OB glue paste technique for establishing nude mouse human gastric cancer orthotopic transplantation models

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OB glue paste technique for establishing nude mouse human gastric cancer orthotopic transplantation models using OB glue paste technique.

METHODS: Using OB glue paste technique, orthotopic transplantation models were established by implanting SGC-7901 and MKN-45 human gastric cancer cell strains into the gastric wall of nude mice. Biological features, growth of the implanted tumors, the success rate of transplantation and the rate of auto-metastasis of the two models were observed.

RESULTS: The success rates of orthotopic transplantation of the two models were 94.20% and 96%. The rates of hepatic metastasis, pulmonary metastasis, peritoneal metastasis, lymphocytic metastasis and splenic metastasis were 42.13% and 94.20%, 48.43% and 57.97%, 30.83% and 36.96%, 67.30% and 84.06%, and 59.75% and 10.53%, respectively. The occurrence of ascites was 47.80% and 36.96%.

CONCLUSION: OB glue paste technique is easy to follow. The biological behaviors of the nude mouse human gastric cancer orthotopic transplantation models established with this technique are similar to the natural processes of growth and metastasis of human gastric cancer, and, therefore, can be used as an ideal model for experimental research of proliferative metastasis of tumors.
Cell strains
SGC-7901 and MKN-45 cell strains were kind gifts from the laboratory of experimental pathology of Shanghai Institute of Tumor Research, CAS. Cancer cells were cultured in RPMI-1640 solution containing 10% bovine serum in a CO₂ thermostatic incubator and passaged routinely.

Establishment and passage of subcutaneously transplanted tumor in nude mice
In vitro cancer cells were collected to the content of $1 \times 10^{7}$/mL. Each nude mouse was injected with 0.2 mL cancer cells under the cervical skin. When the implanted tumor grew to about 1 cm diameter, it was removed out of the mouse. The tumor was cut into 1 mm × 1 mm × 2 mm pieces after scraping off the surrounding fibrous capsule, and implanted directly into the cervical skin of the nude mouse. Each inter-mouse passage used two mice. The third generation subcutaneously transplanted tumor was used as the source of orthotopic transplantation in this study.

Establishment of orthotopic transplantation models
The mice were purchased two days ahead of the experiment for environmental adaptation. The animals were fasted 12 h before operation and anesthetized with 0.4% pentobarbital sodium (60 mg/kg) intraperitoneally. The skin was sterilized routinely and a 1 cm incision was made along the left paramedian line to expose the peritoneum and gastric wall meticulously. The serous layer of the greater curvature of stomach where there are abundant blood vessels, was carefully ruptured with an injection needle until bleeding was visible, into which the tumor tissue was implanted. One to 2 drops of medical OB glue (Cyanacrylate, medical OB 508 series for anastomosis, Guangzhou Bai Yun Medical Glue Co., Ltd., Batch No. 030703) were applied to seal the rupture. After the glue coagulated for about 10 s, the peritoneum was closed with No. 3 suture and the skin was closed with No. 1 suture.

Sacrifice of animals and observation of metastasis of the transplanted tumor
When the mice were seen developing failing signs such as leanness, limited activity and listlessness, they were sacrificed by cervical dislocation and anatomized for comprehensive exploration of the chest and abdominal cavities and macroscopic observation of any transplanted tumor with regard to local growth, ascites, adjacent lymph nodes and distal organ metastasis. The transplanted tumor, enlarged lymph nodes, liver, spleen, pancreas and lungs were excised, and the specimens were fixed, sliced and stained for histopathologic observation under light and electron microscope. The tumor was weighed with an analytical balance and recorded. Four mice which died during the study were treated in the same manner without including into statistics, but for reference and later causative analysis.

Preparation of chromosomal specimens of human gastric cancer cells
Part of the transplanted tumor tissues was sheared with aseptic technique and placed in serum-free 1640 medium for primary culture. When the cells grew vigorously and formed a single layer, the first generation passage was done, for which chromosomal specimens were prepared. The specimens were observed under an × 40 light microscope and photographed for chromosomal metaphasis of one cell with a microscopic camera.

RESULTS

Growth of the orthotopically transplanted tumor
One mouse died from excessive bleeding during establishment of the SGC-7901 orthotopic transplantation model. The skin suture fell off at about day 5 and the wound healed completely in a week. At week 3-4, 4 mm nodules were palpable in the upper abdomen, which grew gradually by week 5-6 and became markedly large by week 8-10. At week 10 some of the tumors were even visible through the wall and as large as 10-20 mm in diameter. The surfaces of these tumors were nodular and hard in consistency. From week 11 on, the animals began presenting failing signs such as leanness, limited activity, listlessness and hypoactivity. One animal died. At week 12 the failing signs were more evident and severe, and tumors in some mice subcutaneously projected out, or the abdomens were bulky looking like a frog abdomen. The animals were sacrificed by cervical dislocation.

