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Expression of CD105 Antigen in Patients with Acute Leukemia, Malignant Lymphoma, and Multiple Myeloma in Active Phase of the Disease

Wysoka ekspresja antygenu CD105 w aktywnej fazie choroby u chorych na ostre białaczki, chłoniaki złośliwe i szpiczaki

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Abstract

Background. CD105 (endoglin) is a proliferation-associated protein abundantly expressed in angiogenic endothelial cells. CD105 is a receptor for TGF (transforming growth factor)-β1 and -β3. It is important for the development of normal vascular architecture and may be associated with tumor angiogenesis. CD105 was strongly expressed in the endothelium of various tumor tissues compared with normal tissues and has been shown to be a useful marker to identify tumor angiogenesis.
Objectives. Determining the expression of CD105 in blood of patients with acute leukemia, malignant lymphoma and multiple myeloma using the cytofluorimetric method. This should help to evaluate the role of CD105 in the development of hematopoietic malignancies. There are few available data in medical literature on this topic.
Material and Methods. The study group consisted of three subgroups of patients with hematopoietic malignancies (20 with acute leukemia, 21 with malignant lymphoma, and 20 with multiple myeloma) and a control group of 30 healthy people. Blood samples were obtained from consecutive patients admitted to the clinic with the newly diagnosed disease before cytostatic treatment was introduced. Cells were prepared, stained with monoclonal antibodies, and analyzed by flow cytometry.
Results. High expression of CD105 was present in patients with ALL (acute lymphoblastic leukemia) and AML (acute myelogenous leukemia) and was significantly higher than in the control group. In the patients with LM (lymphoma malignum) and MM (multiple myeloma) the mean expression was higher than in healthy individuals, but not statistically significantly. No statistically significant correlations were observed between CD105 expression and age, gender, LDH concentration, chromosomal aberrations typical for patients with acute leukemia, or the number of blasts. In blood of patients with acute leukemia, CD105 expression was present on leukemic blast cells. In patients with LM an MM, CD105-positive cells were identified mainly within the population of neoplastic lymphocytes.
Conclusions. These results may suggest a connection between CD105 expression and the pathogenesis of blood neoplasms. The finding of a very high endoglin expression especially in patients with ALL was limited to a small group of patients and needs to be confirmed in a larger group. The mechanisms underlying the role of CD105 action in hematological malignancies still need to be fully elucidated (**Adv Clin Exp Med 2006, 15, 6, 1023–1028**).

Key words: CD105, acute leukemia, malignant lymphoma, multiple myeloma.

Streszczenie

Wprowadzenie. CD105 jest białkiem związanym z proliferacją, które ulega obficie ekspresji na powierzchni komórek śródbłonka. CD105 jest elementem receptora wiążącego się z TGF β-1 i 3 (*transforming growth factor beta*). Endoglina bierze udział w rozwoju prawidłowego unaczynienia i może być związana z angiogenezą nowotworową. Wykazywano wysoką ekspresję CD105 w porównaniu ze zdrowymi tkankami w śródbłonku pochodzącym z unaczynienia różnych typów nowotworów i antygen ten okazał się użyteczny jako marker angiogenezy nowotworowej.

Cel pracy. Oznaczenie ekspresji CD 105 we krwi chorych na ostre białaczki, chłoniaki złośliwe i szpiczaka mnogiego z zastosowaniem metody cytofluorymetrii przepływowej i podkreślenie roli CD105 w powstawaniu nowotworów krwi.

Materiał i metody. Grupa badana składała się z trzech podgrup chorych na nowotwory krwi: 20 chorych na ostrą białaczkę, 21 chorych na chłoniaka złośliwego, 20 chorych na szpiczaka mnogiego i z grupy kontrolnej obejmującej 30 zdrowych ludzi. Próbki krwi pobierano od kolejnych pacjentów przyjmowanych do Kliniki z nowo rozpoznaną chorobą, przed włączeniem leczenia cytostatycznego. Po przygotowaniu do zawiesiny komórek dodano przeciwciała monoklonalne, a następnie analizowano za pomocą cytofluorymetrii przepływowej.

