In vitro cloning of a rat ascites hepatoma cell line, with reference to alpha-fetoprotein synthesis

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SUMMARY

Low alpha-fetoprotein producing clones were isolated from a high AFP producing ascites hepatoma, AH66 cell line which was successively cultured in vitro.

The growth tempo and chromosome constitution were compared between high and low AFP producing clones.

No significant correlation was found between the rate of AFP production and the chromosome number. A slight delay of host survival time was observed in low AFP producing clones.

INTRODUCTION

Almost all hepatoma cases of rats induced by azo-dyes, synthesize α-fetoprotein (AFP). Among the ascites hepatoma cell lines established from azo-dye induced hepatoma and maintained by serial transplantation, AFP was detected in 26 (33%) out of the 78 cell lines[1].

It is not yet well known whether AFP producing cell lines are mixed cell populations or homogeneous populations with reference to AFP production.

In order to clarify this question, 19 clones isolated from AH66 cell line were investigated for AFP production and other biological properties.

MATERIALS AND METHODS

Cultivation

The cells were cultivated and maintained in the TD–15 culture flasks at 37°C.

The basal medium consisted of Eagle's M.E.M. (Nissui Seiyaku Co., Ltd) supplemented with 20% heat-inactivated calf serum. The volume of the medium was 3 ml per flask.

The number of cells inoculated was approximately 5×10^4/ml. The clones of AH66 cell line were isolated by the soft agar method.

Transplantation

The cultured clonal cells were transplanted back to normal male Donryu rats by intraperitoneal injection.

The number of cells for each transplantation was 1×10^7.

Determination of AFP

Qualitative and quantitative examinations of AFP were made employing the immuno-precipitation methods[2] and radioimmunoassay[3].

RESULTS

AFP synthesis in vitro

Nineteen clones were established from AH66 cell lines by the soft

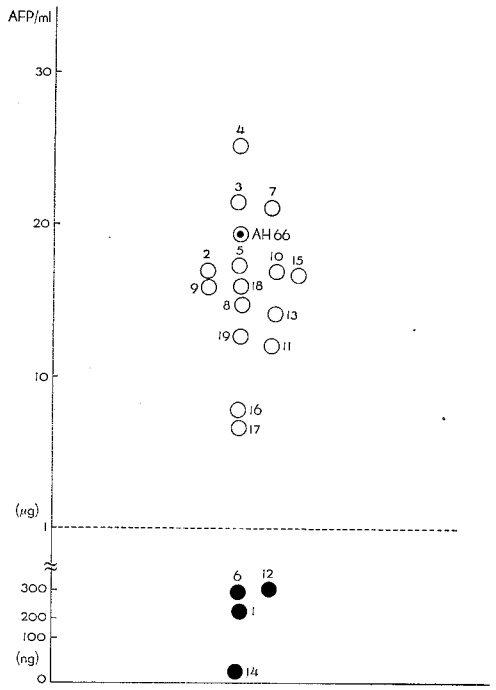

Fig. 1. Concentration of AFP in culture media of the clonal cells. The clonal cells (5×10^4) were inoculated in a flask containing 3 ml of the medium and was cultivated for 7 days. Cell free supernatants of the medium were used for the assay of AFP by single radial immunodiffusion or by the radioimmunoassay.

● low AFP producing clones
○ high AFP producing clones
⊙ original AH66 cell lines

agar method and cultivated in vitro. The amount of AFP secreted into the media in 7 days are shown in Fig. 1.

The original AH66 cell line synthesized 19 μg/ml of AFP. Fifteen clones out of 19 synthesized AFP at various levels in a range of 7 μg/ml to 25 μg/ml.

Four clones out of 19 (clone 2, 6, 12 and 14), however, synthesized extremely low levels of AFP.

The highest AFP producing clone, C-4 synthesized approximately thousand fold AFP over the lowest AFP producing clone, C-14.

Growth and AFP synthesis in vivo

These clonal cells grew well when transplanted intraperitoneally to Donryu rats showing a marked accumulation of ascites with slight hemorrhage. The average survival time of AH66 was 11 days. Most of the high AFP producing clones showed shorter survival times, 8-11 days.

In contrast, the survival time of low AFP producing clones were relatively longer, between 11 to 14 days (Fig. 2).

