Tumor Res. **20**, 33–41 (1985)

Establishment and Characterization of a Human Pancreatic Cancer Cell Line (HPC-1)

Takashi SATO[1,2], Noriyuki SATO[1], Shuji TAKAHASHI[1,2], Hirofumi KOSHIBA[1], Tomoaki USUI[2], Koichi HIRATA[2], Koji SHIRAMATU[2], Hiroshi HAYASAKA[2] and Kokichi KIKUCHI[1]

1. *Department of Pathology (Section 1), Sapporo Medical College*
2. *Department of Surgery (Section 1), Sapporo Medical College*

South 1, West 17, Chuo-ku, Sapporo 060, Japan

SUMMARY

A human pancreatic cancer cell line, designated as HPC-1, was established from the metastatic ascitic fluid of a 73-year-old female with primary pancreatic adenocarcinoma of ductal cell origin. It was shown that HPC-1 grew *in vitro* by 26 hr of doubling time in conventional culture medium supplemented with 10% fetal calf serum, and the cell culture is presently counted over 70 passages *in vitro*. The epithelial characteristics of HPC-1 was verified by light and electron microscopical observations, and chromosome analysis showed a 74 modal number. Furthermore, this line demonstrated an overt anchorage-independent growth in 0.3% soft agar, and progressively developed tumors with a histology of anaplastic carcinoma in the nude mice. The data suggested that although CA19-9, CEA and TPA were not detected in the culture supernatants of HPC-1, this tumorigeneic line served as a new pancreatic cancer cell model for the investigations of biological and therapeutical aspects of pancreatic cancers.

Key words: HPC-1, Human pancreatic cancer line, Tumorigenesis

INTRODUCTION

The incidence of pancreatic cancers has been gradually increasing during past decades, and the establishment of human pancreatic cancer cell lines is required for the investigations of etiological, immunological and therapeutical aspects of this cancer. However, only a few lines of pancreatic cancer origins have been adapted to grow *in vitro*. Currently, six pancreatic cancer cell lines such as CaPa (3), PANC-1 (8), MIA PaCa-2 (11), HGC-25 (1), QGP-1 (6) and HPC-Y1(10) have been established, since Dobrynin (3) reported the first pancreatic cancer line in 1963. There is, however, a lack of information with respect to the malignant potentials or

tumorigenicity *in vitro* and *in vivo*, and to the immunological aspects of these cell lines.

We have succeeded in establishing a new pancreatic cancer cell line (HPC-1) from the metastatic ascitic fluids of a 73-year-old female with primary pancreatic adenocarcinoma. HPC-1 showed a definite malignant potential *in vitro* and *in vivo*, and is suggested to be useful for future investigations of the biological profiles and sensitivity to the therapeutic trials of the pancreatic cancer.

MATERIALS AND METHODS

1. *Cell source and culture*

Tumor cells were obtained from a metastatic ascitic effusion of a 73-year-old female with a primary adenocarcinoma of pancreatic ductal origin. One month prior to the patient's death, 500 m*l* of a bloody ascitic effusion was removed from her abdominal cavity, and this was centrifuged at 250×g for 10 min. The cell pellets were resuspended in 20 m*l* of RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), and cell suspensions were carefully layered on a 20 m*l* of Ficoll-Conray density gradient. After spinning at 1,000×g for 25 min at room temperature, the interface in which tumor cells, mesothelial cells, fibroblasts, macrophages and lymphocytes were concentrated was separated, and washed three times with RPMI 1640 medium. Then the cells were immediately seeded in a 25 cm^2 culture flask (Falcon #3013, Oxnard, CA., U. S. A.), and cultured under 37°C in 5% CO_2. Six hours later, the supernatants containing unattached cell were removed and seeded into other flasks. The supernatants of the each flask were daily reseeded into other flasks for the first week, and in this manner the newly seeded cells were enriched with respect to slowly attaching tumor cells. In contrast, other more rapidly attaching cells, particularly macrophages, mesothelial cells and fibroblasts were selectively reduced. After one month from the initiation of culturing, attaching cells, such as non malignant epithelial cells were almost reduced, and floating cancer cells began to adhere onto the underface of culture flasks. Thereafter the cancer cells began to grow, these were serially transferred by treating with 0.05% trypsin (Sigma Co., St. Louis, MO., U. S. A.) plus 0.02% EDTA in a phosphate buffered saline solution. It is shown that HPC-1 was free of mycoplasma contamination by using a micoplasma stain kit (Flow Laboratories Inc., VA., U. S. A.).

