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Invasion and Metastasis of SY86B Human Gastric Carcinoma Cells in Nude Mice

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A moderately differentiated tubular adenocarcinoma of human stomach, named SY86B, was successfully transplanted subcutaneously to nude mice of different genetic backgrounds (BALB/CA/PBI-*nu*, C57BL/6J.615/PBI-*nu* and ICR·BALB/CA/PBI-*nu*). The tumor has been passaged for 13 generations and the transplantability was 100%. The SY86B cells retained the capacity of invasive and metastatic growth in the nude mice and showed a high rate of metastasis to the regional lymph nodes and to such distant organs as the lungs, liver and pancreas. The overall rate of metastasis was 77.7%. The species of the nude mice, their age and sex apparently did not significantly affect the occurrence of metastasis. Tumor-bearing time and the aggressive character of the tumor cells themselves appeared important for the genesis of metastasis. This experimental model can provide a new approach to basic and clinical studies of cancer metastasis.

Key words: Metastasis — Nude mouse — Human gastric carcinoma

Invasion and metastasis are two of the most important characteristics of malignant tumors. Many workers have tried to establish an experimental model of cancer invasion and metastasis using xenogeneic human tumors in nude mice.[1,2] However, tumors with strong invasive and metastatic potentials in the original host, when transplanted to nude mice, generally showed only a local growth and behaved like benign tumors.[3] These puzzling phenomena have discouraged the use of nude mice for experimental studies of cancer invasion and metastasis. Recent reports have shown that some human tumors can and do metastasize in nude mice,[4,5] but reports on metastasis of human gastro-intestinal carcinomas are unsatisfactory because intraperitoneal[6] or intravenous[7] injection of tumor cells was necessary to generate an organ metastasis or the rate of metastasis was rather low.[8] Recently we preliminarily reported the establishment of a human gastric carcinoma cell line, GC86B, which is transplantable to nude mice and showed a high rate of spontaneous metastasis (55.5%) to the regional lymph nodes and to the lungs of the animals.[9] The cell line is now renamed SY86B to indicate the city (Shenyang) where the cell line was established. Later, through the continuing passages of the tumor cells, it was found that SY86B presents a higher rate of metastasis than we had preliminarily reported and that extra-pulmonary metastasis can occur. The present paper aims to describe the processes of establishing the cell line and to summarize the invasive and metastatic characteristics of the tumor cells in nude mice based on the data obtained through the passages to date.

MATERIALS AND METHODS

Animals Three strains of nude mice with different genetic background (BALB/CA/PBI-*nu*, C57BL/6J.615/PBI-*nu* and ICR·BALB/CA/PBI-*nu*), obtained from the China Institute of Pharmacology & Biological Products, were used. Five- to 32-week-old, male and female animals were available and they were kept in an SPF (specific-pathogen-free) environment. Everything for the mice was autoclaved before being used.

Transplantation of Human Gastric Carcinoma Cells, SY86B The human gastric carcinoma tissue was originally obtained on 17th May, 1986, from a surgical specimen taken from a 58-year-old male suffering from a Borrmann type III lesion. The patient died of systemic metastasis early in 1987. The histological type of the tumor was moderately differentiated tubular adenocarcinoma (Fig. 1). The tissue was cut aseptically into small pieces and placed in an ice-cold culture medium (RPMI-

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1640). One to several tissue fragments of about 2 mm in diameter were transplanted subcutaneously at multiple sites on the back of nude mice using a trocar. The inoculated sites were observed every day and the sizes of individual tumors were measured weekly. When the transplanted tumors reached the size of about 1.0 to 1.5 cm in maximal dimension the animals were killed and serial transplantations were performed by the same method as described above.

Autopsy After the mice were sacrificed a systemic autopsy was carried out. Lungs, liver, heart, pancreas, kidneys, regional lymph nodes as well as the transplanted tumors at the original site of innoculation were removed and fixed in 10% buffered formalin. Paraffin sections were prepared as usual, and histopathological examinations were performed.

RESULTS

Transplantability and Growth Characteristics Since the original transplantation on 17th May in 1986 the SY86B tumor has been passaged for 13 generations. The total transplantability was 100%. Neither infection nor natural death has occurred. The growth of the subcutaneously transplanted tumors was rapid with the mean latent period of 13 days. The tumors were usually ovoid in shape and reached the size of 1 cm in maximal dimension in an average of 25 days. The largest size recorded was 4 cm in the longest diameter at 119 days. No spontaneous regression of the tumors was observed.

