

## LETTERS TO THE EDITOR

### Immunohistochemical Detection of Cyclin D and Cyclin A in Human Vascular Endothelial Cells of the Skin

Sir,

Cyclin was first identified as a protein that is synthesized prior to the splitting of cell, when sea urchin eggs repeat cell splitting after fertilization and disappears rapidly with the completion of splitting. Cyclin, when combined with cdc2, activates the cdc2 kinase and the activated cdc2 kinase phosphorylates various proteins to regulate the cell cycle (1). Such a mechanism is common to all eukaryotic cells. It has been made clear that subtypes exist for both cyclin and cdc2 in mammalian cells and that the advance of the cell cycle is controlled by a combination of these subtypes (2). Up to now, five cyclin subtypes, from A to E, have been confirmed.

There is a possibility that these cell cycle proteins are abnormal in carcinoma cells. In fact, there have been reports showing that cyclin A and cyclin D may be oncogenic proteins. In a case of hepatocellular carcinoma, it has been reported that a

hepatitis B virus gene is inserted into the intron of the cyclin A gene (3). The cyclin D gene was reported first as a gene (PRAD 1) that is activated by chromosome inversion in cells of parathyroid tumors (4). At about the same time, it was reported as a gene (CYL) that is induced in the G1 phase by stimulating macrophage with a growth factor (5) and also as a human gene (cyclin D gene) that can rescue G1 phase deficient yeast cells (6). Furthermore, it has been obvious that the cyclin D gene is overexpressed by chromosome translocation or gene amplification in B cell lymphoma (7), squamous cell carcinoma of the head and neck (8), mammary carcinoma (8) and esophageal cancer (9). These data indicate that cyclin D may be an oncogenic cyclin.

We examined the immunohistochemical localization of cyclin D and cyclin A in normal vascular endothelial cells of the skin, granuloma telangiectaticum with proliferation of vascular endo-

