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Original Article

# Endoglin (CD105) is a more appropriate marker than CD31 for detecting microvessels in carotid artery plaques

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## Abstract

**Background:** Microvascular proliferation is a major risk factor for plaque vulnerability in patients with carotid stenosis. There are several vascular endothelial markers such as CD31 and CD105, but it is unclear which marker is most sensitive for microvessels. This study sought to examine the correlations between CD31 and CD105 expression in microvessels on carotid plaques and clinical manifestations.

**Methods:** We studied 13 lesions in 12 patients. The patients underwent carotid endarterectomy and samples were stained for CD31 and CD105. The numbers of microvessels positive for these markers within a field of view were counted.

**Results:** The average numbers of microvessels were 5.8 ± 5.4 for CD31 and 9.2 ± 9.3 for CD105 ($P = 0.04$). More microvessels were positive for CD105 than there were for CD31 in patients with diabetes mellitus ($P = 0.04$).

**Conclusion:** In patients with carotid artery stenosis, CD105 is more appropriate than CD31 for detecting microvessels in carotid plaques. In patients with diabetes mellitus, CD105 is significantly more highly expressed in microvessels than CD31.

**Key Words:** Carotid stenosis, CD31, endoglin, microvessels

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## INTRODUCTION

Several studies such as the North American Symptomatic Carotid Endarterectomy Trial (NASCET)[4] and the European Carotid Surgery Trial (ECST)[24] have demonstrated that the rate of stenosis is related to the risk of cerebral infarction. However, recent vascular biology studies have indicated that the stability of carotid plaques is correlated with the risk of cerebral infarction.[3,5] Intraplaque hemorrhage makes carotid plaques unstable and is the one of the minor criteria for vulnerable plaques.[19] Intraplaque hemorrhage is usually followed by neovascularization extending from the vasa vasorum of carotid artery.[22]

CD31 is a marker of vascular endotherial cells and is expressed on microvessels in atherosclerotic plaques, but is stained more weakly than normal arteries.[13] CD105 (endoglin) is a marker of tumor microvessels and is associated with tumor progression.[7]

The aim of this study was to compare the expression of CD31 and CD105 in microvessels of carotid artery plaques and to assess the relationships between their expression levels and clinical characteristics.

## MATERIALS AND METHODS

### Patient population

Between November 2009 and March 2011, we performed 13 carotid endarterectomy operations in 12 patients at Fukui Red Cross Hospital. One patient was operated on bilaterally at different times. Digital subtraction angiography was performed for all patients, and the stenosis rate was evaluated according to NASCET criteria.[4]

### Clinical features

Demographic and clinical characteristics were collected from the patients' medical records. Clinical information included symptoms, the presence of risk factors for atherosclerosis (e.g. hypertension, hyperlipidemia, and diabetes mellitus), and the stenosis rate evaluated by digital subtraction angiography.

### Sample preparation

Carotid endarterectomy was performed using conventional surgical techniques. All specimens were fixed in 10% formalin overnight and embedded in paraffin the next day. The specimens were stored at room temperature. In each case, multiple and sequential 3-μm tissue sections were cut from paraffin blocks, deparaffinized in xylene, rehydrated, and prepared for immunohistochemical studies.

### Immunohistochemistry

Endogenous peroxidase activity in the tissue was blocked by incubation in 3% hydrogen peroxide for 15 min at room temperature. Antigen retrieval was performed by autoclaving in citrate buffer heated to 121°C for 5 min. To reduce nonspecific binding of the secondary antibody, tissue sections were incubated for 10 min with a protein blocking agent. Slides with arterial specimens were then incubated with the specified dilution of primary antibody overnight at 4°C. The anti-CD31 (Novocastra) and anti-CD105 (Novocastra) primary antibodies were mouse monoclonal antibodies and were used at a 1:500 dilution. Sections were incubated in biotinylated goat antimouse (DAKO) secondary antibody at room temperature for 10 min. Horseradish-peroxidase-conjugated streptavidin (DAKO) was applied to sections for 10 min at room temperature. Reacted sections were visualized with 3,3′-diaminobenzidine-tetrachloride (DAB), and counterstained with Meyer's hematoxylin for nuclear staining.

