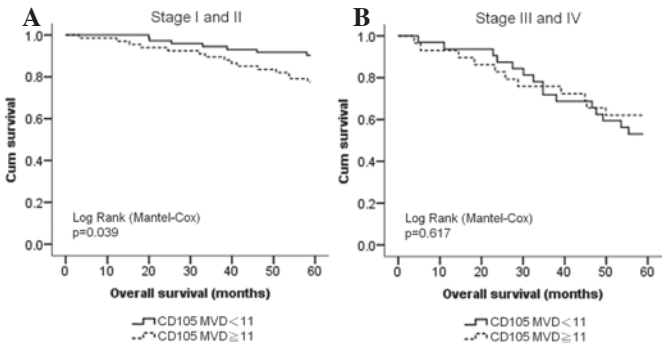

Figure 3. The prognostic effect of CD105 expression in various stages of BC. (A) In stage I and II BCs, lower expression of CD105 correlated with higher overall survival (p=0.039). (B) In stage III and IV BCs, the expression of CD105 did not correlate with OS. MVD, microvascular density; BC, breast cancer.

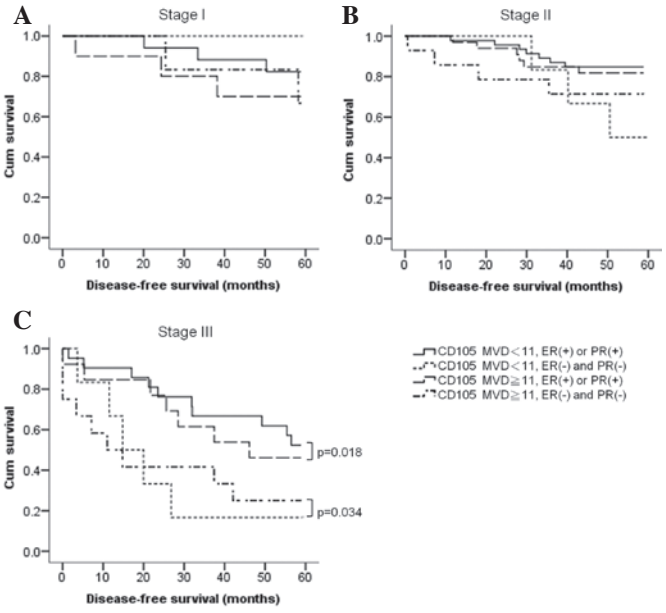

Figure 4. In multivariate analysis, only hormone receptors (HRs) were of prognostic value for the overall survival of (C) stage III patients. For patients with (A and B) early stage cancers, both CD105 and HRs influenced overall survival. MVD, microvascular density; ER, estrogen receptor; PR, progesterone receptor.

VEGF synthesis and thus may increase angiogenesis and metastasis in BC (27). In the current study, we found that the expression of CD105 correlated negatively with HR expression but positively with Her-2 expression, which is compatible with the behavior and prognostic significance of these markers.

Tumorigenesis comprises multiple steps. When a tumor grows to more than 1 mm^3 in size, angiogenesis becomes critical for the tumor to grow and metastasize. However, when the tumor grows beyond this size, the signal transduction pathways become more complicated, with a greater number of overactive pathways which are responsible for tumor growth, survival, angiogenesis, metastasis and drug resistance (28). At an advanced stage, neovascularization may not be the most crucial prognostic factor since there are already several active oncogenic pathways. In clinical practice and animal models, small tumors are more easily controlled by anti-angiogenic agents than larger tumors, which also implies that the role of angiogenesis is less significant when tumors grow larger. In our study, we found that the prognostic effect of MVD was even more considerable when tumors were at their early stages, which might correlate with the aggressiveness of primary tumors and primary resistance to adjuvant therapies.

The poor outcome for the patients with increased MVD as measured by CD105 staining is in line with previously published studies concerning BC and other malignancies (29). Higher MVD correlated with reduced DFS and OS in BC patients, and decreased survival rates in patients with increased CD105 and CD31 counts have also been reported (30).

We report for the first time that MVD measured by CD105 staining is significantly associated with Her-2 molecular subtypes, but is inversely associated with HR expression. In Her-2-overexpressing BC, hormone therapy is less effective than in Her-2-negative BCs, which may be due to cross-talk between HRs and the Her-2 pathway (31,32). Trastuzumab has been proved to be effective on Her-2-overexpressing BC as a monotherapy or as a part of combination therapy with either chemotherapy or endocrine therapy (32), but the reported response rate was less than 70%, and resistance always developed (33). Methods of improving the therapeutic effects of anti-Her-2 agents and preventing the emergence of resistance are necessary.