Wyniki. U chorych na ALL (*acute lymphoblastic leukemia*) i AML (*acute myelogenous leukemia*) obserwowano wysoką ekspresję CD105 i była ona istotnie statystycznie wyższa niż u osób zdrowych. We krwi chorych na LM (*lymphoma malignum*) i MM (*myeloma multiplex*) stwierdzono wyższą ekspresję niż u osób zdrowych, ale nie była to różnica istotna statystycznie. Nie wykazano istotnych zależności między ekspresją CD105 a wiekiem, płcią, stężeniem LDH, obecnością typowych zmian chromosomalnych chromosomalnych chorych na ostre białaczki lub liczbą blastów. We krwi chorych na ostre białaczki ekspresja CD105 była obecna na komórkach białaczkowych. U chorych na LM i MM komórki CD105-dodatnie znajdowały się głównie w populacji limfocytów nowotworowych.

Wnioski. Wyniki badań mogą sugerować istnienie związku między ekspresją CD105 a patogenezą chorób nowotworowych krwi. Wykazanie dużej ekspresji endogliny u chorych na ALL jest ciekawym faktem, wymagającym jednak potwierdzenia na większej grupie chorych. Mechanizmy, z którymi jest związana rola CD105 w powstawaniu nowotworów krwi wciąż nie są dobrze poznane (**Adv Clin Exp Med 2006, 15, 6, 1023–1028**).

Słowa kluczowe: CD105, ostra białaczka, chłoniak złośliwy, szpiczak mnogi.

CD105 (endoglin) is a proliferation-associated and hypoxia-inducible transmembrane protein abundantly expressed in angiogenic endothelial cells. It is a receptor for transforming growth factors (TGF)- β1 and -β3. The human CD105 gene is located on chromosome 9q34 [1]. The exact mechanisms for CD105 regulation of vascular development have not been fully elucidated. A variety of important functions of CD105 have been uncovered. most of which are likely to be associated with TGFβ signalling. CD105 suppresses TGFβ signaling in many cells *in vitro* [2]. CD105 expression increases during angiogenesis, wound healing, and inflammation, all of which are associated with TGFβ signalling and alterations in vascular structure. It is also important for normal vascular architecture. CD105 null mice exhibit multiple vascular and cardiac defects, leading to death at early embryonic stages [3]. From embryonic day 9, the primitive vascular plexus of the yolk sac failed to remodel into mature vessels, causing vascular channel dilation, rupture, and hemorrhage; vessel fragility resulted in the internal bleeding. These severe vascular impairments observed in CD105 null mice suggest that CD105 is required for the formation of mature blood vessels in the extra-embryonic vasculature. Failed endocardial cushion formation, essential for valve development and heart septation, and pericardial edema were also noticed in the CD105 null mice, indicating another crucial role of CD105 in cardiac development. CD105 knock-out mice die from malvascularisation by 11.5 day p.c. [4].

The role of CD105 may be associated with tumor angiogenesis. CD105 was strongly expressed in the endothelium of various tumor tissues compared with normal tissues [5, 6]. It has been shown to be a more useful marker in identifing tumor angiogenesis than panendothelial markers such as CD31 [7]. Studies performed in different laboratories using various antibodies to CD105 have revealed CD105 upregulation in a wide range of tumor endothelia, including those within the colon, breast, brain, lung, prostate, and cervical cancer, suggestive of the possible involvement of CD105 in tumor angiogenesis [5, 8]. In one study, concentrations of CD105 measured in plasma samples were significantly increased in patients who developed distant metastasis compared with disease-free patients and with healthy individuals [9].

The aim of this study was to determine the expression of CD105 in the blood of patients with acute leukemia, malignant lymphoma, and myeloma multiplex with the use of the cytofluorymetric method. The studies may help to evaluate the role of CD105 in the development of hematopoietic malignancies, for there are few data available on this topic in medical literature.