### Immunohistochemical analysis

We counted microvessels using a BX51 microscope (Olympus). After scanning an immunostained section at low magnification (×40), the areas with the greatest number of distinctly highlighted microvessels stained with anti-CD31 were selected, and microvessels were counted at higher power (×100). Microvessels stained with anti-CD105 were counted in an adjacent slice stained with anti-CD31. Vessels were confirmed as microvessels if more than half of the endothelial cells were positive for the marker used. Microvessels were identified based on the architecture, including a lumen lined by endothelial cells as confirmed by staining of an adjacent slice with hematoxylin–eosin.

### Statistical analysis

Numerical data are expressed as means ± SD. The statistical significance of differences was estimated using the Wilcoxon signed rank test or Fisher's exact test. Values of $P < 0.05$ were considered significant. All data were analyzed statistically using R version 2.15.2 for Windows.

## RESULTS

The mean age of the patients at the time of operation was 69.6 ± 7.0 years, and all patients were male. The features of onset were cerebral infarction in four patients, transient ischemic attack in two patients, and amaurosis fugax in one patient. Six patients were asymptomatic. The average stenosis rate was 78 ± 14% (50–95%) [Table 1].

The average numbers of microvessels counted in higher power fields were 5.8 ± 5.4 for CD31 and 9.2 ± 9.3 for CD105 ($P = 0.04$) [Figure 1].

Atherosclerosis risk factors and clinical manifestations for the entire group are shown in Table 2. Ten patients had hypertension and seven patients had diabetes mellitus. All of these patients received appropriate medical treatment [Table 2].

Atherosclerosis risk factors and the numbers of microvessels positive for CD31 and CD105 are shown in Table 3. In the patients with diabetes mellitus, significantly more microvessels were stained with CD105

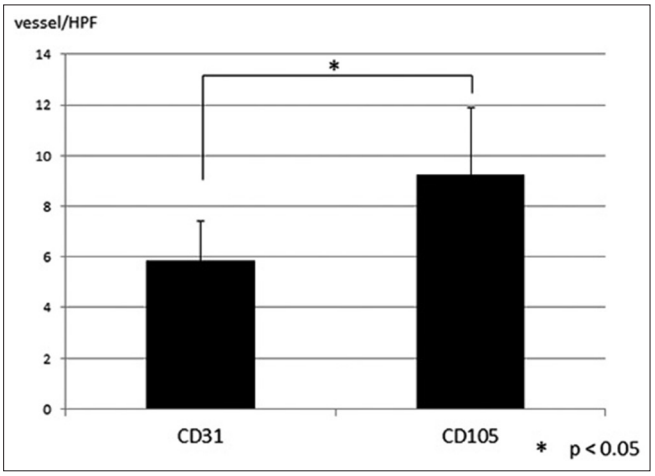


**Figure 1: The average numbers of microvessels counted in higher power fields of sections stained for CD31 and CD105. Significantly more microvessels were detected when sections were stained for CD105. HPF: High-power field, CD: Cluster of differentiation**