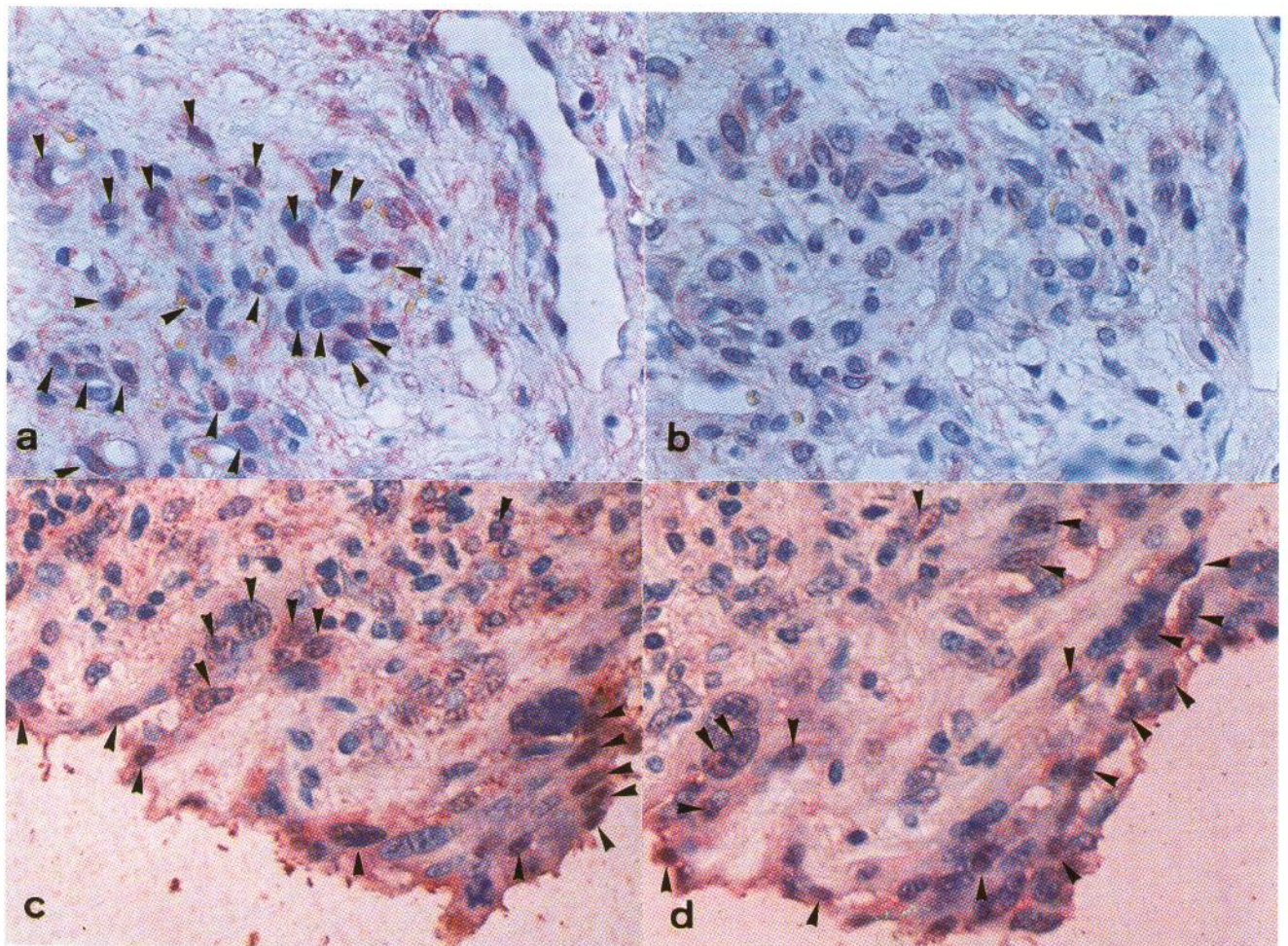


Fig. 1. Immunohistochemical localization of cyclin A (a) and cyclin D (b) in granuloma telangiectaticum and cyclin A (c) and cyclin D (d) in malignant hemangioendothelioma (arrowheads,  $\times 200$ ).



thelial cells and malignant hemangioendothelioma, to decide whether cyclin D and cyclin A are relevant to proliferation and/or carcinogenesis of vascular endothelial cells of the skin.

Normal skin of 5 healthy subjects and skin lesions of 5 granuloma telangiectaticum, 4 malignant hemangioendothelioma were excised surgically. Specimens were formalin-fixed and subsequently embedded in paraffin wax prior to sectioning. Three serial 5- $\mu$ m sections from the specimens were mounted on glass slides, air-dried, then de-waxed and rehydrated through graded ethanol. After hydration in phosphate-buffered saline (PBS), the first serial sections were incubated with anti-cyclin A monoclonal antibody (Upstate Biotechnology Inc., Lake Placid, NY, USA, 10  $\mu$ g/ml) and the second sections were incubated with anti-cyclin D polyclonal antibody (Upstate Biotechnology Inc., 10  $\mu$ g/ml) for 1 h at room temperature. The sections were washed in PBS, followed by the PAP (peroxidase antiperoxidase) detection system (Dako Japan, Kyoto, Japan). Controls included the omission of the primary antibodies and substitution of non-relevant antibodies. Counterstaining was performed with hematoxylin for 30 s.

Cyclin D positive cells were found in 2 out of 4 cases of malignant hemangioendothelioma (Fig. 1d) but not in any of the normal capillary (data not shown) or granuloma telangiectaticum (Fig. 1b). Cyclin A positive cells were found in 3 out of 5 cases of granuloma telangiectaticum (Fig. 1a) and 4 out of 4 cases of malignant hemangio-endothelioma (Fig. 1c), but not in normal capillary (data not shown). Both cyclin D and cyclin A were present in the nuclei in all positive cases (Fig. 1a, c, d).

The present study shows that cyclin D expression is specific to malignant cells of vascular endothelial cells of the skin. This fact is not contradictory to the previous reports, suggesting that cyclin D is a oncogenic protein (7–9). Furthermore, it has been reported that immunohistochemical expression of cyclin D is found only in malignant cells but not in normal cells in various kinds of tissues (10–12).