Material and Methods

The study group consisted of three subgroups with hematopoietic malignancies and a control group. We investigated blood samples of patients with acute leukemia (n = 20), 14 men and 6 women with an average age of 52.55 ± 17.17 years. In this subgroup, 15 patients were identified as having AML and 5 as having ALL. Twenty-one persons were in the subgroup of patients with LM: 14 men and 7 women (mean age 52.0 ± 14.71 years old). The characteristics of this subgroup according to the Ann Arbor classification are shown in Table 1. The subgroup of patients with MM (n = 20) was composed of 11 women and 9 men with an average age of 61.8 ± 11.81 years. Staged by Salmon and Durie criteria, 4 patients had stage II A, 9 patients had III A, and 7 patients had III B. The control group consisted of 30 healthy people without any chronic disease (19 men and 11 women, mean age: 54 ± 16.4 years).

In the study subgroups the mean concentration of lactate dehydrogenase LDH (a well-known marker of progression in blood neoplasms) was 1345 ± 218 U/l in the patients with acute leukemia, 856.05 ± 153 U/l in the patients with LM, and 406 ± 63 U/l in the patients with MM. The percentage of blast cells in the bone marrow in patients with acute leukemia was from 20 to 98%, mean 65 ± 6%, and the mean percentage of blast cells in peripheral blood was 58 ± 5%. In patients with grade IV of LM, bone marrow was infiltrated in all cases by neoplastic cells as were liver, spleen, all palpable lymph nodes, and retroperitoneal lymph nodes. In three of the patients, infiltrations in the mediastinum were present and in one in the facial region (septum of the nose and pharyngeal and palatine tonsils).

Table 1. Characteristics of patients with malignant lymphomas according to the Ann Arbor classification

Tabela 1. Charakterystyka chorych na chłoniaki złośliwe chorych według klasyfikacji Ann Arbor

Stage of lymphoma (Stadium chłoniaka)	Number of patients (Liczba chorych) n = 21
IIB	1
IIIA	1
IIIB	2
IVB	17

Fasting blood samples were obtained from consecutive patients admitted to the clinic with newly diagnosed disease before cytostatic treatment had been introduced. In each patient, puncture of a peripheral vein was carried out and 10 cm^3 of blood was collected. Preparations of samples for flow cytometric analysis were performed by centrifugation, then the cell suspensions were stained for the surface expressions of various markers using FITC-(green), Cyanine 5 (RPE-Cy5, red) fluorochrome, and phycoerythrin (PE, orange) conjugated with mAbs (monoclonal antibodies). R-phycoerythrin-Cyanine5 (RPE--Cy5)-conjugated anti-CD45 mAb (antibody against CD45 leukocyte common antigen) was purchased from Dako (Glostrup, Denmark). Monoclonal antibody against CD105 FITC was obtained from Ancell (Bayport, MN, USA). Mouse IgG1 FITC/PE/RPE-Cy5 negative control mAb (DAKO, Glostrup, Denmark) was used as a negative control. In blood samples from patients with acute leukemia, cells were also stained with CD34 RPE (DAKO, Glostrup, Denmark) antibody, routinely used in diagnosing acute leukemias.

The cell suspensions were incubated with the monoclonal antibodies for 45 minutes on ice with 80 µl of anti-CD105/FITC at a 1:50 dilution (10 mg/ml). The cells were then washed three times and analyzed using a FACS (Partec, Germany). For analysis, the cells were gated on the basis of forward and side scatter. Cells stained positive were compared with the Mouse IgG1 negative control at a similar concentration. Cells were shown as dots in separate quadrants and charts as a percentage of the gated cells.

Statistical analysis was performed with the commercially available package STATISTICA, version 6.0. The results were presented as a mean (X) ± one standard deviation (SD). The distribution of data was examined using the Shapiro-Wilk test. Differences between the analyzed parameters were examined using the non-parametric ANOVA Kruskal-Wallis test. Correlations between the variables were tested using the Spearman correlation coefficient r. For the determination of survival analysis, the Kaplan-Meier estimator was used. A p value less than 0.05 was considered statistically significant.