**Table 1: Clinical and radiological findings and the number of microvessels (vessels/HPF) stained with CD31 or CD105**

| Patients | Age (years), gender | Risk factors for atherosclerosis | Clinical manifestations | Stenosis (%) | CD31 (vessels/HPF) | CD105 (vessels/HPF) |
|---|---|---|---|---|---|---|
| 1 | 78, M | Hypertension, diabetes mellitus | Cerebral infarction | 65 | 18 | 31 |
| 2 | 72, M | Hypertension, hyperlipidemia | Cerebral infarction | 90 | 5 | 6 |
| 3 | 81, M | Diabetes mellitus | Transient ischemic attack | 95 | 16 | 28 |
| 4 | 57, M | Hypertension, diabetes mellitus | Cerebral infarction | 55 | 1 | 6 |
| 5 | 61, M | Hypertension, hyperlipidemia | Amaurosis fugax | 50 | 0 | 3 |
| 6 | 73, M | None | Cerebral infarction | 70 | 7 | 8 |
| 7 | 61, M | Hypertension, hyperlipidemia, diabetes mellitus | Asymptom | 80 | 8 | 12 |
| 8 | 73, M | Hypertension, diabetes mellitus | Transient ischemic attack | 95 | 6 | 0 |
| 9 | 66, M | Hypertension, diabetes mellitus | Asymptom | 85 | 6 | 5 |
| 10 | 63, M | Hypertension | Asymptom | 85 | 1 | 7 |
| 11 | 72, M | Hypertension | Asymptom | 90 | 0 | 0 |
| 12 | 75, M | None | Asymptom | 80 | 3 | 4 |
| 13 | 73, M | Hypertension, diabetes mellitus | Asymptom | 70 | 5 | 10 |

M: Male, HPF: High-power field, CD: Cluster of differentiation

**Table 2: Summary of clinical cases**

| Clinical or radiological feature | No. (%) |
|---|---|
| Age (years) | 69.6±7.0 |
| Male | 13 (100) |
| Atherosclerosis risk factors | |
| Hypertension | 10 (77) |
| Hyperlipidemia | 3 (23) |
| Diabetes mellitus | 7 (54) |
| Clinical manifestations | |
| Cerebral infarction | 4 (31) |
| Transient ischemic attack | 2 (15) |
| Amarousis fugax | 1 (7) |
| Asymptom | 6 (46) |
| Stenosis (%) | 77±14 |

**Table 3: Clinical data and microvessels (vessels/HPF) stained with CD31 or CD105**

| | CD31 | CD105 | *P* value |
|---|---|---|---|
| Symptom | | | |
| Yes | 7.6±6.4 | 11.7±11.5 | 0.13 |
| No | 3.8±2.8 | 6.3±3.9 | 0.10 |
| Hypertension | | | |
| Yes | 5.0±5.1 | 8.0±8.5 | 0.11 |
| No | 8.7±5.4 | 13.3±10.5 | 0.10 |
| Hyperlipidemia | | | |
| Yes | 4.3±3.3 | 7.0±3.7 | 0.25 |
| No | 6.3±5.8 | 9.9±10.3 | 0.10 |
| Diabetes mellitus | | | |
| Yes | 8.6±5.7 | 13.1±11.0 | 0.18 |
| No | 3.8±2.6 | 4.7±2.7 | 0.04* |

*Significant, HPF: High-power field, CD: Cluster of differentiation

than with CD31. In patients with other atherosclerotic risk factors, such as hypertension or hyperlipidemia, more microvessels were stained with CD105, although the difference was not significant. The number of microvessels did not differ significantly according to symptoms.

### Illustrative case

*Case 1* (*Patient 8*)

A 61-year-old male was incidentally found to have left internal carotid artery stenosis. He had hypertension, hyperlipidemia, and diabetes mellitus. Angiography revealed 80% stenosis. He underwent carotid endarterectomy, and the results of pathology are shown in Figure 2. Compared with CD31, CD105 was more strongly expressed in microvessels.

*Case 2* (*Patient 1*)

A 78-year-old male presented left hemiparesis and magnetic resonance imaging (MRI) demonstrated cerebral infarction in the right hemisphere. He had hypertension and diabetes mellitus, and angiography revealed 65% right carotid artery stenosis. He underwent carotid endarterectomy, and the results of pathology are shown in Figure 3. The microvessels were faintly stained with CD31, but strongly expressed CD105 in the whole circumference.

## DISCUSSION

In this study, we found that CD105 was more strongly expressed in microvessels on carotid plaques than CD31.