2. *Giemsa staining*

A conventional Giemsa staining of cells at 30th passage was performed, and the light microscopical morphology of cells was observed.

3. *Electron microscopical feature*

Adherent cultured cells in tissue culture chamber (Lab-Tek ♯4808, MILES Co., IL., U. S. A.) and tumor tissues growing in nude mice were fixed with 0.1 mol cacodylate buffer overnight. Then the cells were treated with 1% OsO_4 in phosphate buffer and washed with 7.5% sucrose for 5 min. The fixed cells were dehydrated in a graded series of ethanol from 50% to 100% and propylene oxide, and were embedded in epon-propylen oxide. The thin sections were stained with 5% uranyl acetate and observed under an electron microscope.

4. *Doubling time of cells*

5×10^4 HPC-1 cells at 34th passage *in vitro* were seeded into 25 cm^2 culture flasks (Falcon ♯3013, Oxnard, CA., U. S. A.). After trypsinizing cell monolayers, the cells were counted at 6 hr intervals for five days, and the doubling time of cells was determined.

5. *Chromosome analysis*

At the 25th passage generation, the chromosome analysis for HPC-1 was undertaken by conventional techniques.

6. *Culture in soft agar*

HPC-1 was brought into the cultivation in soft agar as described previously by several investigators (2. 7). 10^3 HPC-1 or C-C 36 cells were plated in dishes (Falcon ♯3002, Oxnard, CA., U. S. A.), and incubated at 37°C in moistured air. The number of colony formations in soft agar was scored with % plating efficiency after 2 weeks of cultivation. C-C 36 cell line (9) was used for a positive anchorage -independent cell growth control in 0.3% soft agar. This cell line has been shown previously to be highly tumorigeneic *in vitro* and *in viro*.

7. *Transplantation assessment of HPC-1 in nude mice*

HPC-1 cells growing *in vitro* were harvested by trypsinization, and 10^6 cells were injected subcutaneously in the back of five BALB/c nude mice that were obtained from CLEA Japan Co., Shizuoka, Japan. The mice were observed weekly for tumor growth.

8. *Assays for tumor-associated antigens of HPC-1*

10^5 HPC-1 cells were seeded into a tissue culture flask, and were cultured for 72 hr. The cells were multiplied to $1\text{-}2\times10^6$ cells. Then radioimmunoassay was performed to examine CA19-9, CEA and TPA in this supernatant.

RESULTS

1. *Morphological characteristics of cells*

As shown in Fig. 1, most of tumor cells in the ascitic fluid showed a round shape in various sizes with a single or multiple nuclei. Mitoses were seen occasionally. At the primary culture, tumor cells began to grow *in vitro* in RPMI 1640 medium containing 10% FCS in a floating fashion rather than being adherent. After culturing for one month, it was founed that tumor cells propagated on the bottom of the plastic culture flasks, and made up a monolayer of the cells with piling-up foci (Fig. 2). Thereafter, the cells have been continuously multiplying in adherent fashion on the tissue culture flasks. This tumor line of pancreatic origin was named as HPC-1. The histology of HPC-1 tumor developed in nude mice showed undifferenciated or anaplastic carcinoma (Fig. 3).

We examined the ultrastructures of cells growing *in vitro* and tumor tissue that eventually developed in nude mice. Fig. 4 demonstrates that HPC-1 had a scanty round cytoplasma and a large nucleus with clear nucleoli. The HPC-1 tumors developed *in vivo* (Fig. 5) showed desmosome-like junctions and a few microvilli on the cell surface. Mitochondria and rough endoplasmic reticulum were frequently observed. Secretory granules and virus-like particles were not observed electron-microscopically. It seemed that these findings were compatible with the epithelial nature of HPC-1 cells.