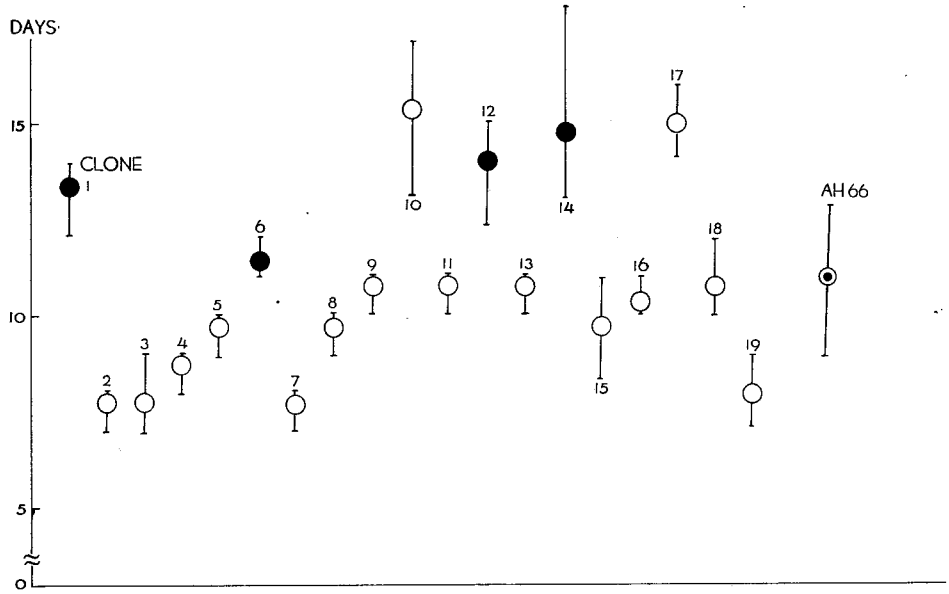

Fig. 2. Survival time of rats transplanted with clonal tumor cells.

- ● low AFP producing clones
- ○ high AFP producing clones
- ⊙ original AH66 cell line clones

The circles indicate the mean survival days of 3 rats inoculated with the clonal cells at a rate of 1×10^7 intraperitoneally. The lines indicate the range.