What is its role in the carcinogenesis of vascular endothelial cells of the skin? It has been suggested that cyclin D, called G1 cyclin, may play a role in the shift from the G0 phase to the G1 phase by phosphorylating Rb protein, a tumor suppressor gene product (2). It is therefore possible that cyclin D accelerates the shift from the G0 phase to the G1 phase in the carcinogenesis of vascular endothelial cells of the skin.

In contrast to cyclin D, expression of cyclin A was not specific to malignant cells of vascular endothelial cells of the skin, since it is positive for both granuloma telangiectaticum (Fig. 1a) and malignant hemangioendothelioma (Fig. 1c). So cyclin A is considered to be related to the proliferation of vascular endothelial cells of the skin. In fact, cyclin A is reported to be concerned with the proliferation of not only malignant cells but also normal cells (13, 14). The reason why cyclin

A could not be found in the cells of normal capillary is not known, but it is possible that the antibody against cyclin A used in the present study has a low affinity to cyclin A protein.

On the basis of the present study, it is suggested that cyclin D may be related to carcinogenesis of vascular endothelial cells of the skin and that cyclin A is related to proliferation of vascular endothelial cells of the skin.

## REFERENCES

1. Norbury C, Nurse P. Animal cell cycles and their control. *Annu Rev Biochem* 1992; 61: 441–470.
2. Sherr CJ. Mammalian G1 cyclins. *Cell* 1993; 73: 1059–1065.
3. Wang J, Chenivresse X, Henglein B, Br  chet C. Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. *Nature* 1990; 343: 555–557.
4. Motokura T, Bloom T, Kim HG, J  ppner H, Ruderman JV, Kronenberg HM, et al. A novel cyclin encoded by bcl-1 linked candidate oncogene. *Nature* 1991; 350: 512–515.
5. Matsushime H, Roussel MF, Ashmun RA, Sherr CJ. Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. *Cell* 1991; 65: 701–713.
6. Lew DJ, Dulic V, Reed DI. Isolation of three novel human cyclins by rescue of G1 cyclin (Cln) function in yeast. *Cell* 1991; 66: 1197–1206.
7. Withers DA, Harvey RC, Faust JB, Melnyk O, Carey K, Meeker TC. Characterization of a candidate bcl-1 gene. *Mol Cell Biol* 1991; 11: 4846–4853.
8. Lammie GA, Fantl V, Smith R, Schuurin E, Brookes S, Michalides R, et al. D11S287, a putative oncogene on chromosome 11q13, is amplified and expressed in squamous cell and mammary carcinomas and linked to bcl-1. *Oncogene* 1991; 6: 439–444.
9. Jiang W, Kahn SM, Tomita N, Zhang Y-J, Lu S-H, Weinstein IB. Amplification and expression of the human cyclin D gene in esophageal cancer. *Cancer Res* 1992; 52: 2980–2983.
10. Zhang Y-J, Jiang W, Chen CJ, Lee CS, Kahn SM, Santella RM, et al. Amplification and overexpression of cyclin D1 in human hepatocellular carcinoma. *Biochem Biophys Res Commun* 1993; 196: 1010–1016.
11. Jiang W, Zhang Y-J, Kahn SM, Hollstein MC, Santella RM, Lu S-H, et al. Altered expression of the cyclin D1 and retinoblastoma genes in human esophageal cancer. *Proc Natl Acad Sci USA* 1993; 90: 9026–9030.
12. Inohara S, Kitano Y. Immunohistochemical detection of cyclin D and cyclin A in human hyperproliferative epidermis. *Arch Dermatol Res* (in press).
13. Paterlini P, Suberville AM, Zindy F, Melle J, Sonnier M, Marie JP, et al. Cyclin A expression in human hematological malignancies: a new marker of cell proliferation. *Cancer Res* 1993; 53: 235–238.
14. Zindy F, Lamas E, Chenivresse X, Sobczak J, Wang J, Fesquet D, et al. Cyclin A is required in S phase in normal epithelial cells. *Biochem Biophys Res Commun* 1992; 182: 1144–1151.

Received April 18, 1994.

Shinichi Inohara, Department of Dermatology, Hyogo College of Medicine, Nishinomiya, Hyogo 663, Japan.