Abstract. Aim: To evaluate the characteristics of a 13762-MAT-B-III model in the rat liver and to assess the adequacy of the model for transarterial embolization (TAE) study. Materials and Methods: One hundred thousand 13762-MAT-B-III rat breast cancer cells were inoculated into the livers of 11 Fisher 344 rats. Natural course via magnetic resonance imaging (MRI) follow-up, histological characteristics and tumor response after TAE were evaluated. Results: The tumor induction rate of the 13762-MAT-B-III hepatoma model was 100%. Except for one tumor that started to regress after 14 days, the 13762-MAT-B-III hepatomas showed rapid growth, up to 13.1±1.4 mm, at 21 days. Peritoneal seeding was observed in all rats. TAE was conducted successfully in three rats on day 11. The TAE-treated hepatomas were significantly smaller on day 21 (p=0.040) and had a significantly greater apoptosis ratio (p=0.046). Conclusion: The 13762-MAT-B-III hepatoma model can be useful in many interventional oncology studies by providing consistent and rapidly growing tumors.

Animal models of hepatoma play crucial roles in the development of interventional oncology (IO) (1-3). In addition to the conventional rabbit tumor model-bearing VX2 carcinoma in the liver, rat hepatoma models have recently garnered interest by providing in vivo results of many IO methods (4-6). Rat models have many advantages over the rabbit VX2 model in terms of ease of breeding, handling of small animals and availability of diverse biochemical analyses, such as immunohistochemistry (6). Recent advances in experimental techniques also make it possible to catheterize the rat hepatic artery with a minimally-invasive method (6-8) that can simulate the transarterial infusion of anticancer agents in humans.

The Morris model (McA-RH7777 cells in Buffalo rats), the Novikoff model (N1S1 cells in Sprague-Dawley rats) and a fusion of the two models (McA-RH7777 cells in Sprague-Dawley rats) are the orthotopic hepatoma models most commonly used in recent studies (5, 9, 10). However, the Novikoff model and the fusion model have a critical drawback, spontaneous tumor regression due to immune responses, which makes them difficult to use in survival studies (11). The three models simulate the hepatocyte-origin tumor in their biological background aspects. As many IO procedures in humans target hepatic metastasis, as well as hepatocellular carcinoma (HCC), another animal model, to mimic hepatic metastasis, is required as well. In this context, Kan et al. (12) conducted a radiological experiment using rats bearing 13762-MAT-B-III adenocarcinoma in the liver. However, no further studies using this tumor model were published after the study by Kan et al. (12). Since many patients with liver metastasis are managed by diverse treatments in practice and many more modalities are being developed as next-generation treatments, well-established metastatic hepatoma models are in high demand in the animal research field.

Therefore, we induced a metastatic hepatoma model using the 13762-MAT-B-III rat breast cancer cell line in Fisher 344 rats. The purposes of this study were to evaluate the basic characteristics of the 13762-MAT-B-III model in the rat liver and to assess the adequacy of the model for transarterial embolization (TAE) study.

Materials and Methods

Tumor cell line. The 13762-MAT-B-III adenocarcinoma cell line (CRL-1666; ATCC, Manassas, VA, USA) originated from rat mammary glands. The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; WelGENE, Daegu, Korea) supplemented with 10% fetal bovine serum (WelGENE) and 1% penicillin-
Animal model. The study was approved by our Institutional animal care and use committee and performed in accordance with Institutional guidelines. Eleven male Fisher 344 rats, initially weighing 300-350 g, were used for this study. The rats were anesthetized using an intramuscular injection of a mixture of zolazepam (5 mg/kg, Zoletil®; Virbac, Carros, France) and xylazine (10 mg/kg, Rompun®; Bayer-Schering Pharma, Berlin, Germany) into the hindlimb. Afterwards, a midline vertical incision was made to expose the left lateral lobe of the liver. The 13762-MAT-B-III cells (1×10^5, prepared in 50 μl of serum-free DMEM) were injected slowly with a six-channel rat body coil (Stark Contrast, Erlangen, Germany). Axial scans of T2-weighted turbo spin echo images (repetition time/echo time=3,800/78 msec; bandwidth=199 Hz/pixel; flip angle=140˚; slice thickness=2 mm; field of view=120×109 mm; matrix=256×197) were obtained for each animal. A radiologist (J.W.C) selected an image slice scanned through the center of the tumor and the largest diameter of each hepatoma. The measurement was conducted using a picture archiving and communication system (INFINITT PACS; INFINITT Healthcare, Seoul, Korea).