One animal in the MKN-45 orthotopic transplantation model died on the second day because of suture failure due to mutual fierce biting of the animals. The situation of the remaining animals was much the same as that of the SGC-7901 model. The only difference in the MKN-45 model was that the tumors grew faster in fewer days. By week 2, hard nodules about 4 mm were palpable in both right and left upper abdomen; the tumors became large gradually by week 3-4, and grew to 10-15 mm in diameter by week 5-6; 3 animals died by week 6-7; and the failing signs became worse by week 8 when giant tumors of 15-20 mm in diameter were palpable. The animals were sacrificed by cervical dislocation.

Invasion of the orthotopically transplanted tumor
A total of 164 cases of models with SGC-7901 were established in this study, including 159 cases of SGC-7901 orthotopic transplantation with a success rate of 97% (159/164). Gross anatomy revealed: the body of stomach was enlarged and the fundus was dilated; grayish fish meat like tumors were seen on the gastric wall; the tumor tissue was parenchymatous with vague margins and round or oval in shape. There were nodular processes on the surface, which infiltrated into the surrounding tissues and adhered with the mesentery, liver, spleen and peritoneum in varying degrees. There

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was bloody ascites in some mice. The tumor sections were grayish, looking like fish meat and homogenous in consistency, on which there were abundant capillaries. In some large tumors, there were small necrotic patches in the center. The mean weight of the tumors was 2.31 ± 0.75 g.

A total of 144 cases of models with MKN-45 were established, including 138 cases of orthotopic transplantation, with a success rate of 96% (138/144). Gross anatomic findings were much the same as those of the SGC-7901 model. The tumors were oval in shape, uneven and hard in consistency. There was a serious necrotic area in the center. The mean weight of the tumors was 2.53 ± 0.84 g (Figure 1).

**Metastasis of the orthotopically transplanted tumor**

Intraperitoneal lymph nodes were enlarged in most mice of the SGC-7901 model. Involvement of the tumors was various. Several small grayish milary nodules were seen in the liver of most tumor-bearing mice, which went into the hepatic parenchyma and were difficult to separate. Pyloric obstruction and bloody ascites were seen in some tumor-bearing mice, and pulmonary, splenic and peritoneal metastases were seen in other animals. The metastasis rates of the liver, lungs, peritoneum, lymph node and spleen were 42.02% (67/159), 48.43% (77/159), 30.82% (49/159), 67.30% (107/159) and 59.75% (95/159), respectively. The prevalence of ascites was 47.80% (76/159).

The situation in the MKN-45 model was similar to that of the SGC-7901 model, where the hepatic focus of metastasis was 2-3 mm (Figure 2). The lung surface was congested in some animals and transparent nodules about 1mm were visible. The metastasis rates of the liver, lungs, peritoneum, lymph node and spleen were 94.20% (130/138), 57.97% (80/138), 36.96% (51/138), 84.06% (116/138) and 10.86% (15/138), respectively. The prevalence of ascites was 31.88% (44/138).

**Histopathologic findings of the orthotopical transplantation models**

SGC-7901 gastric cancer tissue was adenocarcinoma of low differentiation. The tumor cells present as oval shape with large malformed nuclei, most of which were of pathologic mitosis with clear and multiple nucleoli. They were deranged like a nestle with rich sinusoids, in which filtration of lymphocytes was seen.

Histological sections of MKN-45 tumor body was also characterized by poorly differentiated adenocarcinoma, where cells were in patchy, nestle or streaky arrangement and shaped round, oval or irregular. Cell differentiation was poor, with large deep stained nuclei and a large nuclear-cytoplasmic ratio, where karyokinetic phase was seen. Fibrous connective tissue was seen in the mesenchyma, and infiltrative growth of the tumor tissue was seen in the gastric wall (Figure 3A).

The histological structure of the tumor metastatic foci in the liver and lungs was consistent with that of the orthotopic gastric tumor, mostly distributing around blood vessels of the lung parenchyma and hepatic sinusoid and growing infiltratively to the surrounding tissues. Large numbers of cancer cells were seen in the enlarged lymph nodes; they arranged closely, destroying almost all lymphatic structure. Cytohistological morphology of ascites smear was similar to that of the orthotopic tumor.

**Identification of cell chromosome of the orthotopically transplanted tumor**

Under ×40 light microscope, no single chromosome in the metaphase was seen as a telocentric chromosome, indicating that it was a human chromosome. Distribution of the chromosomes was more than 46, indicating that they were malignant tumor cells.

