EXPERIMENTAL METASTASES FROM CE44 TERATOCARCINOMA CELLS

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Teratocarcinomas are tumors that arise most commonly in the gonads and can be experimentally produced from normal early embryos and primordial germ cells transplanted to extrauterine sites of adult syngeneic recipients. Some teratocarcinomas can grow in ascitic form making up solid and cystic aggregates of tumor cells which are called embryoid bodies because they are morphologically similar to morulas and early blastocysts (Stevens, 1959, 1960; Pierce et al. 1960). The cells located inside embryoid bodies are known as embryonal carcinoma cells because of their close cytologic resemblance to the cells of embryonal carcinoma of human testis (Pierce and Dixon, 1959). The embryonal carcinoma cells are the multipotent stem cells of the teratocarcinoma (Pierce, 1961), and the neoplastic equivalent of the cells of the inner mass of blastocysts. The outer cells of the embryoid bodies express features of endoderm (Pierce et al., 1967; Lehman et al., 1974; Teresky et al., 1974; Martin, 1975).

CE44 teratocarcinoma is a tumor line (Gaillard, 1976) derived from the OTT6050 experimental tumor obtained by Dr. Leroy Stevens in 1970 from a graft of a 6-day strain 129 embryo into the testis of strain 129 adult mouse. CE44 teratocarcinoma cells have been mantained intraperitoneally by serial transfers of ascitic fluid to 129 Sv mouse.

This tumor grows in the peritoneal cavity forming embryoid bodies whereas grafting of embryoid bodies in the subcutaneous space results in solid teratocarcinoma (Gaillard, 1976; Monzó et al., 1983; Parchment et al.,1990; Damjanov,1993; Unda et al. 1994). Moreover, CE44 teratocarcinoma does not give rise to spontaneous metastases. This different behavior of CE44 teratocarcinomas could be related to the fact that the malignancy of teratocarcinoma stem cells can be epigenetically regulated (Damjanov et al., 1983). In order to study the behavior of CE44 teratocarcinoma cells in different locations, in the present work we have developed an easy method to obtain experimental metastases.

MATERIALS AND METHODS

Culture conditions

In the present work we have used the CE44 teratocarcinoma. Isogeneic 129/Sv mice bearing 21-day ascitic tumor were killed and the peritoneal cavity immediately washed with Dulbecco´s Modified Eagle Medium (DMEM). The ascitic fluid was centrifuged at 200 g for 5 min at 4° C. Approximately 10^5 embryoid bodies resuspended in 10 ml DMEM were placed into 75 cm^2 tissue culture treated flasks (Costar, USA) at 37° C in a humidified 5% CO_2-air atmosphere. The medium was DMEM supplemented with 10% Fetal Calf Serum (FCS), 200 units/ml penicillin, 200 mg/ml streptomycin and 2,5 μg/ml amphotericin B (Sigma Co. MO, USA). Monolayers were removed with 2mM trypsin/EDTA. Cells, previously washed with FCS, were filtered through 40μm pore nylon filters (Falcon, Becton Dickinson, USA).

Production of experimental metastases

Cells from 6-day monolayers were used to produce liver and lung experimental metastases. Male and female 129/Sv mice (n>20) were anaesthetized with Nembutal (1.2 mg/mouse, i.p.) and then 0.1 ml of a suspension containing $5x10^5$ viable CE44 teratocarcinoma cells was injected into the spleen or a tail lateral vein, respectively. Mice were killed by cervical dislocation 21 to 30 days after injection of tumor cells. Livers and lungs were perfused with 1.5% glutaraldehyde solution and multiple tissue blocks were processed for transmission electron microscopy by conventional methods.

RESULTS AND DISCUSION

In order to obtain experimental metastases we have used monolayer cells from embryoid body cultures (Figure 1). Around 24 hours after set in culture, embryoid bodies attach to the surface of the flask. Cells produces a nest of cells around the attached embryoid body. 48 hours after, an evident crown-like arrangement of the cells can be observed, whereas the embryoid body has reduced markdly in size: after 72 hours almost no embryoid body can be observed. Around the 4^{th}, the culture arranges into a full monolayer.

In semithin sections coming from monolayers (Figure 2) cells with a dispersed chromatin bearing one or more nucleoli and a clear cytoplasm were observed. Other group of cells displayed nuclei with a dense chromatin and a darker cytoplasm. Between these two cell types, a widely range of intermediate shapes were observed, however the most abundant in the culture. The cells showed cytoplasmatic vacuoli not directly related, in appearance, to a specific cell type of those aboved described. Also, mitosis could be observed.

