Increased expression of cytosolic chaperonin CCT in human hepatocellular and colonic carcinoma

Shin-ichi Yokota,^{1,†} Yuzo Yamamoto,² Kenji Shimizu,² Hirohito Momoi,² Tatsuhiko Kamikawa,² Yoshio Yamaoka,² Hideki Yanagi,^{1,‡} Takashi Yura,¹ and Hiroshi Kubota^{1,3}

¹HSP Research Institute, Kyoto Research Park, Shimogyo-ku, Kyoto 600–8813, Japan

²Department of Gastroenterological Surgery, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto 606–8501, Japan

³CREST/JST and Department of Molecular and Cellular Biology, Institute for Frontier Medical Science, Sakyo-ku, Kyoto University, Kyoto 606–8397, Japan

Abstract The chaperonin-containing *t*-complex polypeptide 1 (CCT) is a hetero-oligomeric molecular chaperone that assists in the folding of actin, tubulin, and other cytosolic proteins. We recently reported that the expression level of CCT is closely correlated with growth rates of mammalian cultured cells. Here we examine the levels of CCT subunits and other molecular chaperones in tumor tissues of patients with hepatocellular and colonic carcinoma, and compare them with nontumor tissues in the same patients. Expression levels of CCT β in tumor tissues was significantly higher than in nontumor tissues in all patients with hepatocellular carcinoma ($n = 15$) and 83% of patients with colonic carcinoma ($n = 17$). The increased level of CCT expression in colonic cancer cells was confirmed by immunohistochemistry with anti-CCT β antibody. The levels of CCT β were highly correlated ($r = 0.606$) with those of the proliferating cell nuclear antigen (PCNA), which was used as an indicator of cell growth. CCT α gave similar results, although the correlation with PCNA levels was weaker. Other cytosolic and endoplasmic reticulum chaperones also showed higher expression in significant numbers of tumor tissues but less frequently than that observed with CCT. These results suggest that CCT is up-regulated in rapidly proliferating tumor cells *in vivo* to effectively produce proteins required for growth, and may serve as a useful tumor marker because it is widely distributed in the cytosol.

INTRODUCTION

The chaperonin-containing *t*-complex polypeptide 1 (CCT), also called TRiC or c-cpn, mediates the folding of proteins in the cytosol (reviewed by Kubota et al 1995; Lewis et al 1996; Willison and Horwich 1996). CCT is a member of the chaperonin family that includes mitochondrial 60-kDa heat shock protein (HSP60), bacterial GroEL, plastid Rubisco subunit binding protein, and archaea group II chaperonins. CCT has been shown to assist in the folding of actin and tubulin in the presence of ATP *in vitro* (Tian et al 1995; Frydman and Hartl 1996; Farr et al 1997) and to bind newly synthesized actin and tu-

bulin *in vivo* (Thulasiraman et al 1999). Several other proteins were also reported as possible CCT substrates. CCT is required for the maturation of cyclin E (Won et al 1998); and myosin II (Srikakulam and Winkelmann 1999), α -transducin (Farr et al 1997), von Hippel Lindau protein (Feldman et al 1999) and firefly luciferase (Frydman and Hartl 1996; Gebauer et al 1998) can be folded with the assistance of CCT. In addition, more than 70 unidentified newly synthesized proteins can be immunoprecipitated with CCT in mammalian cells (Thulasiraman et al 1999). These observations indicate that CCT plays essential roles in protein folding in eukaryotic cytosol.

Electron microscopic analysis revealed that CCT has a double-torus-like structure with 8-fold rotational symmetry assembled from 16 subunits (Llorca et al 1999). In mammalian somatic cells, CCT is composed of 8 different subunits of approximately 60 kDa each: α , β , γ , δ , ϵ , ζ -1, η , and θ (Kubota et al 1995). These subunits are encoded by independent genes and show approximately 30% iden-

Received 26 October 2000; Revised 17 May 2001; Accepted 30 May 2001.

Correspondence to: Hiroshi Kubota, Tel: 81 75 751-4606; Fax: 81 75 751-4646; E-mail: hkubota@frontier.kyoto-u.ac.jp.

[†]Present address: Department of Microbiology, Sapporo Medical University School of Medicine, Chuo-ku, Sapporo 060-8556, Japan.

