ESTABLISHMENT OF A MURINE ASCITES HEPATOMA CELL LINE
H22-F25/L AND ITS BIOLOGICAL CHARACTERISTICS

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Having been passed for 160 generations, a cell line designated as H22-F25/L was established from a murine tumor lymphatic metastatic model H22-F25 which had been set up in our college. The cell line was in suspension culture with a rapid proliferation and stable growth. The peak time of cell division and proliferation was 48 and 96 hours after culture. In a week, the cell number was increased by 25 times. H22-F25/L still keeps the features of a poorly differentiated cancer. Its tumor inducing rate (in vivo) was 100% in 615 mice. Lymph node metastasis rate was 50% and pulmonary metastasis rate 10%. H22-F25/L is a population of heterogenetlc tumor cells including 2 stem cell lines (the model number of chromosomes being 43 in 40% tumor cells and 86 in 32%) and some side lines. The common marker chromosomes M1, M2, M3 and M4 were present in all stem and side lines.

Murine ascites hepatoma (H22) was transformed from H22 solid tumor by Pharmaceutical Institute of Chinese Science Academy in 1963, transplanting in KM mice and was wildly used in domestic research. Since 1979, it was transplanted into abdomic cavity of syngeneic 615 mice in our lab. After multiple generations of transplanting it became stable. We used it in lymphatic metastatic experiments and established a lymphatic metastatic model of murine tumor. Successively we established a H22-H25 cultured cell line named as H22-F25/L and has been transplanted in vitro for one and half year (160 generations). The tumor cells grow stably and rapidly. The establishment of H22-F25/L and its biological features are as following.

MATERIALS AND METHODS

Original Tumor Cells and Culture Medium

Ascitic fluid of 615 mice after being transplanted with H22-F25 for 7th days appeared opalescent and turbid. Tumor cells showed obviously allotypy. Complete medium; RPMI-1640 with 20% bovine serum, glutamine 300 µg/ml, penicillin G and streptomycin as usual, pH 7.2—7.4. Other supplement; insulin 0.05 µg/ml media, hydrocortisone 0.2 µg/ml media.

Primary Culture

Ascitic fluid 0.1 ml was drawn sterilly, wash 2 times with PBS, removed supernatant, the tumor cells were suspended in complete medium with other supplement. The cells were grown at 27℃ in a
humidified atmosphere (98 in humidity) containing 5% CO2/95% air.

Mitotic Index

The nicely growing 75th generation culture was made for cell suspension with a density $5 \times 10^6$/ml, each bottle 1.5 ml separately standing cultured in 37°C incubator. 3 bottles were taken out for every 24 hour, release 0.8 ml supernatant, the rest was smeared and H.E. stained, 1000 cells each bottle was counted with high magnifying objective, the mitotic index of that day was the average value of the 3 bottle.

Growth Curve

Cell suspension was made from 75th generation culture, separately planted in 21 bottles (25 ml in volume each bottle) and incubated. 3 samples were taken out for every 24 hour and count the mean number of the cell as vertical parameter, cultured days as horizontal parameter, growth curve was drawn up on a semilog paper. The doubling time of cell population was then identified.

Chromosome Assay

69th generation of H22-F25/L culture was taken for chromosome assay. Slides were made as usual, G- banding, 25 — 50 good spreading metaphase was counted and 3—5 among them was photographed.

Abbrerations:

- TEM: trans electron microscope
- SEM: screening electron microscope
- LM: light microscope

Ultrastructure Exam of the Tumor Cell

Also 69th generation of H22-F25/L culture was taken for ultrastructure exam, TEM specimen was prepared as usual, XDT-10 TEM was used. For SEM the specimen was taken from 78th generation culture.

Tumorigenicity

The 110th generation culture was used for tumorigenic test. $5 \times 10^6$ cells/each animal was transplanted under right armpit skin of 20 of 615 mice and observe the tumor growing and metastatic capacity.

RESULTS

Establishment of Cell Line

The primary culture showed suspension growth, and about 1/3 of the cells were half-attached. A multitude of cells are small round. Half medium was exchanged and transferred during every 3—4 days since the 7th day. The cells grew rapidly at 30 days (7th generation). Large and median size cells can be seen as well as megatoma cells. (Figure 1). The transferred it every 2—3 days. Supplement were cancelled from medium since the 35th generation (80 days) and bovine serum was reduced to 15%, but the tumor cells were still grew quickly. Up to 160th generation, there was no detectable change in morphology or cell cycle. During this period the cell line was frozen and resuscitated for several times and transplanted intraperitoneally and subcutaneously into 615 mice, it grow nicely either.