Autopsy Findings Generally the tumors at the site of inoculation were covered by a thin semi-transparent skin tissue with rich vascularity. Larger tumors show ulcerations and central necroses. Some tumors adhered to the adjacent soft tissues. Tumor metastasis was detected in the axillary and/or inguinal lymph nodes in the animals killed after 59 days. The lymph nodes with metastasis often reached a size of over 5 mm in maximal dimension and were almost totally replaced by the tumor. Distant metastases to the lungs were often seen, but most of them were microscopic in size. Metastases to the liver and the pancreas also appeared in some of the animals (Table I). The liver metastases at the 7th passage were grossly visible large tumors, each replacing almost a whole lobe of the liver (Fig. 5). In one case at the 9th passage, a tumor located at the lower-left site of the back was found to have penetrated the peritoneal membrane and to be growing in the abdominal cavity, causing a large amount of bloody ascites. Invasion of psoas muscles was also observed. Histologically, the original tumor of human stomach *in situ* showed an infiltrative

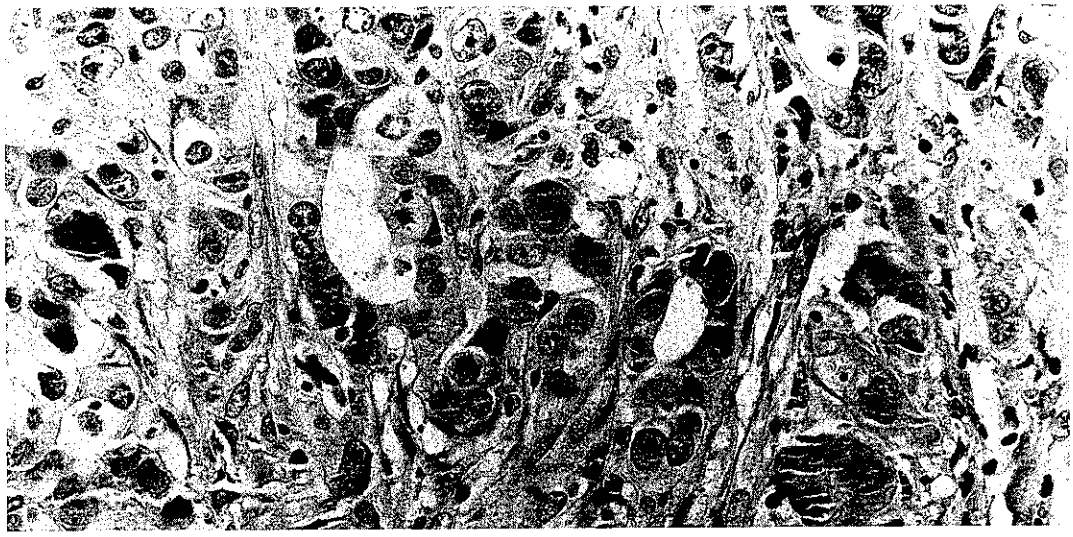

Fig. 1. The original histology of gastric carcinoma of a 58-year-old male suffering from a Borrmann type III lesion. The tumor shows irregular tubular or cord structures of pleomorphic dark cells with irregular, large, hyperchromatic nuclei.

growth consisting of irregular tubular or cord structures of pleomorphic dark cells with irregular, large, hyperchromatic nuclei. Mitotic figures were frequently seen (Fig. 1). The transplanted tumor at the site of subcutaneous inoculation showed an infiltrative growth consisting of papillo-tubular structures with a thin fibro-vascular stroma linded by pleo-

