Site-dependence of invasiveness of ECA109 human oesophageal carcinoma cells in nude mice

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ECA109 human oesophageal carcinoma cells were injected either subcutaneously or intraperitoneally into BALB/CATc 1-nu/nu mice. After 23 weeks tumours were examined histologically and by scanning electron microscopy. Subcutaneous ECA109 tumours were well-delineated without signs of invasion. By contrast, intra-abdominal tumours invaded into the abdominal wall and abdominal organs. This result provides us with another example of site-dependence of invasion in vivo.

Introduction

Cancer of the oesophagus is responsible for approximately 20 per cent of the total number of deaths in the Linhsien county of North China [18]. This tumour is invasive in early stages of its development [1], and invasion followed by dysphagia is usually the cause of the fatal outcome. To study mechanisms of invasion in oesophageal carcinoma we have used the ECA109 human oesophageal cancer cell line [2]. One of the factors governing the spread of experimental tumours is the site of implantation (review in [12]). We thought that transplantation of ECA109 cells at different sites might be a useful approach to some aspects of their invasiveness. Therefore, we have injected ECA109 cells either subcutaneously (s.c.) or intraperitoneally (i.p.) into nude mice and examined resulting tumours by light and by scanning electron microscopy.

Materials and methods

The ECA109 cell line (obtained from the Department of Cytobiology, Cancer Institute, Chinese Academy of Medical Sciences [2]) was derived from a human oesophageal carcinoma in 1973 and maintained in tissue culture with Medium 199 plus double distilled water (Nissui Seiyaku Co. Ltd, Tokyo, Japan) supplemented with 20 per cent new born calf serum.

Female BALB/CATc1-nu/nu mice (obtained from the Experimental Animal Centre, Chinese Academy of Medical Sciences, Beijing, China) were kept in specific
pathogen-free conditions on a standard diet with acidic (pH 2.5) drinking water. ECA109 single cell suspensions were prepared from cultures on glass surfaces by treatment with trypsin (Difco, Detroit, U.S.A., 0.5 per cent) and ethylene diamino tetraacetic acid (0.2 per cent in Ca\(^{+}\)- and Mg\(^{+}\)-free balanced salt solution) for 10 min at 37°C each. Suspended cells were centrifuged at 90 g for 15 min and resuspended in Medium 199. Aliquots (0.2 ml) of 1 x 10\(^7\) ECA109 cells from the same batch were injected either s.c. or i.p. into respectively ten and eight 6-week-old mice. All animals were killed 23 weeks after injection.

For light microscopy tumours were fixed in Bouin’s solution during 2 days and processed for embedding in paraffin. They were serially sectioned into 5–8 \(\mu\)m thick sections. After every three or six sections, in accordance with the size of the tumours, ten sections were discarded.

Sections were stained with hematoxylin and eosin. For scanning electron microscopy (Cam Scan 3-30 ACV, Cambridge, U.K.) specimens were fixed in glutaraldehyde (3 per cent in cacodylate buffer, pH 7.2 at 4°C) for 2–4 hours followed by post-fixation in osmium tetroxide (1 per cent in the same buffer) for 1 hour and treatment with tannic acid (2 per cent in distilled water) for 1 hour. Fixed specimens were dehydrated through graded series of alcohols, dried by the CO\(_2\) critical point method, and coated with gold–palladium. To visualize the interior parts of the specimen freeze cracking was done [17]. Therefore, tumours were fixed in osmium tetroxide for 1 hour followed by immersion in dimethylsulphoxide 25 and 50 per cent for 30 min each. After freeze cracking specimens were passed through 50 and 25 per cent dimethylsulphoxide for 20 min each, rinsed with balanced salt solution and fixed again in 1 per cent osmium tetroxide. Further processing, starting with tannic acid, was as described above.

**Results**

**Subcutaneous tumours**

Tumours appeared about 1 month after s.c. injection in most mice. During the next 11 weeks the increase in size of these tumours was relatively slow as compared to their increase during the last 8 weeks. In one mouse tumour regression started after 10 weeks. Macroscopically (figure 1) tumours were well-delineated, and were easily removed from the neighbouring tissues.

Light microscopy of s.c. tumours showed the general features of a squamous cell carcinoma. Tumours were surrounded by a multilayered fibrous capsule focally infiltrated by leucocytes (figure 2). Solid parts and cysts were found in all tumours. In the solid parts areas of typical carcinoma were separated by stroma. Walls of cysts were of varying thickness. In solid parts as well as in cysts various degrees of keratinization were observed (figures 3 and 4). Invasion through the fibrous capsule into the surrounding normal tissues was never seen.

Scanning electron microscopy showed nest of ECA109 cells in contact with each other via filopodia and microvilli. Usually, a space was present between the tumour cells and the fibrous capsule (figure 5). Occasionally, contacts between the tumour and the fibrous capsule through filopodia were observed. Sometimes, isolated ECA109 cells appeared to be attached to the host tissue (figure 6).

**Intraperitoneal tumours**

About 16 weeks after i.p. injection of ECA109 cells tumours became palpable in the abdomen. Opening of the abdomen revealed tumours adhering to the abdominal