[‡]Sumitomo Pharmaceuticals Research Center, Konohana-ku, Osaka 554-0022, Japan.

tity at the amino acid sequence level (Kubota et al 1994, 1999).

Recently, we found that CCT expression levels correlate closely with growth rates in mammalian cultured cells, and that the expression of CCT is markedly up-regulated around early S phase of the cell cycle (Yokota et al 1999). Because cell cycle-dependent proteins such as proliferating cell nuclear antigen (PCNA) and MIB-1 (Ki-67) antigen are frequently used as tumor markers (Risio 1994; Elias 1997; Schipper et al 1998), we postulated that CCT could also be useful as a tumor marker. Here we analyze CCT levels in primary tumor tissues obtained from patients with hepatocellular and colonic carcinoma and compare them with nontumor tissues from the same patients. We also examined the expression levels in tumor and nontumor tissues of other chaperone proteins, including the 70-kDa heat shock protein (HSP70), cognate of HSP70 (HSC70), and the 78-kDa and 94-kDa endoplasmic reticulum (ER) glucose-regulated proteins (GRP78 and GRP94, respectively). Of all the molecular chaperones tested, CCT was the most frequently up-regulated in tumor tissues.

MATERIALS AND METHODS

Tissue sampling

Between July 1997 and August 1999, 32 patients were enrolled in this study at the Department of Gastroenterological Surgery, Kyoto University Graduate School of Medicine. Informed consent was obtained from all patients, and 15 primary hepatocellular carcinomas and 17 colonic cancers (2 ascending, 2 transverse, 2 descending, 7 sigmoid, and 4 rectal cancers) were included. Samples of primary tumors and vicinal nonneoplastic tissues were obtained after excision of surgical specimens. Tissue samples were immediately frozen and stored at -80°C until analysis.

Antibodies

Rat monoclonal antibodies to CCT α (clone 84a) and HSC70 (1B5), and mouse monoclonal antibodies to HSP70 (C92F3A-5) and KDEL (10C3) were purchased from StressGen (Victoria, British Columbia, Canada). The anti-KDEL antibody was used to detect GRP78 and GRP94. Mouse monoclonal antibodies to PCNA (PC10) and actin (C4) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Chemicon (Temecula, CA, USA), respectively. Rabbit polyclonal antibody against CCT β (BC-1; Hynes et al 1995) was kindly provided by Prof K.R. Willison.

Western blotting

Tissue specimens were homogenized in a buffer containing 1% Triton X-100, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 2 mM ethylenediamine tetraacetic acid, 0.1 IU/mL aprotinin, and 10 mM Hepes-NaOH (pH 7.5), and centrifuged at $25\,000 \times g$ for 20 minutes at 4°C . The protein concentration of the resulting supernatant was determined by the Bradford method (1976) using bovine serum albumin as a standard. The extracted proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) using 10% gels (for PCNA) or 7.5% gels (for other proteins; Laemmli 1970), and blotted onto polyvinylidene difluoride filters according to the method of Towbin et al. (Towbin et al 1979). Detection and quantitation of proteins on the filters using specific antibodies was carried out as described previously (Yokota et al 1999). Briefly, the filters were incubated with an appropriate primary antibody and then with alkaline phosphatase-conjugated goat antibody against rabbit, rat, or mouse immunoglobulins. Immunoreactive bands were visualized by developing with the solution containing tetrazolium bromochloroindolylphosphate and nitrobluetetrazolium. Digital images of the resulting blots were obtained with a flatbed scanner and analyzed using the public domain NIH Image program (U.S. National Institutes of Health, Bethesda, MD, USA). Experiments were carried out three times, and mean values and standard deviations were calculated.

Immunohistochemistry

Tissue samples were fixed in 4% formaldehyde and immunohistochemical staining of paraffin sections (4 μm) was carried out using an LSAB2/HRP kit (Dako, Via Real Carpinteria, CA, USA) according to the manufacturer's instructions. Briefly, after blocking endogenous peroxidase activity and nonspecific protein binding, sections were incubated with anti-CCT β antibody (1:100). Sections were then incubated with biotinylated anti-rabbit immunoglobulin and peroxidase-conjugated streptavidin, and developed with 3-amino-9-ethyl carbasol. Developed sections were counterstained with hematoxylin.