Results

High expression of CD105 was present in patients with ALL and AML (mean ± SD: 36.2 ± ± 27.6% and 17.8 ± 11.3%, respectively). The expression of CD105 in the group with ALL and AML was significantly higher than in the control group (1.15 ± 0.45%; p = 0.002 for ALL and p = = 0.4 for AML). In LM and MM patients the mean expressions were 6.35 ± 4.31% and 4.03 ± 2.03%. These results were not statistically significant in

Table 2. CD105 expression in the study subgroups

Tabela 2. Ekspresja CD105 w badanych podgrupach chorych

Disease (Choroba)	CD105 expression (Ekspresja CD105) %
AML	36.2 ± 27.6
ALL	17.8 ± 11.3
LM	6.35 ± 4.31
MM	4.03 ± 2.03

comparison with healthy individuals (Table 2). In patients with ALL and high CD105 expression, a high percentage of blasts in bone marrow (93–97%) was observed. These patients were found to be in poor clinical condition, with high levels of lactate dehydrogenase and the extremely increased levels of D-dimer. However, no statistically significant correlations were observed between CD105 expression and age, gender, LDH concentration, typical chromosome aberrations, or blast cells in bone marrow or peripheral blood for patients with acute leukemia. In patients with AML, CD105 expression was diverse and ranged from several to 30% (Fig. 1).

There were no statistically significant differences in CD105 expression between patients with higher (above median) and lower (below median) concentrations of LDH in any of the studied subgroups (Fig. 2). No correlations were found between advanced stage of disease in patients with LM (IV) and increased CD105 expression. However, the relatively small number of patients and

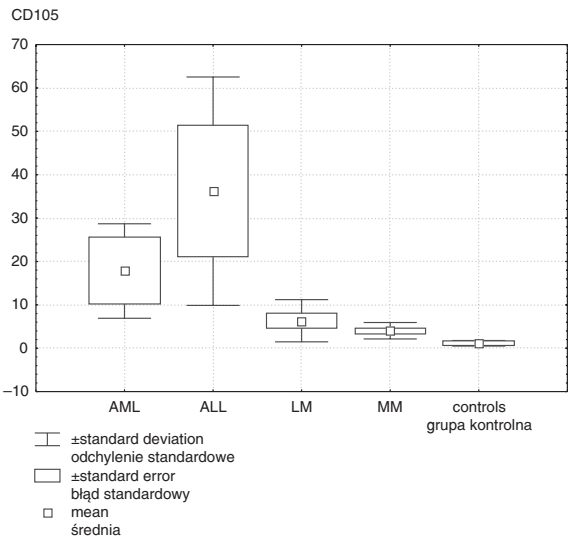

Fig. 1. Comparison of CD105 expressions in patients with acute leukemia, malignant lymphoma, myeloma multiplex, and the control group

Ryc. 1. Porównanie ekspresji CD105 u chorych na ostrą białaczkę, chłoniaka złośliwego, szpiczaka mnogiego i w grupie kontrolnej

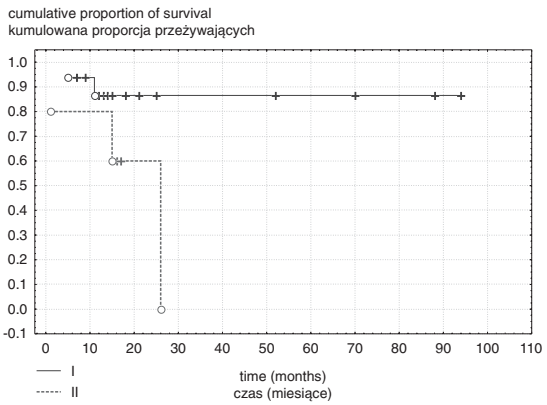

Fig. 2. Kaplan-Meier survival analysis according to the median expression of CD105 (I – below median, II – above median) in patients with lymphoma malignum ($p = 0.025$)

Ryc. 2. Krzywe przeżycia Kaplana-Meiera w zależności od mediany ekspresji CD105 (I – poniżej mediany, II – powyżej mediany) u chorych na chłoniaka złośliwego (p = 0,025)

the heterogeneous character of the subgroup could make statistical analysis in this matter inaccurate.