MRI monitoring. MRI scans were performed using a 3.0-Tesla clinical MRI unit (TrioTim; Siemens Medical Solutions, Erlangen, Germany) with a six-channel rat body coil (Stark Contrast, Erlangen, Germany). The rats underwent MRI ten days after tumor cell inoculation to confirm tumor formation and TAE was conducted the next day (day 11). The treatment responses were evaluated by follow-up MRI ten days after TAE (day 21 after tumor cell inoculation), after which the rats were euthanized for histological analysis.

Statistical analyses. The Mann–Whitney U-test was used to compare the differences in tumor size and apoptosis ratio between the eight untreated rats and the three TAE-treated rats. A p-value less than 0.05 indicated a statistical significance. All statistical analyses were performed using the MedCalc software (MedCalc, version 14.10.2; MedCalc Software, Ostend, Belgium).

Results

Characteristics of the tumor model. Liver tumors were identified as round and T2-hyperintense lesions on the initial MRI scan of all of the rats (on day 7 in the eight untreated rats and day 10 in the three treated rats). In the eight rats used to address the natural course of the 13762-MAT-B-III model, the mean±standard deviation of the tumor size on day 7 was 4.4±0.4 mm (range=3.9-5.0 mm). During the serial MRI follow-up, seven of the eight tumors (87.5%) gradually increased; thus, the tumor sizes on day 14 and day 21 were 10.8±1.8 mm (range=8.5-13.1 mm) and 13.1±1.4 mm (range=11.2-14.6 mm), respectively. One tumor, measuring 3.9 mm on day 7 (the smallest among the 11 tumors), had increased up to 7.5 mm on day 21. Immediately after undergoing MRI on day 21, unintended death occurred in two rats who demonstrated extensive peritoneal tumor seeding on autopsy and MRI. The remaining six rats were euthanized the same day (day 21), as scheduled. Autopsy revealed peritoneal seeding in those six rats as well; however, the severity was milder than that of the two rats that died unexpectedly. The MVD of the eight untreated tumors was 280.8±61.0 microvessels/1.65 mm^2. A representative subject is shown in Figure 1.

Tumor response after TAE. Tumor size in the three rats that underwent TAE ranged from 6.1 mm to 6.9 mm on day 10. TAE with internal carotid artery approach was successfully conducted in all three rats on day 11. During the procedure, hypervascular tumors were identified with distinct contrast-agent staining (Figure 2). One rat died the next day of TAE.
Figure 1. Representative rat model of 13762-MAT-B-III hepatoma. The tumor presented as a hyperintense lesion (arrowheads) on T2-weighted MRI taken at seven days (A) and 21 days (B) after tumor cell inoculation. The tumor (arrowheads) was also grossly identified in the explanted liver (C). Hematoxylin and eosin staining (×100 optical magnification) (D) showed the hypercellular viable portion (black asterisk) and necrotic portion (white asterisk) of the tumor. TUNEL staining (×50 optical magnification) (E) also showed the viable portion (black asterisk) and necrotic portion (white asterisk) of the tumor (arrowheads). CD34 staining (×100 optical magnification) (F), as a marker of angiogenesis, showed hyperdense microvessels (brown) in the tumor.
and the autopsy revealed peritoneal tumor seeding. On day 21, the remaining two tumors were measured at 9.8 mm and 8.9 mm. The autopsies of those two rats revealed peritoneal seeding as well, but the severity was much milder than that of the rat that died of TAE. Comparing the TAE-treated tumors (n=2) and untreated tumors (n=8), the TAE-treated hepatomas were significantly smaller on day 21 \((p=0.040)\) and showed a significantly higher apoptosis ratio \((p=0.046)\) (Table I).