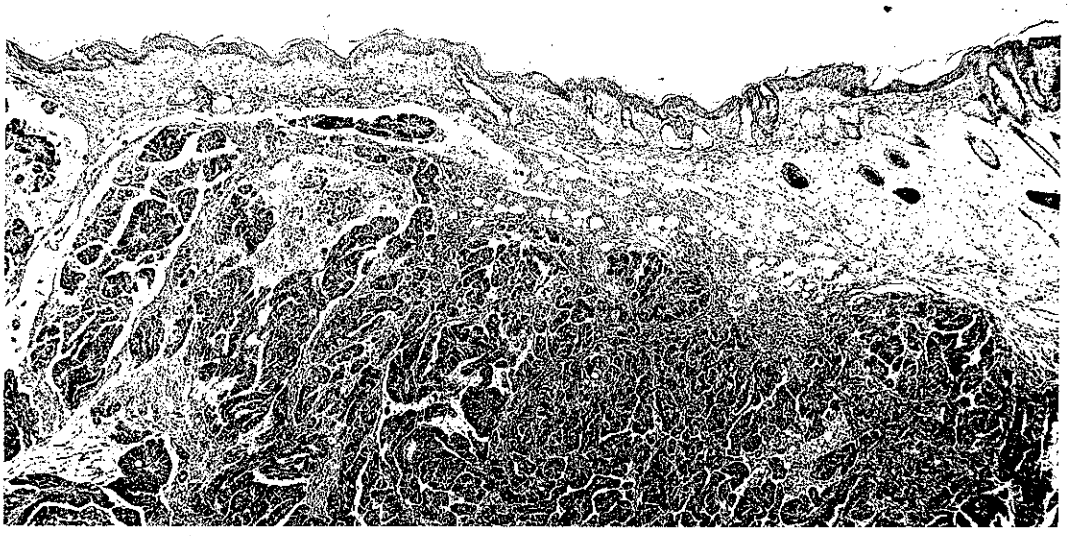

Fig. 2. The transplanted tumor at the site of subcutaneous inoculation in a nude mouse. The tumor shows infiltrative growth, consisting of papillo-tubular structures of pleomorphic columnar cells. Necrotic areas are occasionally seen.

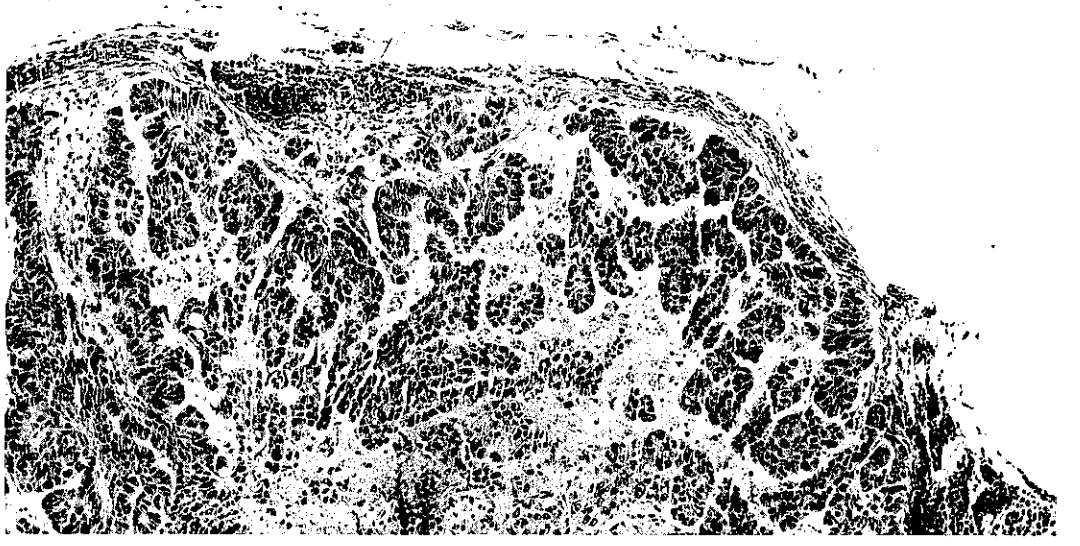

Fig. 3. Tumor metastasis in an axillary lymph node. Tumor cells in paracortical sinuses and their extension to cortical areas are seen. Central areas are necrotic.

morphic dark columnar cells with irregular, large, hyperchromatic nuclei (Fig. 2). Mitotic figures were frequently detected. Areas of necroses were often seen. In the lymph nodes with metastasis tumor cells were characteristically seen in the paracortical sinuses with an extension to the cortex and medullary parts (Fig. 3). Central areas were often necrotic. In the lungs, various histological stages of cancer metastasis were observed. Firstly, the tumor