RESULTS

Up-regulation of molecular chaperones in tumor tissues

Tumor tissues and surrounding nontumor tissues from the same patients with hepatocellular ($n = 15$) or colonic ($n = 17$) carcinoma were obtained at the time of surgery, and the protein expression levels of cytosolic molecular chaperones CCT, HSP70, and HSC70, and ER molecular chaperones GRP78 and GRP94 in these tissues were an-

Fig. 1. Protein expression levels of CCT (α and β subunits), HSP70, HSC70, GRP78, GRP94, PCNA, and actin in tumor and nontumor tissues derived from patients with hepatocellular and colonic carcinoma. Soluble proteins were extracted from tumor and nontumor tissues from the same patients and separated by SDS-PAGE (total protein loaded: 10 μ g/lane for CCT α , CCT β , HSP70, and HSC70; and 5 μ g/lane for GRP78, GRP94, PCNA, and actin). These proteins were then analyzed by Western blotting using specific antibodies. Tissue samples from 15 patients with hepatocellular carcinoma and 17 patients with colonic carcinoma were tested, and representative data are shown. N, nontumor tissue; T, tumor tissue.

alyzed by Western blot analysis. In addition, the levels of PCNA (a marker of rapid cell growth) and actin (a control for intracellular protein) were determined; representative results are shown in Figure 1. The intensity of each band was quantified, and tumor:nontumor ratios of individual proteins expressed in the same patients were determined (Fig. 2 and Table 1). In all patients with hepatocellular and colonic carcinoma, the expression levels of CCT (α and β subunits), GRP78, and GRP94 were frequently (73%–100%) enhanced in tumor, as was the expression level of PCNA (80%–82%). Of the molecular chaperone proteins examined, CCT β was the most frequently up-regulated in tumor tissue (82%–100%), closely followed by CCT α (76%–93%). HSP70 was frequently up-regulated in hepatocellular carcinoma (87%) but not in colonic carcinoma (29%). In contrast, HSC70 levels were frequently increased in colonic carcinoma (82%), but much less often in hepatocellular carcinoma (45%). Actin expression levels were not up-regulated in tumor tissues from a significant number of patients (only 35%–40% of cases showed actin up-regulation).

Immunohistochemical staining of CCT β in colonic carcinoma and surrounding normal tissues indicated that CCT β protein is abundant in cytosolic portions of malignant epithelial tissue (Fig. 3A). In contrast, the degree of CCT β staining in normal epithelial tissue (Fig. 3B) or connective tissues was much weaker than that in colonic carcinoma tissue. Immunohistochemical staining of microwave-treated sections with anti-CCT γ antibody (GC-1; Hynes et al 1995) exhibited similar staining patterns (data

not shown). These results confirmed that the increased expression of CCT occurred primarily in the cytosol of tumor cells.

Correlation between expression levels of different proteins

The degree of up-regulation of PCNA in tumor tissue correlated strongly ($r = 0.606$, $P = 0.0002$) with that of CCT β (Fig. 4), and to a lower but significant degree ($r = 0.367$, $P = 0.038$) with that of CCT α . The degree of up-regulation of HSC70 showed significant correlation ($r = 0.589$, $P = 0.0003$) with that of PCNA, whereas that of HSP70 did not ($r = -0.020$). Up-regulation of GRP78 showed a weak but significant correlation with that of PCNA ($r = 0.354$, $P = 0.046$), whereas the degree of up-regulation of GRP94 did not show significant correlation with that of PCNA ($r = 0.218$). Consistent with the fact that CCT α and CCT β are components of the same complex (Kubota et al 1995), the up-regulation of these 2 proteins correlated very strongly ($r = 0.686$, $P < 0.0001$). The up-regulation of GRP78 and GRP94 also correlated very strongly ($r = 0.690$, $P < 0.001$), suggesting that the “unfolded protein response,” by which ER chaperones are induced (McMillan et al 1994), occurred in an appreciable number of tumor tissues. Actin expression did not show any significant correlation with the other proteins tested. The cases examined contained 15 moderately differentiated and 3 well-differentiated colonic carcinomas, and 7 poorly differentiated, 3 moderately differentiated, and 5