CD105 is a transmembrane glucoprotein expressed on activated vascular endothelial cells,[14] and is an accessory protein for the transforming growth factor-β (TGF-β) receptor system.[1] A number of studies have shown that CD105 is expressed on endothelial cells of both mature and immature blood vessels[8,25] and that it is

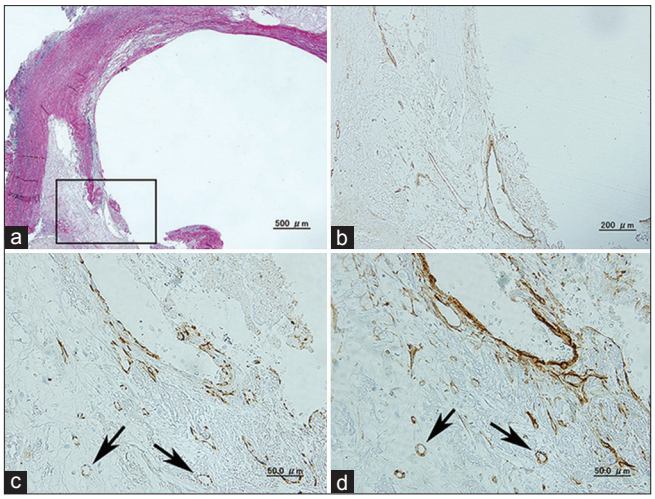


**Figure 2: Histological and immunohistological findings of the carotid artery plaque from patient 8. (a) Elastica von Gieson stain, (b) CD105 stain (×100). (c) CD31 stain (×400). The microvascular endothelial cells are stained faintly. (d) CD105 stain (×400). The microvascular endothelial cells are strongly stained in the whole circumference. The arrows show the microvessels stained negatively for CD31 and positively for CD105**

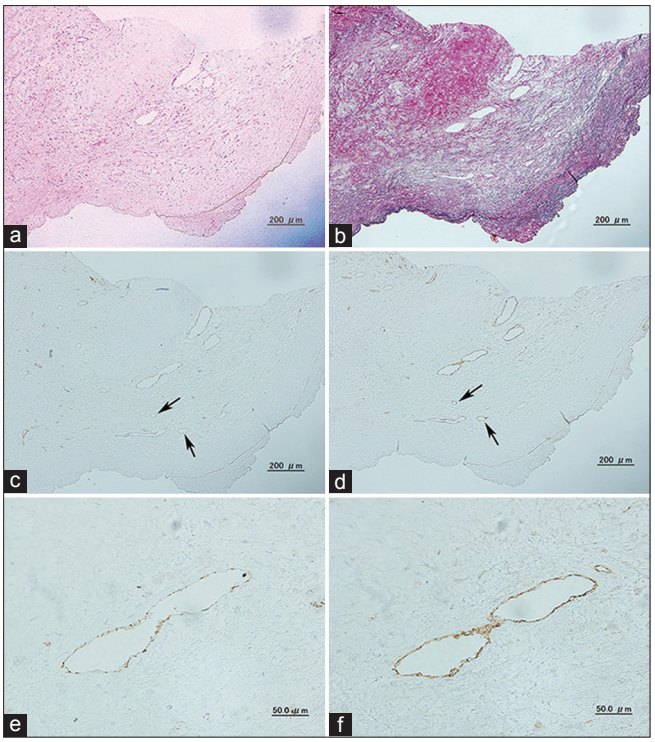


**Figure 3: Histological and immunohistological findings in the carotid artery plaque from patient 1. (a) H and E stain, (b) Elastica von Gieson stain, (c) CD31 stain (×100), (d) CD105 stain (×100), (e) CD31 stain (×400). The microvascular endothelial cells are stained faintly, (f) CD105 stain (×400). The microvascular endothelial cells are strongly stained in the whole circumference. The arrows show the microvessels stained negatively for CD31 and positively for CD105**

overexpressed in vascular endothelial tissues undergoing angiogenesis.[6,17,25] CD105 is the most suitable marker available to quantify tumor angiogenesis,[8,25] and the density of microvessels stained strongly with CD105 correlates with prognosis in cancer patients.[8,11,20,25] In patients with atherosclerosis, CD105 is a better marker than CD31 to assess the vulnerability of plaques on the coronary artery.[15]