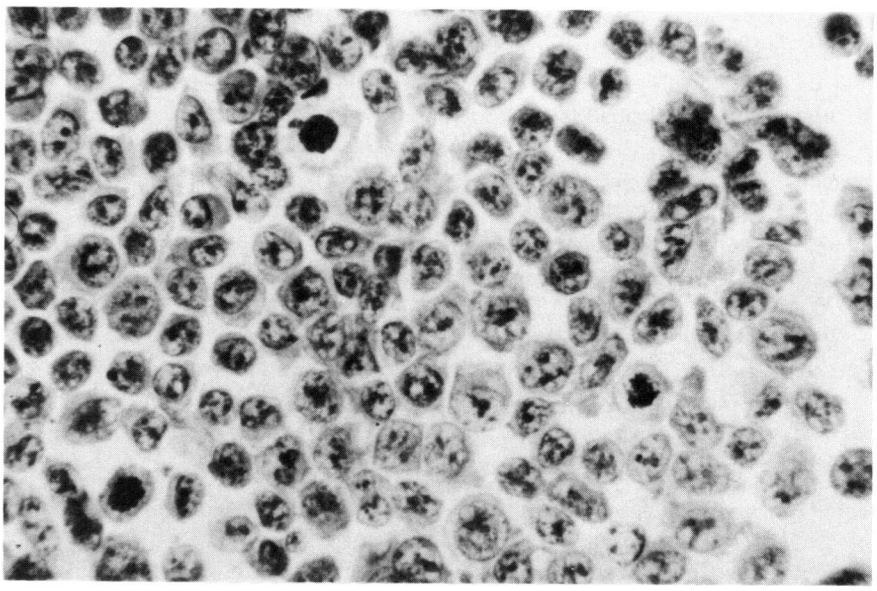

Fig. 1 A microscopic view of tumor cells in the primary culture. Giemsa stain ×400

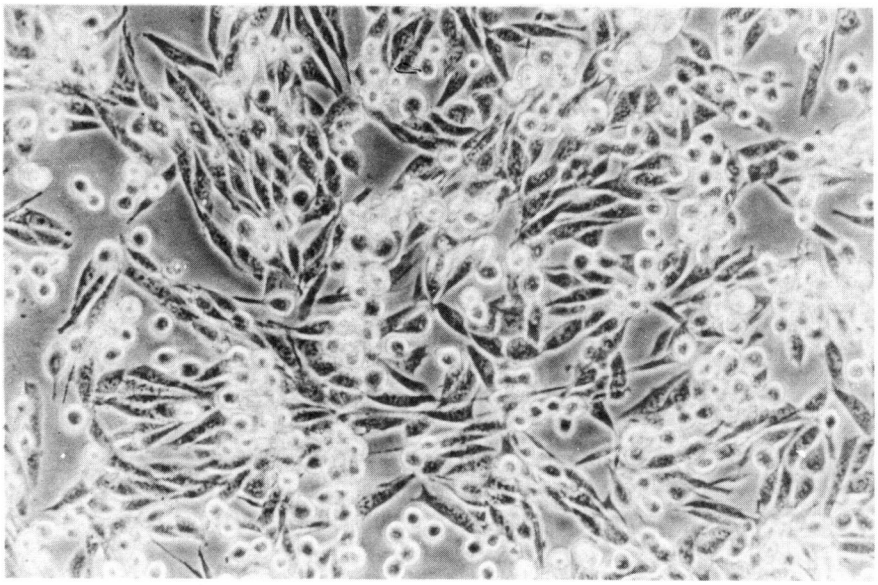

Fig. 2 A phase contrast microscopic view of HPC-1 cells cultured for one month demonstrating a monolayer of cells. ×200