In the group of patients with LM, a significant correlation between the expression of CD105 and fibrinogen concentration was noted ($r = 0.81$, $p < 0.05$). In this group, a negative correlation between CD105 expression and hemoglobin concentration was also present ($r = –0.61$, $p < 0.05$).

Analysis with two-color flow cytometry enabled determining in which part of CD45-positive cells (a marker characteristic for leukocytes) CD105 was co-expressed. In the blood of patients with acute leukemia the expression of CD105 was present on leukemic blast cells. There was no statistically significant correlation between a high (above median) percentage of blast cells and high expression of CD105 antigen in these patients.

In the subgroups of patients with LM an MM, CD105-positive cells were identified mainly within the population of neoplastic lymphocytes.

In a group with acute leukemia, staining with marker CD34, typically used for the routine diagnosis of AML and ALL, revealed a high co-expression with CD105 in patients with ALL, ranging from 25 to 43% (Fig. 3).

The highest statistically significant rate of mortality was present in patients with LM and a high level of CD105 (above median) in comparison with patients with lower expression ($p < 0.02$).

Discussion

In these studies it was revealed that the expression of CD105, a well-known marker of neopastic angiogenesis, is relatively high in patients with

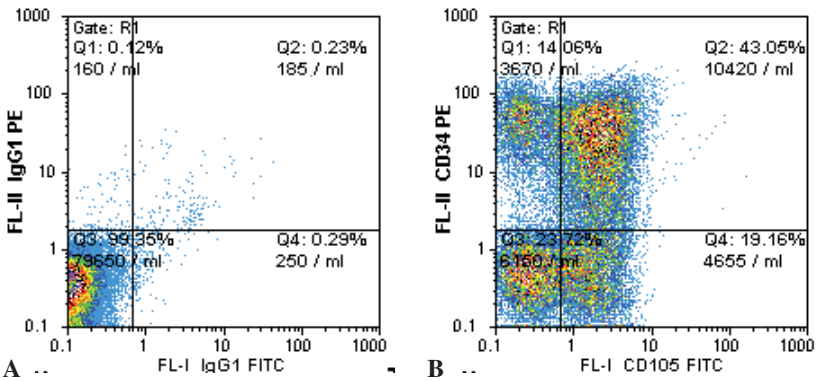

Fig. 3. CD105 expression by flow cytometric analysis in a patient with ALL (A – negative control, B – co-expression of CD105 and CD34)

Ryc. 3. Ekspresja CD105 w badaniu na cytofluorymetrze przepływowym u chorych na ALL (A – kontrola ujemna, B – koekspresja CD105 i CD34)

acute leukemia, especially those with ALL. Moderately high expressions were also noted in patients with malignant lymphomas and multiple myeloma, although these were not statistically significant. The results correspond with data presented by Calabro and colleagues [10], who found increased serum levels of CD105 in patients with acute leukemia and also those with CMD (chronic myeloproliferative disorders). However, the present experiments enabled the measurement of CD105 not as a soluble molecule in serum or plasma, but as a molecule directly expressed on the surface of CD45-positive cells (leukocytes). The fact that the highest expression of CD105 was found in the population of blast cells suggests a connection with the pathogenesis of the disease. Unfortunately, the interesting finding of a very high CD105 expression in patients with ALL was limited to a small group of five individuals. In the medical literature no reports about the expression of CD105 in ALL were found. These interesting data need to be confirmed in larger groups of patients. The blood samples were collected from consecutive patients with acute leukemia, so the number of patients with ALL was random.