The components of carotid artery plaques have a large impact on the risk of cerebral infarction.[3,5] Naghavi *et al.*[19] described some of the criteria of vulnerable plaques. These criteria included a large lipid core, a thin fibrous cap, outward remodeling, and intraplaque hemorrhage. An MRI study showed that carotid artery stenoses with intraplaque hemorrhages were associated with a higher stroke recurrence rate than those without intraplaque hemorrhage.[12]

A pathological study revealed a microvascular network (the vasa vasorum) extending from the adventitia through the media and into the thickened intima, and found that nonatherosclerotic vessels rarely had a vasa vasorum.[10] Intraplaque hemorrhage is believed to arise from the disruption of thin-walled microvessels that are lined by a discontinuous endothelium without supporting smooth-muscle cells.[23] Several studies have suggested that intraplaque hemorrhage and rupture of the fibrous cap are associated with an increased density of microvessels.[2,16,18] Therefore, it is important to find a useful marker for detecting fragile microvessels.

CD105, CD31, and CD34 are all used as vascular endothelial markers. CD31 and CD34 are expressed in various tumor microvessels, but they can react with both normal vessels and activated vessels, whereas CD105 has a greater affinity for activated endothelial cells.[21] In the coronary artery, CD31 is more strongly expressed in vascular endothelial cells of normal arteries than in those of arteries with atherosclerosis.[13] These findings suggest that CD105 is a useful marker to examine genuine fragile vessels on carotid artery plaques.

In the present study, the difference in the numbers of microvessels in patients with hypertension and hyperlipidemia was not significant. The difference was only significant for patients with diabetes mellitus. A recent study indicated that, in diabetes patients, angiogenesis is induced and arteriogenesis is impaired in atherosclerotic plaques,[9] so it is suggested that more immature and fragile microvessels are present on carotid atherosclerotic plaques in patients with diabetes mellitus.

## CONCLUSION

In patients with carotid artery stenosis, CD105 is more suitable than CD31 for detecting microvessels in carotid plaques. In patients with diabetes mellitus, CD105 is significantly more highly expressed in microvessels than CD31.