The targeting of TGF-β signaling leads to the inhibition of angiogenesis and reverses a stem cell phenotype to a more differentiated luminal phenotype (34). These effects are potentially inhibited by a dual blockade of the TGF-β and CD105 pathways (35). Moreover, the anti-CD105 antibody as a single agent as well as CD105 as a target for an oral DNA vaccine have also been reported to be effective agents for suppressing or preventing tumor progression and prolonging survival in *in vitro* models (36-38).

Since overactivity of the Her-2 pathway promotes angiogenesis and neovascularization contributes to the progression and metastasis of Her-2-overexpressing BC, a combination of anti-Her-2 and anti-angiogenic treatments may be feasible This combination may not only increase the therapeutic effect but also delay the emergence of resistance (39). The targeting of tumor neovascularization with vaccines against CD105 in a Her-2-driven BC cell line has been reported (38). In our study, we found that MVD measured by CD105 staining correlated with Her-2 expression, which indicates that combination therapy may be of benefit in the treatment of Her-2-overexpressing BC which also has high CD105 expression.

In conclusion, we found that MVD measured by CD105 staining correlated positively with Her-2 expression, but negatively with HR expression. The significance of MVD on OS is greater for early stage BCs.

Acknowledgements

We thank J.K. Hsiao for his technical support. This study was supported by a grant from the Chang Gung Memorial Hospital (CMRPD890111).