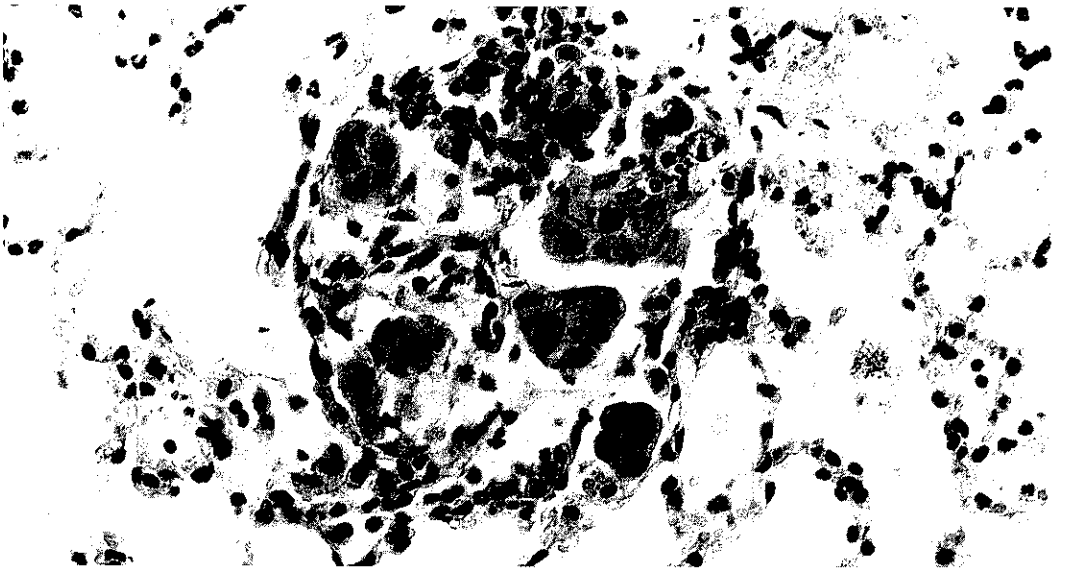

Fig. 4. A focus of tumor metastasis in the lung showing clusters of tumor cells in alveolar spaces.

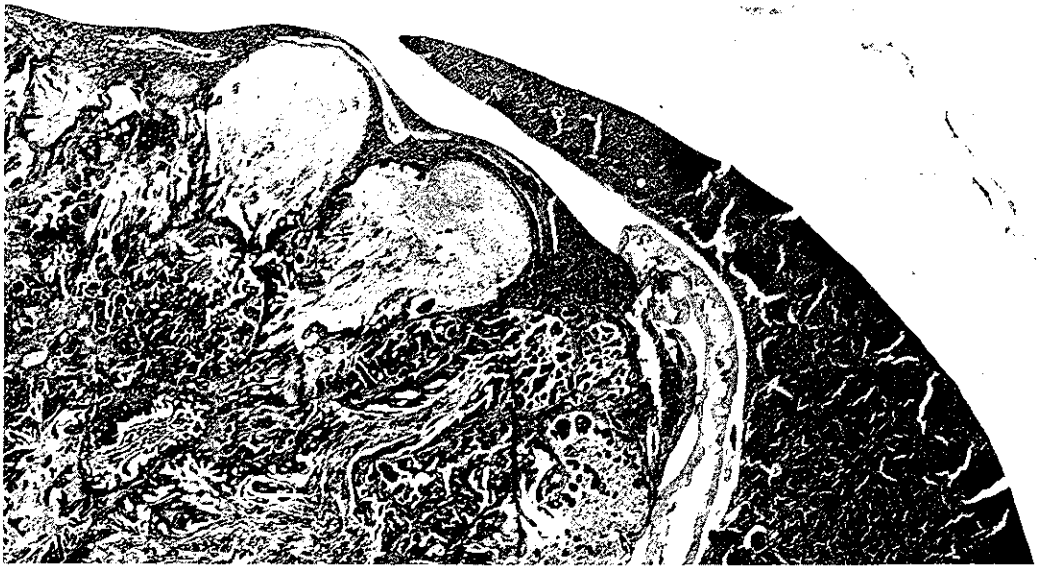

Fig. 5. A tumor metastasis in the liver showing extensive and infiltrative growth almost totally replacing a lobe. Areas of necrosis are often seen. The tumor shows papillo-tubular structures almost the same as those of the transplanted subcutaneous tumor (Fig. 1).

Table I. Passages of SY86B Cells and Metastasis of the Tumor in Nude Mice

Passage No.	Mice No.	Genetic background	Sex	Age (weeks)	Tumor-bearing time (days)	Organs with metastases			
						Lymph node	Lungs	Liver	Pancreas
1	A	BALB/CA	F	5	26	−	−	−	−
	B	C57BL/6J	F	5	43	−	−	−	−
	C[a]	C57BL/6J	F	5	58				
2	A	BALB/CA	F	10	23	−	−	−	−
	B	C57BL/6J	F	10	59	+	−	−	−
3	A	BALB/CA	F	7	85	+	−	−	−
	B	C57BL/6J	F	14	70	+	+	−	−
4	A	BALB/CA	M	11	24	−	−	−	−
	B	ICR·BALB/CA	M	18	71	+	+	−	−
5	A	BALB/CA	M	6	119	+	+	−	−
	B	ICR·BALB/CA	M	6	81	+	+	−	−
6	A	BALB/CA	F	32	84	+	+	−	−
	B[b]	C57BL/6J	F	10	87				
7	A	BALB/CA	F	13	93	+	+	+	−
	B	C57BL/6J	F	13	102	+	+	−	−
	C	ICR·BALB/CA	F	5	87	+	+	−	−
8	A	BALB/CA	M	9	42	−	−	−	−
	B	C57BL/6J	M	9	53	+	−	−	−
	C	ICR·BALB/CA	M	10	60	+	+	−	−
9	A	BALB/CA	F	12	54	−	−	−	−
	B	C57BL/6J	F	13	71	+	+	−	−
10	A	BALB/CA	F	6	70	−	+	−	−
	B	BALB/CA	F	6	82	+	+	−	−
11	A	BALB/CA	F	11	73	+	+	+	+
	B	BALB/CA	F	11	91	−	+	−	−
12	A	BALB/CA	F	5	69	−	+	−	−
	B	BALB/CA	F	5	91	+	+	+	−
13	A	BALB/CA	F	10	90	−	+	−	−
	B	BALB/CA	F	10	82	+	+	−	−

