Immunohistochemical demonstration of *c-myc* gene product in tumors induced in nude mice by human hepatoblastoma: A study with antiserum to a related synthetic peptide

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ABSTRACT

Antiserum to synthetic pentadecapeptide corresponding to the 423-437 sequence of putative human *c-myc* gene product stained cells in a human hepatoblastoma transplanted in athymic nude mice. The immunostained cells were found in clusters and mainly distributed in areas adjacent to the blood vessels and lakes. The immunoreactive elements were localized in the cytoplasm of the tumor cells.

Cellular *myc* (*c-myc*) gene is a homolog of avian MC29 viral transforming gene (*v-myc*). The nucleotide sequence of human *c-myc* oncogene has been determined by the gene cloning technique (2, 12) and the protein coded by the gene was deduced to consist of 439 amino acid residues. On the basis of the structural information of human *c-myc* gene product, we synthesized a pentadecapeptide corresponding to the C-terminal 423-437 sequence of the predicted protein and produced an antiserum against the synthetic peptide. With use of the antiserum the presence of *c-myc*-related protein was demonstrated immunohistochemically in a human hepatoblastoma heterotransplanted in athymic nude mice.

A pentadecapeptide corresponding to the 423-437 sequence of human *c-myc* protein, H-Arg-Arg-Glu-Gln-Leu-Lys-His-Lys-Leu-Glu-Gln-Leu-Arg-Asn-Ser-OH, was prepared by a solid-phase technique (7) with a Beckman

model 990B peptide synthesizer. After extensive purification by gel filtration and reverse-phase high performance liquid chromatography, purity of the peptide was assessed by routine analytical criteria. An antiserum (R5452) was produced in a rabbit using the synthetic pentadecapeptide conjugated with *Ascaris* protein as immunogen. A radioimmunoassay was developed with use of the antiserum and radioiodinated synthetic pentadecapeptide as tracer. For comparison, antisera were also produced in rabbits against synthetic tetradecapeptide corresponding to the 166-179 sequence of *c-Ki-ras* gene protein (9) (antiserum R4203) and synthetic pentadecapeptide corresponding to the 166-180 sequence of *c-Ha-ras* gene protein (1) (antiserum R4199). Each of the synthetic peptides related to *c-ras* gene products was used as immunogen in the form of *Ascaris* protein conjugate. The anti-synthetic peptide sera R5452, R4203 and R4199 were pretreated with *Ascaris* carrier protein before their use for immunostaining.

Hepatoblastoma, HBL-2, poorly differentiated type derived from a 19-month-old male

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Fig. 1 Immunostaining with antiserum R5452 of a cryostat section of the tumor tissue from a nude mouse transplanted human hepatoblastoma. $\times 200$
 Fig. 2 Immunostaining with antiserum R5452 of a paraffin section of the tumor tissue from a nude mouse transplanted human hepatoblastoma. $\times 100$
 Fig. 3 Immunostaining with antiserum R5452 (a) and negative control staining (b) of serial paraffin sections of the tumor tissue from a nude mouse transplanted human hepatoblastoma. $\times 200$. For the negative control staining, antiserum R5452 was preincubated with synthetic *c-myc* related pentadecapeptide.

infant has been serially heterotransplanted in athymic nude mice for 1 year. Radioimmunologically determined serum α -fetoprotein level of the tumor-bearing mice ranged from 1,200 to 1,400 $\mu\text{g}/\text{mg}$. Extirpated tumor tissues at the 5th and 6th passage generation were fixed in 10% neutral formalin and processed for paraffin wax embedding. Tumor tissues were also treated for cryostat sectioning. Dewaxed paraffin sections (4 μm thick) and cryostat sections (12 μm thick) were stained according to the peroxidase-antiperoxidase (PAP) method using antiserum R5452 (dilution, 1:2,000), antiserum R4203 (1:1,000) or

antiserum R4199 (1:1,000).

We used in this study synthetic pentadecapeptide related to the C-terminal 423–437 part of the predicted protein sequence as haptenic immunogen, in order to obtain C-terminal specific anti-human *c-myc* protein sera. The extremely C-terminal Cys-Ala in positions 438 and 439 of the protein were not included purposely in the synthesized peptide immunogen because of the functional side chain of Cys residue. A radioimmunoassay with the antiserum recognized solely a 60–65 kDa peak component in Sephadex G150 gel filtration of rat liver extract. Giallongo *et al.* (3) have demon-

strated that the antiserum to a synthetic peptide corresponding to the 428–439 human *c-myc* protein sequence immunoprecipitated a 48 kDa protein from normal and malignant human and mouse B cells.

The present anti-*c-myc* peptide antiserum R5452 has already been shown to stain numerous cells in a human giant cell carcinoma of the lung transplanted in nude mice, Lu-65, in which amplification of *c-myc* gene had been proved (6). In this study we observed that antiserum R5452 stains cells in small cell clusters in both cryostat and paraffin sections of the tumor tissues from athymic nude mice transplanted human hepatoblastoma (Figs. 1, 2 and 3a). The staining was completely abolished by pretreatment of the antiserum with the synthetic *c-myc* related pentadecapeptide (Fig. 3b). The frequency of immunostained cells was $22.89 \pm 1.03\%$ in the cryostat sections and $7.47 \pm 2.71\%$ in the paraffin sections. The clusters of immunostained cells distributed mainly in areas adjacent to the dilated capillaries (Fig. 2) and blood lakes (Fig. 3a). The staining was observed almost exclusively in the cytoplasm of the tumor cells in both sections.

No immunoreactive cells were detected with anti-*ras* related peptide sera (R4203 and R4199) in either paraffin or cryostat sections of the tumor tissues.

Immunohistochemistry with use of the present antiserum to *c-myc* peptide provided an additional example of the presence of *c-myc* gene product or the related protein in tumor cells. The expression of the oncogene seemed to be heterogeneous among cells in the transplanted hepatoblastoma, as in the case of *ras* gene expression in human mammary and colon carcinomas (4). Conflicting data have been presented with respect to the localization of *c-myc* gene product in cell nucleus or cytoplasm (5, 6, 8, 10, 11). The present immunostaining localized *c-myc* related protein in the cytoplasm. The intracellular localization of the *c-myc* gene product still remains to be clarified.

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