**Color doppler imaging of the orthotopically transplanted tumor**

B-ultrasound detected low echo masses in the upper abdomen of the nude mice, which grew into the abdominal cavity (Figure 3B). Most blood flow of the tumors was in the periphery, or mixed blood flow was seen both inside and around the tumor. The course of vessels was irregular and the vessels were various in size and deranged, forming winding, fork-like and net-like blood flows. Blood flow was more abundant and faster in the periphery than in the center of the tumor (Figure 3B).

**DISCUSSION**

Human gastric cancer fresh tissue orthotopic transplantation is the main means of model establishment. The
The present study used OB glue paste technique to establish the human gastric cancer nude mouse orthotopic transplantation model since 2003, when the technique was first attempted in our department. The present study used OB glue paste technique to establish two tumor strain orthotopic transplantation models. Observations showed that although different tumor strains grew at different rates, infiltrative growth and multi-organ metastases are common features of the two models, and these features are similar to the clinical presentation of invasive metastasis. Chromosomal identification also demonstrated that both orthotopic and metastatic tumors came from the human gastric cancer implanted. This technique is an ideal means of model establishment owing to its easier manipulation, shorter operating time, less blood loss, quicker postoperative recovery, and higher survival of experimental animals and avoidance of tumor falling off.

The success rate was not 100% in both cases. Anatomy of the mice that failed to bear tumors showed that there was local organ adhesion arising from manipulation, and part of the transplanted tumor tissues stop growing. With our experience, the following points are worthy of mention with regard to factors affecting the success rate: (1) The amount of glue should be appropriate, 1-2 drops are enough, too much glue would envelope the implant, halting its growth; (2) It is best to wait for 10 seconds or so before closing the abdomen so that the glue can coagulate sufficiently; or it may cause extensive adhesion of the surrounding tissues; (3) It is preferable to use the seromuscular layer near the antrum of the greater curvature, because rich blood flow there facilitates tumor growth and metastasis; (4) Rupture of the seromuscular layer should not be too superficial, and bleeding is the hallmark. In addition, the tumor tissue to be implanted should be placed into the ruptured site before dropping OB glue. Smooth forceps can be used to push the rupture inward to form a denture before implanting the tumor tissues, if necessary; and (5) It is not preferable to leave too much suture outside the abdomen to avoid suturing failure due to fierce biting between animals.

**REFERENCES**

1. Zhu H, Xian LP, Zhang YM, Li XD, Zou DF. Human Cancer of the Stomach Transplanting Tumour Changes the Model of Nude Mouse Research General Situation. *Kexue Jiuba Ya Gongcheng* 2007; 7: 6031-6034

2. Takao S, Shimazu H, Maenohara S, Hokita S, Aikou T. Tumorigenicity, invasion, and metastasis of human gastric cancer in nude mice. *J Cancer Res Clin Oncol* 1991; 117;
Critical factors in the biology of human cancer metastasis: twenty-eighth G.H.A. Clowes memorial award lecture. Cancer Res 1990; 50: 6130-6138

Heterotransplantation of a human malignant tumour to "nude" mice. 1969. *APMIS* 2007; 115: 604-606; discussion 607-608

Optimization of a metastasizing human gastric cancer model in nude mice. *Microsurgery* 2003; 23: 508-512

Yasoshima T, Denno R, Shishido T, Hata M, Furukawa T, Kubota T, Watanabe M, Kitajima M, Wei PK, Xu L, Qin ZF, Shi J. Anticancer effect of Matrix metalloproteinase inhibitor BB-94 (batimastat) in Nude mouse metastatic models of human gastric carcinoma in nude mice. *Clin Exp Metastasis* 2004; 21: 7-12

Yashiro M, Chung YS, Nishimura S, Inoue T, Sowa M. Peritoneal metastatic model for human scirrhous gastric carcinoma in nude mice. *Clin Exp Metastasis* 1996; 14: 43-54

Yamaguchi K, Ura H, Yasoshima T, Shishido T, Denno R, Hirata K. Liver metastatic model for human gastric cancer established by orthotropic tumor cell implantation. *World J Surg* 2001; 25: 131-137

Nakanishi H, Mochizuki Y, Koderia Y, Ito S, Yamamura Y, Ito K, Akiyama S, Nakao A, Tatematsu M. Chemosensitivity of peritoneal micrometastases as evaluated using a green fluorescence protein (GFP)-tagged human gastric cancer cell line. *Cancer Sci* 2003; 94: 112-118