Endoglin is a prognostic marker in patients with prostate cancer [11]. High levels of soluble sCD105 were found in women with breast cancer compared with healthy people, especially in patients with metastases [9] and those with cancer of the large intestine [12]. In the studies of Pruneri et al., anti-CD105 monoclonal antibody was very sensitive in the determination of increased angiogenesis in bone marrow samples from patients with myeloma multiplex [13]. The same authors reported increased CD105 expression in bone marrow samples with hairy cell leukemia, one of the types of lymphoma [14].

There are only a few reports supporting some connections between CD105 and the development of hematolological malignancies and the mechanisms of it. It is known that CD105 antagonizes the inhibitory effect of TGFβ1 on human and murine endothelial cells [15]. TGFβ is a cytokine with an inhibitory effect on lymphoma cells in animal studies [16]. It is possible that the complex system of cytokines and their interactions with CD105 have an important influence on lymphoma development. Miller et al. revealed connections between the expression of endoglin and proliferation markers such as cyclin A and Ki-67 protein in lung cancer, which suggests an influence on the cell cycle [17]. Preliminary studies done by Guo et al. showed that the role of CD105 is not limited to being a receptor for TGFβ, but also includes participation in adhesion, migration, and survival of cells [18]. TGFβ inhibits EC proliferation, migration, and the formation of microvessels, whereas CD105 counteracts these actions, thereby promoting angiogenesis.

CD105 is a promising target that can be used for tumor imaging and prognosis and it possesses a therapeutic potential in patients with solid tumors and other neoplastic diseases with increased angiogenesis. Increased CD105 levels may be useful as an indicator of disease progression and to identify patients at risk of recurrence and/or metastasis, especially in women with breast cancer. Additionally, endoglin could serve to monitor the effects of treatment, because the level of CD105 decreased after effective treatment in patients with neoplastic diseases [3, 9].

CD105 represents an ideal target for antiangiogenic therapy and a good marker for tumor prognosis. However, the mechanisms underlying the pro-angiogenic action of CD105 have not been fully elucidated. Studies have demonstrated long-lasting complete abrogation of human breast tumors in SCID mice using CD105 mab (anti-CD105 antibodies) that has been conjugated with immunotoxins [19] and growth suppression of human solid tumors using a radio-labeled mab to CD105 [12]. Endoglin is not a fully specific marker of neoplastic vascularity [20]. Slight amounts of CD105 were identified within normal vessels and in stroma. In future, the use of monoclonal antibodies should be thoroughly considered. The results of this study support the important role of CD105 in angiogenesis and in tumor progression.

References

[1] Fernandez-Ruiz E, St.-Jacques S, Bellon T, Letarte M, Bernabeu C: Assignment of the human endoglin gene (END) to 9q34-qter. Cytogenet Cell Genet 1993, 64, 204–207.

[2] Li C, Hampson I, Hampson L, Kumar P, Bernabéu C, Kumar S: CD105 antagonizes the inhibitory signaling of transforming growth factor β1 on human vascular endothelial cells. FASEB J 2000, 15, 55–64.

[3] Arthur H M, Ure J, Smith A, Renforth G, Wilson D, Torsney E, Charlton R, Parums D, Jowett T, Marchuk D et al.: Endoglin, an ancillary TGFβ receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. Dev Biol 2000, 5, 42–53.

[4] Li D, Sorensen L, Brooke B, Urness L, Davis E, Taylor D, Boak B, Wendel D: Defective angiogenesis in mice lacking endoglin. Science 1999, 284,1534–1537.

[5] Burrows F, Derbyshire E, Tazzari, P, Amlot P, Gazdar A, King S, Letarte M, Vitetta E, Thorpe P: Up-regulation of endoglin on vascular endothelial cells in human solid tumors: implications for diagnosis and therapy. Clin Cancer Res 1995, 1, 1623–1634.

[6] Wang J, Kumar S, Pye D, van Agthoven A, Krupinski J, Hunter R: A monoclonal antibody detects heterogeneity in vascular endothelium of tumours and normal tissues. Int J Cancer 1993, 54, 363–370.