References

1. Jemal A, Siegel R, Xu J and Ward E: Cancer statistics, 2010. CA Cancer J Clin 60: 277-300, 2010.
2. Cianfrocca M and Gradishar W: New molecular classifications of breast cancer. CA Cancer J Clin 59: 303-313, 2009.
3. Folkman J, Watson K, Ingber D and Hanahan D: Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 339: 58-61, 1989.
4. Gasparini G: Clinical significance of the determination of angiogenesis in human breast cancer: update of the biological background and overview of the Vicenza studies. Eur J Cancer 32A: 2485-2493, 1996.
5. Uzzan B, Nicolas P, Cucherat M and Perret GY: Microvessel density as a prognostic factor in women with breast cancer: a systematic review of the literature and meta-analysis. Cancer Res 64: 2941-2955, 2004.
6. Nico B, Benagiano V, Mangieri D, Maruotti N, Vacca A and Ribatti D: Evaluation of microvascular density in tumors: pro and contra. Histol Histopathol 23: 601-607, 2008.
7. Fox SB, Gasparini G and Harris AL: Angiogenesis: pathological, prognostic, and growth-factor pathways and their link to trial design and anticancer drugs. Lancet Oncol 2: 278-289, 2001.
8. Woodfin A, Voisin MB and Nourshargh S: PECAM-1: a multifunctional molecule in inflammation and vascular biology. Arterioscler Thromb Vasc Biol 27: 2514-2523, 2007.
9. Mineo TC, Ambrogi V, Baldi A, *et al*: Prognostic impact of VEGF, CD31, CD34, and CD105 expression and tumour vessel invasion after radical surgery for IB-IIA non-small cell lung cancer. J Clin Pathol 57: 591-597, 2004.
10. da Silva BB, Lopes-Costa PV, dos Santos AR, *et al*: Comparison of three vascular endothelial markers in the evaluation of microvessel density in breast cancer. Eur J Gynaecol Oncol 30: 285-288, 2009.
11. Tanaka F, Otake Y, Yanagihara K, *et al*: Evaluation of angiogenesis in non-small cell lung cancer: comparison between anti-CD34 antibody and anti-CD105 antibody. Clin Cancer Res 7: 3410-3415, 2001.
12. Fonsatti E, Del Vecchio L, Altomonte M, *et al*: Endoglin: an accessory component of the TGF-beta-binding receptor-complex with diagnostic, prognostic, and bioimmunotherapeutic potential in human malignancies. J Cell Physiol 188: 1-7, 2001.
13. Kumar S, Ghellal A, Li C, *et al*: Breast carcinoma: vascular density determined using CD105 antibody correlates with tumor prognosis. Cancer Res 59: 856-861, 1999.
14. Dallas NA, Samuel S, Xia L, *et al*: Endoglin (CD105): a marker of tumor vasculature and potential target for therapy. Clin Cancer Res 14: 1931-1937, 2008.
15. Yao Y, Kubota T, Takeuchi H and Sato K: Prognostic significance of microvessel density determined by an anti-CD105/endoglin monoclonal antibody in astrocytic tumors: comparison with an anti-CD31 monoclonal antibody. Neuropathology 25: 201-206, 2005.
16. Elliott RL and Blobe GC: Role of transforming growth factor beta in human cancer. J Clin Oncol 23: 2078-2093, 2005.
17. Yamamoto T, Kozawa O, Tanabe K, *et al*: Involvement of p38 MAP kinase in TGF-beta-stimulated VEGF synthesis in aortic smooth muscle cells. J Cell Biochem 82: 591-598, 2001.
18. Li C, Hampson IN, Hampson L, Kumar P, Bernabeu C and Kumar S: CD105 antagonizes the inhibitory signaling of transforming growth factor beta1 on human vascular endothelial cells. FASEB J 14: 55-64, 2000.
19. Seon BK, Haba A, Matsuno F, *et al*: Endoglin-targeted cancer therapy. Curr Drug Deliv 8: 135-143, 2011.
20. Marinho A, Soares R, Ferro J, Lacerda M and Schmitt FC: Angiogenesis in breast cancer is related to age but not to other prognostic parameters. Pathol Res Pract 193: 267-273, 1997.
21. Sørlie T, Perou CM, Tibshirani R, *et al*: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 98: 10869-10874, 2001.
22. Liedtke C and Kiesel L: Current issues of targeted therapy in metastatic triple-negative breast cancer. Breast Care (Basel) 6: 234-239, 2011.
23. Niers TM, Richel DJ, Meijers JC and Schlingemann RO: Vascular endothelial growth factor in the circulation in cancer patients may not be a relevant biomarker. PLoS One 6: e19873, 2011.
24. Pang R and Poon RT: Angiogenesis and antiangiogenic therapy in hepatocellular carcinoma. Cancer Lett 242: 151-167, 2006.
25. Webber K, Cooper A, Kleiven H, Yip D and Goldstein D: Management of metastatic renal cell carcinoma in the era of targeted therapies. Intern Med J 41: 594-605, 2011.
26. Kesisis G, Kontovinis LF, Gennatas K and Kortsaris AH: Biological markers in breast cancer prognosis and treatment. J BUON 15: 447-454, 2010.
27. Klos KS, Wyszomierski SL, Sun M, *et al*: ErbB2 increases vascular endothelial growth factor protein synthesis via activation of mammalian target of rapamycin/p70S6K leading to increased angiogenesis and spontaneous metastasis of human breast cancer cells. Cancer Res 66: 2028-2037, 2006.
28. Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell 144: 646-674, 2011.
29. Chien CY, Su CY, Hwang CF, *et al*: Clinicopathologic significance of CD105 expression in squamous cell carcinoma of the hypopharynx. Head Neck 28: 441-446, 2006.
30. Dales JP, Garcia S, Andrac L, *et al*: Prognostic significance of angiogenesis evaluated by CD105 expression compared to CD31 in 905 breast carcinomas: Correlation with long-term patient outcome. Int J Oncol 24: 1197-1204, 2004.
31. Rau KM, Kang HY, Cha TL, Miller SA and Hung MC: The mechanisms and managements of hormone-therapy resistance in breast and prostate cancers. Endocr Relat Cancer 12: 511-532, 2005.
32. Davoli A, Hocevar BA and Brown TL: Progression and treatment of HER2-positive breast cancer. Cancer Chemother Pharmacol 65: 611-623, 2010.
33. Roy V and Perez EA: Beyond trastuzumab: small molecule tyrosine kinase inhibitors in HER-2-positive breast cancer. Oncologist 14: 1061-1069, 2009.
34. Nam JS, Terabe M, Mamura M, *et al*: An anti-transforming growth factor beta antibody suppresses metastasis via cooperative effects on multiple cell compartments. Cancer Res 68: 3835-3843, 2007.
35. She X, Matsuno F, Harada N, Tsai H and Seon BK: Synergy between anti-endoglin (CD105) monoclonal antibodies and TGF-beta in suppression of growth of human endothelial cells. Int J Cancer 108: 251-257, 2004.
36. Uneda S, Toi H, Tsujie T, *et al*: Anti-endoglin monoclonal antibodies are effective for suppressing metastasis and the primary tumors by targeting tumor vasculature. Int J Cancer 125: 1446-1453, 2009.
37. Lee SH, Mizutani N, Mizutani M, *et al*: Endoglin (CD105) is a target for an oral DNA vaccine against breast cancer. Cancer Immunol Immunother 55: 1565-1574, 2006.
38. Wood LM, Pan ZK, Guirnalda P, Tsai P, Seavey M and Paterson Y: Targeting tumor vasculature with novel Listeria-based vaccines directed against CD105. Cancer Immunol Immunother 60: 931-942, 2011.
39. Foy KC, Liu Z, Phillips G, Miller M and Kaumaya PT: Combination treatment with HER-2 and VEGF peptide mimics induces potent anti-tumor and anti-angiogenic responses in vitro and in vivo. J Biol Chem 286: 13626-13637, 2011.