

Studies on a New Human Cell Line (SiHa) Derived from Carcinoma of Uterus. I. Its Establishment and Morphology¹ (35091)

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The widespread application of HeLa cells, established originally from a human epithelial neoplasm of the cervix uteri in 1953 by Scherer and his associates (1), to the studies of virology and oncology and its subsequent contribution to the field needs little comment. On the contrary, it is rather a peculiar fact that only a few reports can be found in the literature on attempts to establish new cell lines from the human uterine carcinoma. During our course of a study to pursue the possible presence of "known" viral antigens in the human cancerous tissues by means of *in vitro* culture of the cells and application of the immunofluorescence techniques, we have

been able to establish a cell line, designated as SiHa, from a cell culture derived from a human carcinoma of the cervix. The cells have been in culture for over 15 months and still retain their epithelial morphology with orderly growth patterns when grown as a monolayer. The procedure of isolation and establishment as well as some morphological features of the cell line are reported herein.

Materials and Methods. Neoplastic tissue. The starting materials from which the cultures had been attempted were obtained from 10 Japanese female patients. Their age varied from 37 to 58 years.

Culture medium. The primary cultures

TABLE I. Attempts to Establish Cell Lines from Human Uterine Carcinomas.

Expt. no.	Patient		Histology	Days in culture	Growth ^b	
	Name	Age			Epithelial	Fibro-blastic
1	NoAi	50	Squamous cell carcinoma grade II	17 ^a	+	+
2	TiSu	58	Squamous cell carcinoma grade II	30	++	+
3	HaLw	39	Squamous cell carcinoma grade II	29	+	++
4	OuTo	43	Scirrhus adenocarcinoma	14	+	++
5	SiHa	55	Squamous cell carcinoma grade II	>450	++++	+
6	ImHa	46	Squamous cell carcinoma grade II	21	+	+++
7	MaFu	42	Squamous cell carcinoma partly adenocarcinoma	7 ^a	±	+
8	NiTa	53	Squamous cell carcinoma grade I	11 ^a	±	+
9	KaKe	37	Squamous cell carcinoma grade III	9	±	+
10	OhKi		Adenocarcinoma	7	±	±

^a Bacterial contamination became apparent at that time.

^b ± = little or no detectable growth; ++++ = confluent growth around the explant; +- + + + indicates the grades of growth of cells between ± and ++++.

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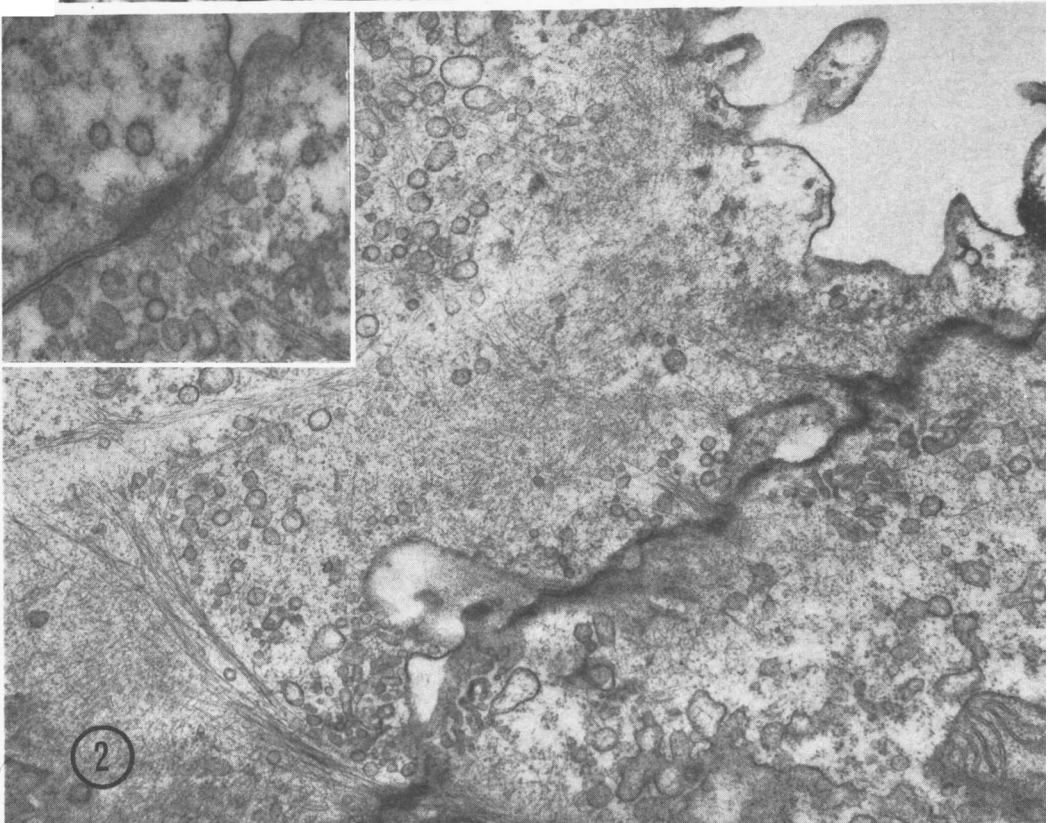
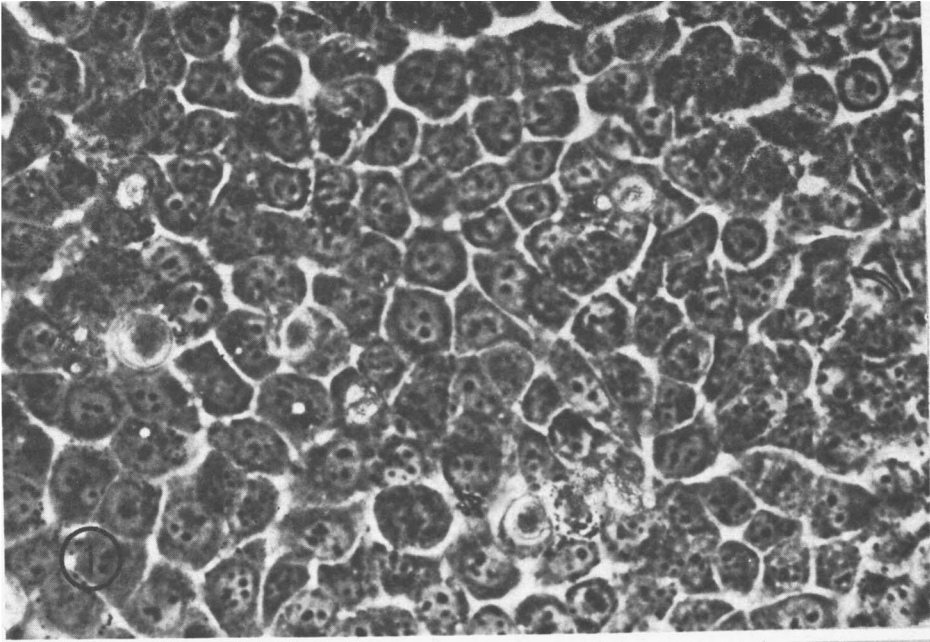