[7] Saad R, El-Gohary Y, Memari E, Liu Y, Silverman J: Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in esophageal adenocarcinoma. Hum Pathol 2005, 36, 955–961.

[8] Bodey B, Bodey B, Siegel S, Kaiser H: Overexpression of endoglin (CD105): a marker of breast carcinoma-induced neo-vascularization. Anticancer Res 1998, 18, 3621–3628.

[9] Li C, Guo B, Wilson P, Stewart A, Byrne G, Bundred N, Kumar S: Plasma levels of soluble CD105 correlate with metastasis in patients with breast cancer. Int J Cancer 2000, 89, 122–126.

[10] Calabro L, Fonsatti E, Bellomo G, Alonci A, Colizzi F, Sigalotti L, Altomonte M, Musolino C, Maio M: Differential levels of soluble endoglin (CD105) in myeloid malignancies. J Cell Physiol 2003, 194, 171–175.

[11] Wilkstrom P, Lissbrant I, Stattin P, Egevad L, Bergh A: Endoglin (CD105) is expressed on immature blood vessels and is a marker for survival in prostate cancer. Prostate 2002, 268–275.

[12] Takahashi N, Kawanishi-Tabata R, Haba A, Tabata M, Haruta Y, Tsai H Seon, B: Association of serum endoglin with metastasis in patients with colorectal, breast, and other solid tumors, and suppressive effect of chemotherapy on the serum endoglin. Clin Cancer Res 2001, 7, 524–532.

[13] Pruneri G, Ponzoni M, Ferreri AJ, Decarli N, Tresoldi M, Raggi F, Baldessari C, Freschi M, Baldini L, Goldaniga M, Neri A, Carboni N, Bertolini F, Viale G: Microvessel density, a surrogate marker of angiogenesis, is significantly related to survival in multiple myeloma patients. Br J Haematol 2002, 118, 817–820.

[14] Pruneri G, Bertolini F, Baldini L, Valentini S, Goldaniga M, Soligo D, Carboni N, Viale G, Lambertenghi-Deliliers G: Angiogenesis occurs in hairy cell leukaemia (HCL) and in NOD/SCID mice transplanted with the HCL line Bonna-12. Br J Haematol 2003, 120, 695–698.

[15] Warrington K, Hillarby MC, Li C, Letarte M, Kumar S: Functional role of CD105 in TGF-beta1 signalling in murine and human endothelial cells. Anticancer Res 2005, 25, 1851–1864.

[16] Buske C, Hannig H, Schneider EM, Blaschke S, Hunsmann G, Bodemer W, Hiddemann W: Transforming growth factor beta is a growth-inhibitory cytokine of B cell lymphoma in SIV-infected macaques. AIDS Res Hum Retroviruses 1999, 15, 1477–1485.

[17] Miller DW, Graulich W, Karges B, Stahl S, Ernst M, Ramaswamy A, Sedlacek H, Muller R, Adamkiewicz J: Elevated expression of endoglin, a component of the TGF-beta-receptor complex, correlates with proliferation of tumor endothelial cells. Int J Cancer 1999, 81, 568–572.

[18] Guo B, Rooney P, Slevin M, Li C, Parameshwar S, Liu D, Kumar P, Bernabeu C, Kumar S: Overexpression of CD105 in rat myoblasts: role of CD105 in cell attachment, spreading and survival. Int J Oncol 2004, 2, 285–291.

[19] Matsuno F, Haruta Y, Kondo M, Tsai H, Barcos M, Seon B: Induction of lasting complete regression of preformed distinct solid tumors by targeting the tumor vasculature using two new anti-endoglin monoclonal antibodies. Clin Cancer Res 1999, 5, 371–382.

[20] Balza E, Castellani P, Zulstra A, Neri D, Zardi L, Siri A: Lack of specificity of endoglin expression for tumor blood vessels. Int J Cancer 2001, 94,579–585.

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