Moriyama K, Walker SM, Nakajima M, Pathak S, Jessup JM, Fidler IJ. Influence of organ environment on the growth, selection, and metastasis of human colon carcinoma cells in nude mice. *Cancer Res* 1988; 48: 6863-6874

Luo MH, Wang WP, Huang PG, Cai QZ, Li FH, Mo MY. Biological behavior of orthotopic implantation model of human gastric carcinoma in nude mice. *Shijie Huaren Xixuehua Zazhi* 1998; 6: 887-890

Matsuoka T, Yashiro M, Sawada T, Ishikawa T, Ohira M, Hirakawa K, Chung YS. Effect of a matrix metalloproteinase inhibitor on a lymph node metastatic model of gastric cancer cells passed by orthotopic implantation. *J Exp Clin Cancer Res* 2001; 20: 213-218

Furukawa T, Fu X, Kubota T, Watanabe M, Kitajima Y, Hoffman RM. Nude mouse metastatic models of human stomach cancer constructed using orthotopic implantation of histologically intact tissue. *Cancer Res* 1993; 53: 1204-1208

Lin Q, Zhou SF. Establishment of Three Orthotopic Implant and Metastatic Models of Human Stomach Cancer in Nude Mice. *Jpn J Cancer Res* 1993; 84: 1023-1028

Liu QZ, Nakajima M, Pathak S, Jessup JM, Fidler IJ. Anticancer effect of jinlongshe granules on human gastric cancer cells orthotopically transplanted in nude mice by S-1, a novel oral derivative of 5-Fluourouracil. *Clin Exp Metastasis* 2004; 21: 1076-1081

Li YJ, Chen YL, Su XM, Wei PK. Study On Nude Mouse Metastatic Models of Human Gastric Carcinoma Constructed by Using Orthotopic Transplantation. *Jpn J Cancer Res* 2004; 95: 6130-6138

Liu QZ, Tuo CW, Zhang N, Zhang D, Ming CR. The high metastatic models of human gastric carcinoma established in nude mice by orthotopic transplantation. *Zhonghua Xiaxue Waike Za Zhi* 2002; 1: 89-92

Bai E, Guo X, Yang L, Wang J, Shi Y, Zhang F, Zhai H, Lu Y, Xie H, Wu K, Fan D. Establishment and characterization of a high metastatic potential in the peritoneum for human gastric cancer by orthotopic tumor cell implantation. *Dig Dis Sci* 2007; 52: 1571-1578

Ma JY, Zhang WB, Guo X, Yang L, Wang J, Shi Y, Zhang F, Zhai H, Lu Y, Xie H, Wu K. Establishment and improvement of orthotopic transplantation of human gastric tumor metastasis model in nude mice. *Ai Zheng* 1997; 16: 343-347

Liu QZ, Tuo CW, Zhang N, Zhang D, Ming CR. The high metastatic models of human gastric carcinoma established in nude mice by orthotopic transplantation. *Zhonghua Xiaxue Waike Za Zhi* 2003; 10: 476-478

Yu ZH, Wei PK, Xu L, Qin ZF, Shi J. Anticancer effect of jinlongshe granules on in situ-transplanted human MKN-45 gastric cancer in nude mice and xenografted sarcoma 180 in Kunning mice and its mechanism. *World J Gastroenterol* 2006; 12: 2890-2894

Yu ZH, Wei PK, Xu L, Qin ZF, Shi J, Xiao Y, Lin HM. [Effects of jinlongshe granules on apoptosis of MKN-45 human gastric cancer cells orthotopically transplanted in nude mice] *Zhongguo Zhongxiyi Jiehe Za Zhi* 2006; 4: 275-280

Xiao Y, Wei PK, Li J, Shi J, Yu ZH, Lin HM. [Effects of Rhizoma kaempferiae volatile oil on tumor growth and cell cycle of MKN-45 human gastric cancer cells orthotopically transplanted in nude mice] *Zhongguo Zhongxiyi Jiehe Xuebao* 2006; 4: 384-387

Sun YQ, Zhang H, Wang Q, Sun X, Xiao ZB. Establishment and improvement of orthotopic transplantation of human gastric tumor metastasis model in nude mice. *Nanjing Xuebao* (Medical Sciences) 2005; 25: 169-170, 173

Wang N, Wang B, Wang YJ. [Effect of vascular endothelial growth factor antibody Avastin on angiogenesis of human gastric cancer growing orthotopically in nude mice] *Ai Zheng* 2005; 25: 1076-1081

Li YJ, He Yan, Wang QM, Liu M, Jin XM. Establishment of Orthotopic Implant M odel of Human Gastric Cancer in Nude Mice Using Glue Paste Technique. *Shoudei Xuebao* 2007; 15: 369-371

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