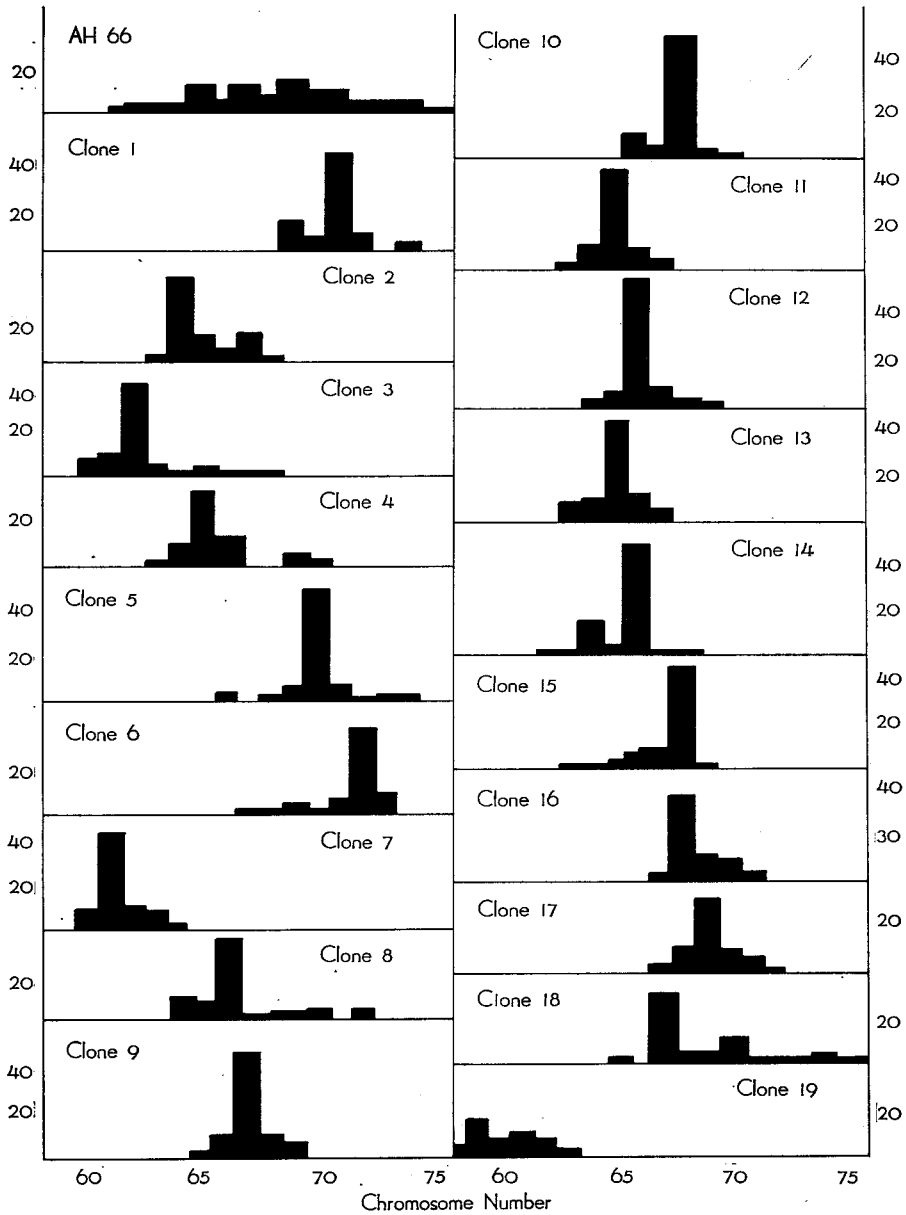

Fig. 3. Frequency of chromosome number. Chromosome examinations were made on the Giemsa-stained specimens prepared by the air-drying technique.

Clonal cells used were 3rd generation. Chromosome number were culculated in 100 cells in metaphase.

AFP production in vivo was also determined. The rate of AFP production was comparable to that in vitro.

The average amount of AFP production in vivo and in vitro was 2.3×10^{-9} and 2.9×10^{-8} mg/cell/day, respectively. The amount of AFP production by AH66 cells in vitro was about 12 times higher than that by AH66 cells in vivo.

Chromosome Analysis

The original AH66 cell line, with no predominant chromosome number, showed a wide distribution, although the most frequent number observed was from 66 to 72 as previously described by Isaka.

The chromosome numbers of the clonal cells differed from clone to clone. The mode of each clone was distributed from 59 to 72.

No correlation between the amount of AFP production and the modal number was observed (Fig. 3).

DISCUSSION

Ishidate[5] demonstrated the heterogeneity of the cell population of a hepatoma nodule in a single rat with respect to chromosome number and some other cytological properties.

Previously Watabe *et al.*[1] reported that slow growing variants of Yoshida Sarcoma (YS) designated as LY strain produced AFP. The original YS strain has been shown to be a typical AFP non-producing strain. Some of the clonal cells of the YS cell line cultured in vitro showed AFP production[6].

Some low AFP producing clones were obtained from a high AFP producing cell line, AH66 and in contrast, some AFP producing clones were obtained from an AFP non-producing cell line, Yoshida sarcoma. The heterogeneity of cell population was thus clearly demonstrated.

The heterogeneity of the population was also demonstrated by chromosome analysis. Most of the clones showed different modal distributions of chromosomes (Fig. 3).

The fluorescent antibody technique applied to different clones producing varying amounts of AFP also demonstrated the heterogeneity of various cell populations. Only a few cells of a AFP producing cell line were stained by fluorescence while some others were not[7]. This phenomenon was also observed in human hepatoma, and may partly be explained by the phase of cell cycle (AFP synthetic phase measured by synchronous culture of C-4 clone, corresponds to about one-third of the doubling time), but is probably mainly explicable in terms of the mosaic structure of the hepatoma.

AFP levels in blood from hepatoma patients are very widely distri-

buted[3,8,9]. This wide distribution may be explained by the fact that the hepatoma is a mixture of high AFP-producing and low or non-producing cells as postulated by Hirai *et al.*[10].

The amount of AFP production of AH66 cells was about 12 times higher *in vitro* than *in vivo*. The culture medium may provide more favorable conditions for the tumor cells than *in vivo* conditions, as far as AFP synthesis is concerned.

These findings suggest that the tumor cells are a mixed cell population and that selection or adaptation takes place in the population of the tumor cells.

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