FIG. 1. Phase-contrast micrograph of a monolayer culture of SiHa cells in 20th transfer generation.

FIG. 2. Electron micrograph of thin section of SiHa cells. Notice the tight junction with many desmosomes accompanied with tonofilaments and abundance of the filaments in the cytoplasm; $\times 20,000$. (insert) A typical desmosome seen at the cellular junction; $\times 37,500$.

were grown in 2-oz prescription bottles using Eagle's minimum essential medium (MEM) plus 30% fetal calf serum and 0.06% Difco bacto-peptone. Later, the cells were maintained in MEM and 20% calf serum.

Cell culture. A piece of cancerous tissue from the uterus was transferred to the isolation room within 15 min after removal of the organ during surgery. After rinsing the material twice in MEM, the tumorous tissue was dissected carefully in the culture medium to exclude the necrotic mass. The seemingly healthy portion of the cancerous tissue was cut into small fragments with a pair of surgical scissors and was rinsed three times in MEM. Subsequently, the fragments were transferred to TD 40 culture flasks and fixed on to the glass surface by keeping them in an incubator at 37° for 45 min. Next, the culture medium was added and the cultures were incubated at 37°. The medium was changed every 2 to 3 days. Outgrowth of the cells from the explants was ordinarily seen on days 7 to 10.

Electron microscopy. The cells grown in plastic petri dishes were immersed in 2% osmium tetroxide in Millonig's buffer for 40 min. Then, they were dehydrated through the graduated ethanol series and embedded in Epon 812 (Luft). Ultrathin sections were cut with an LKB ultratome, stained with a solution of uranyl and a mixture of lead citrate, lead acetate, and lead nitrate (2), and examined in an HS-7 electron microscope.

Results. Establishment of cell line. As listed in Table I, 10 attempts were made and only one cell line was successfully established from carcinomatous tissue specimens. The tissue, from which the cell line originated, came from a patient with a squamous cell carcinoma. The name of the patient from whom the carcinoma had been removed was abbreviated SiHa.

Morphology of SiHa cells. Although the SiHa cells have not as yet been cloned, the culture consists of rather homogeneous cells with apparent epithelial morphology. A phase-contrast micrograph of the culture in its 20th generation is shown in Fig. 1. The cells show high nucleocytoplasmic ratios with conspicuous nucleoli. Electron microscopic observations have revealed the presence of typical desmosomes at the cellular junction and abundance of tonofilaments in the cytoplasm (Fig. 2a, b). No virus-like particles have been detected in the cells.

Summary. A human cell line designated as SiHa has been established from cancerous tissues of the cervix uteri. The studies with light and electron microscopy have revealed the definite epithelial characteristics of the SiHa cells.

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1. Scherer, W. F., Syverton, J. T., and Gey, G. O., *J. Exp. Med.* **97**, 695 (1953).
 2. Sato, T., *J. Electronmicrosc.* **16**, 195, 1967.
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