Fig. 2. Relative expression levels of CCT α , CCT β , HSP70, HSC70, GRP78, GRP94, and actin in tumor tissues. Expression levels of proteins in tumor and nontumor tissues were analyzed by Western blotting as described in Figure 1 and quantified by digital image analysis after scanning the blots. Tumor:nontumor ratios were calculated using the data derived from the same patients with hepatocellular ($n = 15$) and colonic ($n = 17$) carcinoma. Experiments were carried out in triplicate and the results are presented as mean values (standard deviations were less than 15%). Dotted lines indicate a tumor:nontumor ratio of 1.2. Mean values with standard errors for each group are indicated under the plots. HCC, hepatocellular carcinoma; CC, colonic carcinoma.

well-differentiated hepatocellular carcinomas. However, no significant correlation was observed between the degree of differentiation and the expression levels of molecular chaperones. Larger numbers of tumors should be examined to address whether expression of molecular chaperones correlate with the degree of differentiation in hepatocellular or colon cancer. These results indicate that CCT subunit proteins are frequently up-regulated in hepatocellular and colonic carcinoma tissues. The up-regulation of CCT in these tumor tissues may be related to the rapid growth of tumor cells.

DISCUSSION

In this study, we presented evidence that levels of the cytosolic chaperonin, CCT, are frequently up-regulated in hepatocellular and colonic carcinomas relative to corresponding normal tissues in the same patients. We recently reported that the expression level of CCT is dependent on cell growth rate, regardless of cell type, as a result of preferential synthesis at the G1/S transition through early S phase in the cell cycle (Yokota et al 1999). CCT is a hetero-oligomeric complex composed of 8 or 9 subunit species (Kubota et al 1995), of which 2, the α and β subunits, were examined in the present study. The levels of up-regulation of CCT α and CCT β in tumor tissues correlated strongly with each other, although they were not identical. CCT β showed more frequent up-regulation in tumors and a higher correlation with the up-regulation of PCNA than did CCT α . The observed difference between the α and β subunits may be related to our recent observation that the turnover rates of these subunits are significantly different, and that CCT α is less stable than CCT β in the cell (submitted for publication). These results suggest that CCT is up-regulated in tumor cells to meet the increased requirement for assistance in the folding of proteins that are actively synthesized during cell growth. Such target proteins may include tubulins (Yokota et al 1999) and cyclin E (Won et al 1998).

We also examined several other molecular chaperones and observed frequent up-regulation of GRP78, GRP94, HSP70 (only in hepatocellular carcinoma), and HSC70 (only in colonic carcinoma) in tumor tissues. GRP78 and GRP94 mediate protein folding in the ER, and are induced by glucose starvation or accumulation of abnormal proteins in the ER (the so-called "unfolded protein response"; Lee 1992; McMillan et al 1994). HSP70 is the major cytosolic protein induced by heat shock and chemical stress, and assists in the refolding of denatured proteins (Hightower 1991; Morimoto 1993). HSC70 (cognate of HSP70) is a cytosolic molecular chaperone that is important for the folding of newly synthesized polypeptides; HSC70 cooperates with CCT for folding proteins in vitro (Gebauer et al 1998). Several molecular chaperones,

Table 1 Number of patients with increased expression of molecular chaperones and proliferating cell nuclear antigen (PCNA) in hepatocellular and colonic carcinomas

Tumor type	Total no.	No. of cases with increased levels of the indicated proteins in tumor tissue ^a							
		CCT α	CCT β	Hsp70	Hsc70	GRP78	GRP94	PCNA	Actin ^b
Hepatocellular carcinoma	15	14 (93%)	15 (100%)	13 (87%)	7 (45%)	11 (73%)	11 (73%)	12 (80%)	6 (40%)
Colonic carcinoma	17	13 (76%)	14 (82%)	5 (29%)	14 (82%)	13 (76%)	14 (82%)	14 (82%)	6 (35%)

CCT, chaperonin-containing *t*-complex polypeptide 1; HSP, heat shock protein; HSC, heat shock protein cognate; GRP, glucose-regulated proteins.

^a The data were taken from Figure 2, and cases were scored positive for increased protein levels when the tumor-nontumor ratios exceeded 1:1.2.

^b Actin level was examined as a control.