## REFERENCES


1. Barbara NP, Wrana JL, Letarte M. Endoglin is an accessory protein that interacts with the signaling receptor complex of multiple members of the transforming growth factor-β superfamily. J Biol Chem 1999;274:584-94.
2. Burke AP, Farb A, Malcom GT, Liang Y, Smialek JE, Virmani, R. Plaque rupture and sudden death related to exertion in men with coronary artery disease. JAMA 1999;281:921-6.
3. Carr S, Farb A, Pearce WH, Virmani R, Yao JS. Atherosclerotic plaque rupture in symptomatic carotid artery stenosis. J Vasc Surg 1996;23:755-65.
4. Collaborators NASCET. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. N Engl J Med 1991;325:445-53.
5. Falk E. Stable versus unstable atherosclerosis: Clinical aspects. Am Heart J 1999;138 Suppl 5:S421-5.
6. Fonsatti E, Jekunen AP, Kairemo KJ, Coral S, Snellman M, Nicotra MR, *et al*. Endoglin is a suitable target for efficient imaging of solid tumors: *In vivo* evidence in a canine mammary carcinoma model. Clin Cancer Res 2000;6:2037-43.
7. Fonsatti E, Nicolay HJ, Altomonte M, Covre A, Maio M. Targeting cancer vasculature via endoglin/CD105: A novel antibody-based diagnostic and therapeutic strategy in solid tumours. Cardiovasc Res 2010;86:12-9.
8. Fonsatti E, Vecchio LD, Altomonte M, Sigalotti L, Nicotra MR, Coral S, *et al*. Endoglin: An accessory component of the TGF-β-binding receptor-complex with diagnostic, prognostic, and bioimmunotherapeutic potential in human malignancies. J Cell Physiol 2001;188:1-7.
9. Hayden MR, Tyagi SC. Vasa vasorum in plaque angiogenesis, metabolic syndrome, type 2 diabetes mellitus, and atheroscleropathy: A malignant transformation. Cardiovasc Diabetol 2004;3:1.
10. Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, *et al*. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med 2003;349:2316-25.
11. Kumar S, Ghellal A, Li C, Byrne G, Haboubi N, Wang JM, *et al*. Breast carcinoma vascular density determined using CD105 antibody correlates with tumor prognosis. Cancer Res 1999;59:856-61.
12. Kurosaki Y, Yoshida K, Endo H, Chin M, Yamagata S. Association between carotid atherosclerosis plaque with high signal intensity on T1-weighted imaging and subsequent ipsilateral ischemic events. Neurosurgery 2011;68:62-7.
13. Laszik ZG, Zhou XJ, Ferrell GL, Silva FG, Esmon CT. Down-regulation of endothelial expression of endothelial cell protein C receptor and thrombomodulin in coronary atherosclerosis. Am J Pathol 2001;159:797-802.
14. Letamendía A, Lastres P, Botella LM, Raab U, Langa C, Velasco B, *et al*. Role of endoglin in cellular responses to transforming growth factor-β: A comparative study with betaglycan. J Biol Chem 1998;273:33011-9.
15. Li X, van der Meer JJ, van der Loos CM, Ploegmakers HJ, de Boer OJ, de Winter RJ, *et al*. Microvascular endoglin (CD105) expression correlates with tissue markers for atherosclerotic plaque vulnerability in an ageing population with multivessel coronary artery disease. Histopathology 2012;61:88-97.
16. McCarthy MJ, Loftus IM, Thompson MM, Jones L, London NJM, Bell PR, *et al*. Angiogenesis and the atherosclerotic carotid plaque: An association between symptomatology and plaque morphology. J Vasc Surg 1999;30:261-8.
17. Miller DW, Graulich W, Karges B, Stahl S, Ernst M, Ramaswamy A, *et al*. Elevated expression of endoglin, a component of the TGF-β-receptor complex, correlates with proliferation of tumor endothelial cells. Int J Cancer 1999;81:568-72.
18. Mofidi R, Crotty TB, McCarthy P, Sheehan SJ, Mehigan D, Keaveny TV. Association between plaque instability, angiogenesis and symptomatic carotid occlusive disease. Br J Surg 2001;88:945-50.
19. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, *et al*. From vulnerable plaque to vulnerable patient. Circulation 2003;108:1664-72.
20. Shariat SF, Karam JA, Walz J, Roehrborn CG, Montorsi F, Margulis V, *et al*. Improved prediction of disease relapse after radical prostatectomy through a panel of preoperative blood-based biomarkers. Clin Cancer Res 2008;14:3785-91.
21. Tanaka F, Otake Y, Yanagihara K, Kawano Y, Miyahara R, Li M, *et al*. Evaluation of angiogenesis in non-small cell lung cancer: Comparison between anti-CD34 antibody and anti-CD105 antibody. Clin Cancer Res 2001;7:3410-5.
22. Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, *et al*. Atherosclerotic plaque progression and vulnerability to rupture: Angiogenesis as a source of intraplaque hemorrhage. Arterioscler Thromb Vasc Biol 2005;25:2054-61.
23. Virmani R, Narula J, Farb A. When neoangiogenesis ricochets. Am Heart J 1998;136:937-9.
24. Warlow C. MRC European Carotid Surgery Trial: Interim results for symptomatic patients with severe (70-99%) or with mild (0-29%) carotid stenosis. European Carotid Surgery Trialists' Collaborative Group. Lancet 1991;337:1235-43.
25. Wikstrom P, Lissbrant IF, 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;51:268-75.


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