a) Died of thirst.
b) Whole body fixation.
F, Female; M, male.

cells were embolized in the alveolar capillaries, secondly the cells tended to grow outside the capillaries and formed microscopic clusters of tumor cells in the alveolar spaces (Fig. 4), and thirdly formation of a grossly visible, small metastatic tumor nodule in the lung parenchyma. Metastatic tumors in the liver and the pancreas showed almost the same histological features as those seen in the transplanted subcutaneous tumors. Large areas of necrosis were seen (Fig. 5).

The Rate of Metastasis of SY86B in Nude Mice Twenty-one out of 27 tumor-bearing animals in the 1–13th passages were found to have spontaneous metastases, and thus the overall rate of metastasis was 77.7% (21/27). Three cases had only a lymph node metastasis, 11 cases had both lymph node and pulmonary metastases and two cases had lymph node, pulmonary and liver metastases. In addition to the metastasis to the regional lymph nodes, lung and liver, metastasis to the pancreas was

found in mouse A of the 11th passage. The nude mice with metastasis were different in genetic background, age, sex and tumor-bearing time. The data are summarized in the table.

Discussion

The use of nude mice for experimental studies of cancer metastasis is an important challenge in cancer research. However, most reports have emphasized the rareness of metastasis of malignant tumors in nude mice.[3] Sharkey and Fogh transplanted 106 kinds of human tumor cell lines into 1054 nude mice and obtained only 14 cases with metastasis.[1] Ezaki *et al.* transplanted tissues of human gastric carcinomas into nude mice and obtained 100% transplantability, but metastasis from the subcutaneous transplanted tumors was not detected during 20 passages.[6] More recent studies reported higher incidences of spontaneous metastasis in human melanomas[4, 10, 11] and in neuroblastoma.[5] However, metastasis of gastro-intestinal carcinomas showed a rather low rate of occurrence[8] or required an intravenous injection[7] of an established cell line or an intraperitoneal injection[6] of tumor cell suspensions made from solid, subcutaneously transplanted tumors at the site of inoculation.

In the present study the metastatic incidence of SY86B cells was comparatively very high, reaching 77.7%. Human gastric origin of the tumor cells has been proven in our previous paper by the positive reactivities of the cells to monoclonal antibodies against human gastric carcinomas and to anti-human CEA antibody.[9] It is worth noting that the genesis of metastasis in nude mice was not artificial but spontaneous from the subcutaneous tumors at the site of transplantation to regional lymph nodes and to such distant organs as the lungs, liver and pancreas.

Since metastasis was found constantly in nude mice after the second passage, it can be said that SY86B cells possess a stable metastatic property. It was observed that all the nude mice having a metastasis had borne tumors for more than 8 weeks and that more organs tended to be involved in metastasis as the tumor-bearing time was prolonged. These observations indicated that the tumor-bearing time is one of the important factors for the genesis of metastasis. Keeping the tumor-bearing animals alive as long as possible may enhance the rate of metastasis.

Since the nude mice that showed metastasis were different in genetic background, age and sex, it is thought that these factors are less important in the genesis of metastasis.

Histopathological studies disclosed that SY86B cells possessed capabilities for all three types of tumor spread in nude mice, i.e., direct invasion as shown in the subcutaneously transplanted tumors, lymph-born metastasis as typically shown in the regional lymph nodes, and blood-born metastasis as consecutively shown in the lungs. These properties of SY86B cells can make them an ideal experimental model of cancer metastasis. Investigations of the cytological characteristics of SY86B cells are the next step of our research.

In summary, the human gastric carcinoma cells SY86B are transplantable to nude mice and can reproduce in the animals the malignant behavior of the original tumor, showing invasion and a high rate of spontaneous metastasis in regional lymph nodes and in distant organs. This model can provide a new approach to basic and clinical studies of cancer metastasis.

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