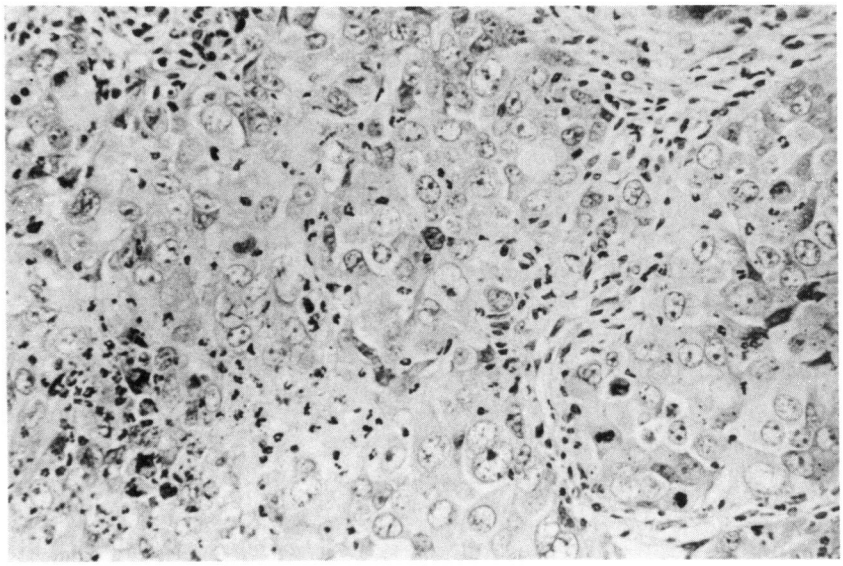

Fig. 3 Histology of HPC-1 tumor developing in nude mouse. H. E. stain ×200

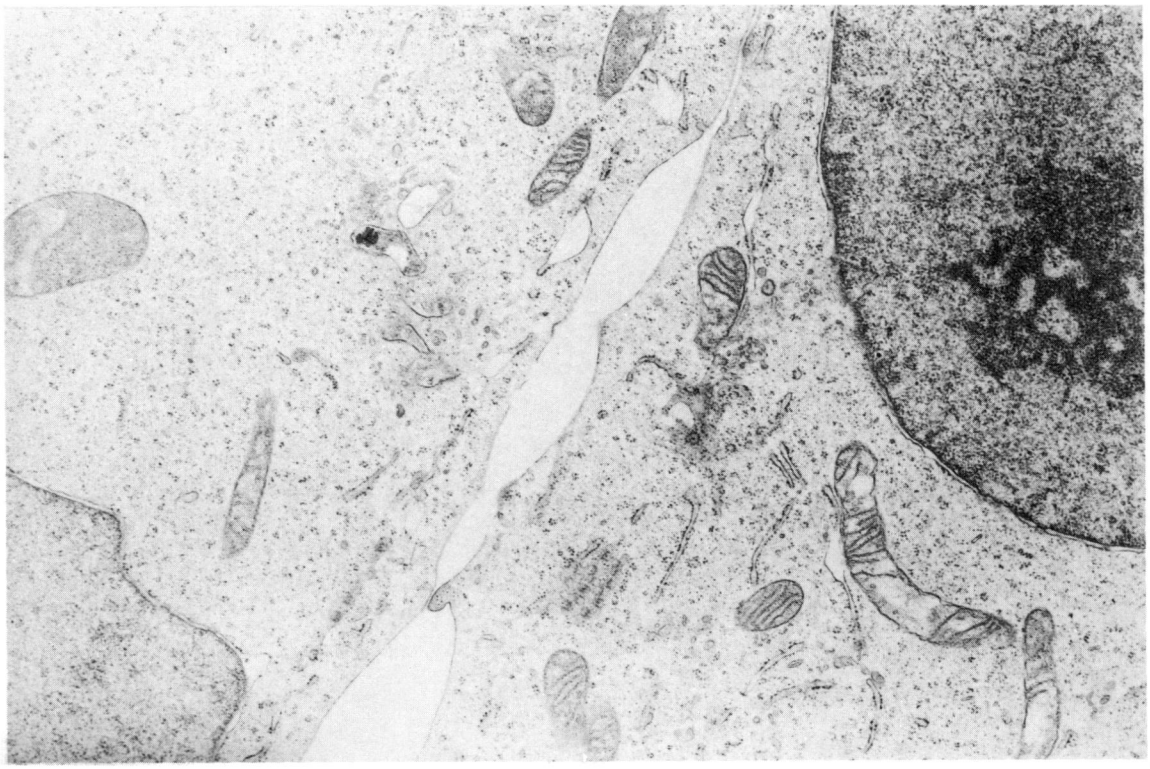

Fig. 4 An electron micrograph of the confluent monolayer of HPC-1. ×8,000

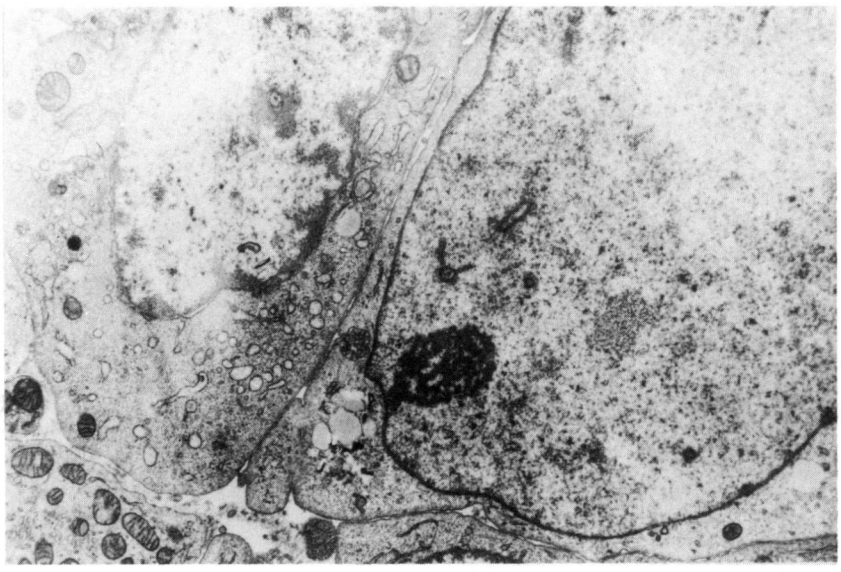

Fig. 5 An electron micrograph of a HPC-1 tumor developing in nude mouse. ×8,000

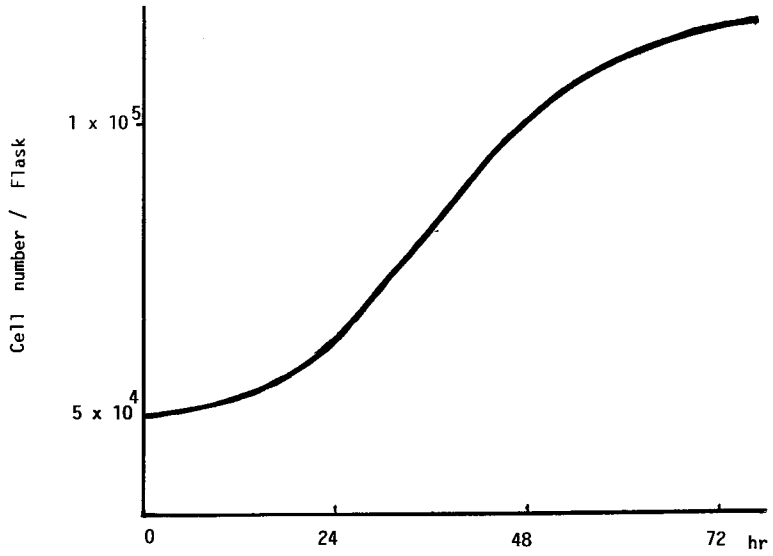

Fig. 6 A growth curve of HPC-1 cells cultured *in vitro*.

2. *Doubling time*

The doubling time was demonstrated during a exponential phase of cell growth (Fig. 6). It was shown that HPC-1 cells had an approximately 26 hr doubling time.

3. *Chromosome analysis*

Chromosome studies of HPC-1 cells showed that it was a human chromosome with a 74 modal number.

4. *Anchorage-independent growth of HPC-1 cells*

The growth capability of the HPC-1 cell on soft agar was studied. It was shown in Table 1 that HPC-1 formed colonies with a plating efficiency of 13%,

Table 1 *Anchorage-independent colony growth at 2nd week of culture of HPC-1 cells inoculated in soft agar.*

Cell lines	Inoculum (cells/9.6 cm² dish)	Plating efficiency (%)[a]
HPC-1	10^3	13.7±3.3[b]
C-C 36	10^3	33.8±7.1

[a] %of plating efficiency

$$=\frac{\text{No. of clusters}-\text{No. of original cell aggregates}}{\text{No. of viable nucleated cells plated}}\times 100$$

[b] Mean±SE.

whereas C-C 36 showed 33% of plating efficiency when 10^3 cells per dish were cultured for 2 weeks.

5. *Tumorigenicity in nude mice*

To examine the tumorigenicity *in vivo* of HPC-1, 10^6 HPC-1 cells were inoculated subcutaneously into five nude mice. The mice were observed weekly for tumor growth. Three out of five mice showed tumor development up till 13th week after inoculation. The first growing tumor was seen at 6th week after inoculation.