Fig. 3. Immunohistochemical staining of CCT β in colonic carcinoma. Sections of colonic carcinoma (A) and surrounding normal colon tissue (B) from the same patient were incubated with anti-CCT β antibody (BC-1). CCT β protein specifically recognized by the antibody was stained red by peroxidase reaction with 3-amino-9-ethyl carbasol. Sections were counterstained with hematoxylin for nuclear staining in blue. Bar = 50 μ m.

including HSPs (Ferrarini et al 1992; Gress et al 1994; Kaur and Ralhan 1995; Ralhan and Kaur 1995) and GRPs (Bini et al 1997; Gazit et al 1999), have been shown to be up-regulated in malignant cancers or in cell lines derived from them, which has led to a discussion of their etiological roles (Soti and Csermely 1998; Jäättelä 1999). Increased expression of these chaperones in tumor cells

Fig. 4. Correlation between CCT β and PCNA expression levels in tumor tissues from the same patients with hepatocellular (circle) or colonic (triangle) carcinoma. Data are taken from Figure 2.

may contribute to survival and growth under stressful conditions in the tumor (Soti and Csermely 1998; Jäättelä 1999), which is in agreement with our results showing increased expression of GRP78 and GRP94. However, the observed up-regulation of these chaperones was less frequent than that of CCT. The levels of up-regulation of CCT β and HSC70 in tumor tissue correlated strongly with that of PCNA, which is used as a marker of cell growth (Woods et al 1991; Yu et al 1991), suggesting that a number of molecular chaperones are up-regulated in tumor cells not only to cope with stressful conditions but also to actively produce proteins for cell growth.

In conclusion, our present results suggest that the cytosolic chaperonin, CCT, may be useful as a cytosolic protein marker of rapidly growing tumors (including hepatocellular and colonic cancers), in addition to conventional tumor markers localized in the nucleus such as PCNA

and MIB-1 (Ki-67; Risio 1994; Elias 1997; Schipper et al 1998).

ACKNOWLEDGMENTS

We thank Prof K.R. Willison for the antibody to CCT β . We also thank Drs H. Shirane, T. Tanaka, and K. Sugita for helpful comments on immunohistochemical experiments.