Mice inoculated into a tail lateral vein showed a high rate of lung metastases, which consisted of solid nodules of tumor cells resembling the internal cells of the embryoid bodies (Figure 3). Cells displayed a clear cytoplasm and a dispersed chromatin nuclei, bearing one or more nucleoli. No differentiated tissues were observed.

On the other hand, mice inoculated intrasplenically with CE44 teratocarcinoma cells showed a splenic tumor. Liver metastases (Figure 4) were formed by tumor cells similar to those observed in the lung. However, the presence of a necrosis zone was more frequently observed inside the center of the metastatic nodules.

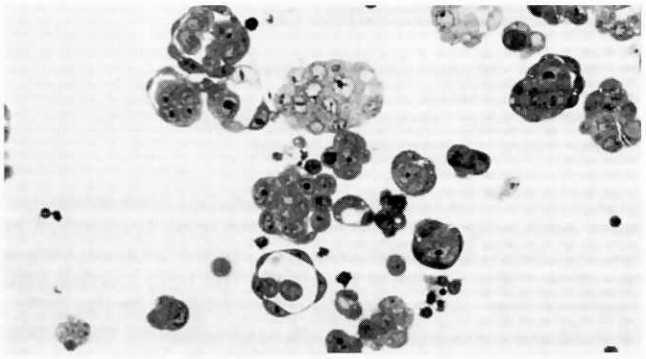

Figure 1. Semithin section of CE44 teratocarcinoma embryoid bodies showing simple and cystic structures.

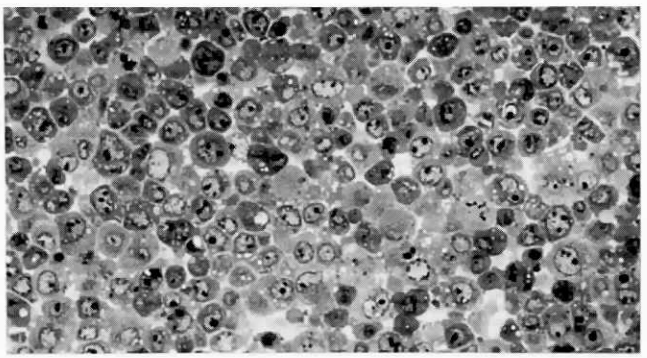

Figure 2. Semithin section of CE44 teratocarcinoma cell monolayer from 6-day culture .

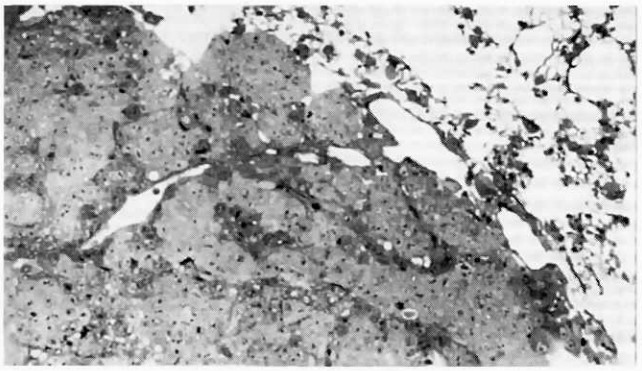

Figure 3. Nodular image of a lung metastasis constituted by densely arranged tumor cells.

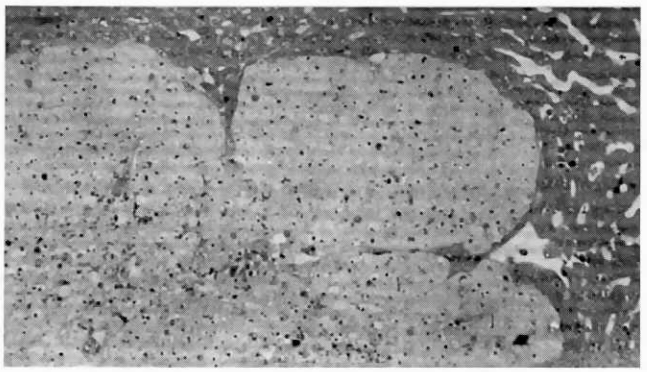

Figure 4. Liver metastasis constituted by nodules of tumor cells, in which the existence of necrosis can be observed.

Our results show that the use of CE44 teratocarcinoma monolayer cells is an adecuate model to produce experimental metastases both in liver and lung. In the present work we used the whole of monolayer cells to produce experimental metastases. Nevertheless, the various population may play a differential role in the production of metastases.

ACKNOWLEDGMENTS

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