6. *Tumor-associated antigens*

Since HPC-1 was derived from a pancreatic cancer, we examined the production of CA19-9 antigen, CEA and TPA by HPC-1 cells. 10^5 HPC-1 cells were cultured for 3 days in 5 m*l* medium, and the culture supernatant was assessed by radioimmunoassay for the presence of tumor antigens. It was found that HPC -1 cells did not have these antigens in the culture supernatant.

DISCUSSION

We succeeded in establishing a continuous tumor cell line, named as HPC-1, from a human pancreatic adenocarcinoma. The data showed that HPC-1 is clearly an epithelial cell, and presumably of a pancreatic tumor origin, while HPC-1 might not have the tumor antigens associated with the panreatic cancers such as CA19-9, CEA and TPA (6).

There have already been several pancreatic tumor lines established previously (1, 3, 6, 8, 10, 11), and it has been demonstrated that these lines have different biological characteristics, including various grades of the cell differentiation and the tumor associated antigens (6). However, the information as to the tumorigenicity *in vivo* and *in vitro* of these cells have been limited. It is also not clear whether these lines did show high tumorigenicity from the very early passage generations. On the other hand, recent studies showed that the anchorage-independet growth potentials of cells in the soft agar were a very reliable parameter to indicate the neoplastic nature of cells (5, 9). In fact, NIH 3T3 cells transformed by various oncogenes but not nontransformed NIH 3T3 cells grew anchorage-independently, and developed macroscopic colony formations on soft agar (4).

HPC-1 showed malignant characteristics *in vitro* and *in vivo* of cells. Although the plating efficiency of HPC-1 was lower than that of extremely high tumorigenic murine colon tumor line C-C36, HPC-1 grew fairly well in 0.3% soft agar. Furthermore, this line developed tumors in nude mice when injected subcutaneously with HPC-1 cells. Thus. the data suggests that HPC-1 maintained its neoplastic nature of the cells well.

It is very important to understand the characteristics of tumor specific or tumor associated antigens that are closely correlated with the neoplastic transformation of the cells. HPC-1 may be useful for these studies because this line shows a tumorigenic nature *in vitro* as well as *in vivo*. Moreover, this line could be employed for a wide variety of studies including drug sensitivity test and other therapeutic trials in the pancreatic cancers.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Shuichi ABE of the Chromosome Research Unit of Hokkaido University for his chromosomal analysis. This work was supported by a Grant-in-Aid for a Special Research Project in the field of Biotechnology.

REFERENCES

1. AKAGI, T. and KIMOTO, T.: Acta Pathol. Jpn. **27**, 51-58 (1977).
2. BRATTAIN, M. G., STROBEL-STEVENS, J., FINE, D., WEBB, M. and SARRIF, A. M.: Cancer Res. **40**, 2142-2146 (1980).
3. DOBRYNIN, Y. V.: J. Natl. Cancer Inst. **31**, 1173-1195 (1963).
4. DREBIN, J. A., LINK, V. C., STERN,, D. F., WEINBERG, R. A. and GREENE, M. I.: Cell **41**, 695-706 (1985).
5. FREEDMAN, V. and SHIN, S.: Cell **3**, 355-358 (1974).
6. KAKU, M., NISHIYAMA, T., YAGAWA, K. and ABE, M.: Gann **71**, 596-601 (1980).
7. KIMBALL, P. M., BRATTAIN, M. G. and PITTS, A. M.: Br. J. Cancer **37**, 1015-1019 (1978).
8. LIEBER, M., MAZZETTA, J., NELSON-REES, W., KAPLAN, M. and TODARO, G.: Int. J. Cancer **15**, 741-747 (1975).
9. SATO, N., SATO, T., TAKAHASHI, S. and KIKUCHI, K.: Cancer Res. **46**, 362-366 (1986).
10. YAMAGUCHI, N., SUGIMOTO, M., UOZUMI, G., IKEZAWA, T., AOCHI, S. and KAWAI, K.: Jpn. J. Gastroenterol. (Nippon Shokakibyo Gakkai Zasshi: Tokyo) **80**, 1662 (1983).
11. YUNIS, A. A., ARIMURA, G. K. and RUSSIN, D. J.: Int. J. Cancer **19**, 128-135 (1977).