REFERENCES

- Bini L, Magi B, Marzocchi B, et al. 1997. Protein expression profiles in human breast ductal carcinoma and histologically normal tissue. *Electrophoresis* 18: 2832–2841.
- Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254.
- Elias JM. 1997. Cell proliferation indexes: a biomarker in solid tumors. *Biotech Histochem* 72: 78–85.
- Farr GW, Scharl EC, Schumacher RJ, et al. 1997. Chaperonin-mediated folding in the eukaryotic cytosol proceeds through rounds of release of native and nonnative forms. *Cell* 89: 927–937.
- Feldman DE, Thulasiraman V, Ferreyra RG, et al. 1999. Formation of the VHL-elongin BC tumor suppressor complex is mediated by the chaperonin TRiC. *Mol Cell* 4: 1051–1061.
- Ferrarini M, Heltai S, Zocchi MR, et al. 1992. Unusual expression and localization of heat-shock proteins in human tumor cells. *Int J Cancer* 51: 613–619.
- Frydman J, Hartl FU. 1996. Principles of chaperone-assisted protein folding: difference between in vitro and in vivo mechanisms. *Science* 272: 1497–1502.
- Gazit G, Lu J, Lee AS. 1999. De-regulation of GRP stress protein expression in human breast cancer cell lines. *Breast Cancer Res Treat* 54: 135–146.
- Gebauer M, Melki R, Gehring U. 1998. The chaperone cofactor Hop/p60 interacts with the cytosolic chaperonin-containing TCP-1 and affects its nucleotide exchange and protein folding activities. *J Biol Chem* 273: 29475–29480.
- Gress TM, Muller-Pillasch F, Weber C, et al. 1994. Differential expression of heat shock proteins in pancreatic carcinoma. *Cancer Res* 54: 547–551.
- Hightower LE. 1991. Heat shock, stress proteins, chaperones, and proteotoxicity. *Cell* 66: 191–197.
- Hynes G, Kubota H, Willison KR. 1995. Antibody characterisation of two distinct conformations of the chaperonin-containing TCP-1 from mouse testis. *FEBS Lett* 358: 129–132.
- Jäättelä M. 1999. Escaping cell death: survival proteins in cancer. *Exp Cell Res* 248: 30–43.
- Kaur J, Ralhan R. 1995. Differential expression of 70-kDa heat shock-protein in human oral tumorigenesis. *Int J Cancer* 63: 774–779.
- Kubota H, Hynes G, Carne A, et al. 1994. Identification of six *Tcp-1*-related genes encoding divergent subunits of the TCP-1-containing chaperonin. *Curr Biol* 4: 89–99.
- Kubota H, Hynes G, Willison K. 1995. The chaperonin containing *t*-complex polypeptide 1 (TCP-1): multisubunit machinery assisting in protein folding and assembly in the eukaryotic cytosol. *Eur J Biochem* 230: 3–16.
- Kubota H, Yokota S, Yanagi H, et al. 1999. Structures and coregulated expression of the genes encoding mouse cytosolic chaperonin CCT subunits. *Eur J Biochem* 262: 492–500.
- Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685.
- Lee AS. 1992. Mammalian stress response: induction of the glucose-regulated protein family. *Curr Opin Cell Biol* 4: 267–273.
- Lewis SA, Tian G, Vainberg IE, et al. 1996. Chaperonin-mediated folding of actin and tubulin. *J Cell Biol* 132: 1–4.
- Llorca O, McCormack EA, Hynes G, et al. 1999. Eukaryotic type II chaperonin CCT interacts with actin through specific subunits. *Nature* 402: 693–696.
- McMillan DR, Gething MJ, Sambrook J. 1994. The cellular response to unfolded proteins: intercompartmental signaling. *Curr Opin Biotech* 5: 540–545.
- Morimoto RI. 1993. Cells in stress: transcriptional activation of heat shock genes. *Science* 259: 1409–1410.
- Ralhan R, Kaur J. 1995. Differential expression of Mr 70,000 heat shock protein in normal, premalignant, and malignant human uterine cervix. *Clin Cancer Res* 1: 1217–1222.
- Risio M. 1994. Methodological aspects of using immunohistochemical cell proliferation biomarkers in colorectal carcinoma chemoprevention. *J Cell Biochem Suppl* 19: 61–67.
- Schipper DL, Wagenmans MJM, Peters WHM, et al. 1998. Significance of cell proliferation measurement in gastric cancer. *Eur J Cancer* 34: 781–790.
- Soti C, Csermely P. 1998. Molecular chaperones in the etiology and therapy of cancer. *Pathol Oncol Res* 4: 316–321.
- Srikakulam R, Winkelmann DA. 1999. Myosin II folding is mediated by a molecular chaperonin. *J Biol Chem* 274: 27265–27273.
- Thulasiraman V, Yang C-F, Frydman J. 1999. In vivo newly translated polypeptides are sequestered in a protected folding environment. *EMBO J* 18: 85–95.
- Tian G, Vainberg IE, Tap WD, et al. 1995. Specificity in chaperonin-mediated protein folding. *Nature* 375: 250–253.
- Towbin H, Staehelin T, Gordon J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76: 4350–4354.
- Willison KR, Horwich AL. 1996. Structure and function of chaperonins in archaeobacteria and eukaryotic cytosol. In: *The Chaperonins*, ed Ellis RJ. Academic Press, San Diego, 107–136.
- Won K-A, Schumacher RJ, Farr GW, et al. 1998. Maturation of human cyclin E requires the function of eukaryotic chaperonin CCT. *Mol Cell Biol* 18: 7584–7589.
- Woods AL, Hall PA, Shepherd NA, et al. 1991. The assessment of proliferating cell nuclear antigen (PCNA) immunostaining in primary gastrointestinal lymphomas and its relationship to histological grade, S+G2+M phase fraction (flow cytometric analysis) and prognosis. *Histopathology* 19: 21–27.
- Yokota S, Yanagi H, Yura T, et al. 1999. Cytosolic chaperonin is up-regulated during cell growth: preferential expression and binding to tubulin at G1/S transition through early S phase. *J Biol Chem* 274: 37070–37078.
- Yu CC, Hall PA, Fletcher CD, et al. 1991. Haemangiopericytomas: the prognostic value of immunohistochemical staining with a monoclonal antibody to proliferating cell nuclear antigen (PCNA). *Histopathology* 19: 29–33.