**Discussion**

Our study demonstrated that a highly malignant, metastatic hepatoma model could be established by inoculating 13762-MAT-B-III rat breast cancer cells into the livers of Fisher rats. This cell line was originally developed by oral administration of 7,12-dimethylbenz[a]anthracene in rats (13). Although this cell line is used in diverse experimental studies due to its highly malignant characteristics (14, 15), its usefulness in forming a hepatoma has hardly been evaluated. In our study, the liver tumors formed by inoculating 13762-MAT-B-III cells into the livers of Fisher rats were hypervascular, as confirmed by angiography and CD34 staining. This finding is noteworthy, considering that many metastatic liver tumors, which usually originate from colon cancer, breast cancer and neuroendocrine cancer, are hypervascular and that the feature is a key rationale of some treatments, such as TAE and anti-angiogenic agents. Our tumor model was also well-responded by TAE, which is similar to that of human patients in practice.
B-III cells (1×10^5 cells) were injected into the liver peritoneal seeding, a relatively small number of 13762-MAT-untreated group and one in the treated group). To minimize might have led to the three unintended deaths (two in the rats had peritoneal seeding, both mild and severe, which growth rate and high peritoneal seeding rate. In our study, all follow-up studies might be difficult due to the rapid tumor progression. Long-term use of 13762-MAT-B-III hepatoma model. The stability of our model seems satisfactory enough over other tumor models (11). Another advantage of consistent tumor induction is that the treatment–response relationship can be clear. If spontaneous tumor regression occurs frequently, it could be difficult to determine whether the tumor response comes solely from the anticancer effects of a treatment. If too long time is required to form a mass, other variables, such as immunity, can dominantly affect tumor growth. Therefore, the 13762-MAT-B-III model might be more useful in survival studies following anticancer treatments than other hepatoma models.

Our tumor model displayed an 100% tumor induction rate and rapid growth after induction. However, the Novikoff hepatoma model (N1S1 cells in Sprague–Dawley rats) and the fusion model (McA-RH7777 cells in Sprague–Dawley rats) presented complete regression of some tumors three weeks after tumor induction and all tumors disappeared five to six weeks after induction (11). Although one out of eight tumors (12.5%) in our study demonstrated spontaneous regression after two weeks, the stability of our model seems satisfactory enough over other tumor models (11). Another advantage of consistent tumor induction is that the treatment–response relationship can be clear. If spontaneous tumor regression occurs frequently, it could be difficult to determine whether the tumor response comes solely from the anticancer effects of a treatment. If too long time is required to form a mass, other variables, such as immunity, can dominantly affect tumor growth. Therefore, the 13762-MAT-B-III model might be more useful in survival studies following anticancer treatments than other hepatoma models.

The 13762-MAT-B-III hepatoma was visible on MRI. While some hepatic tumors are difficult to recognize with non-invasive imaging (16), all our tumors were depicted as hyperintense lesions on T2-weighted MRI. This feature implies that our model can be monitored non-invasively after induction. However, frequent peritoneal seeding could be an obstacle to the use of 13762-MAT-B-III hepatoma model. Long-term follow-up studies might be difficult due to the rapid tumor growth rate and high peritoneal seeding rate. In our study, all rats had peritoneal seeding, both mild and severe, which might have led to the three unintended deaths (two in the untreated group and one in the treated group). To minimize peritoneal seeding, a relatively small number of 13762-MAT-B-III cells (1×10^5 cells) were injected into the liver compared to other hepatoma models (11, 17). This strategy was feasible because 13762-MAT-B-III cells exhibit a very rapid tumor growth, taking only two weeks to increase about 10 mm in diameter. The experimental technique used was electrocautery followed by slow and gentle inoculation with 13762-MAT-B-III cells. In our preliminary study, undertaken without electrocautery, peritoneal seeding developed at an earlier stage and progressed rapidly. Considering the rapid tumor growth rate of 13762-MAT-B-III cells, even a small amount of cell leakage into the peritoneal space can induce extensive peritoneal metastasis in the early stages. Therefore, the liver entry site was ablated by electrocautery, even though heating can damage tumor cells. As a result, the severity of the peritoneal seeding was able to be minimized without compromising the tumor induction rate (100%).

There were a few limitations to our study. The natural course of the 13762-MAT-B-III tumor model was evaluated in a small number of rats for up to 21 days. To conduct experiments that require longer follow-up periods, researchers should consider other aspects of our tumor model, such as distant metastasis, tumor necrosis and immune response. In addition, the tumor chip implantation method used in the study by Kan et al. (12) was not examined in our study. We used the tumor cell inoculation method because it is easy to prepare and apply with any amount of cells. However, the tumor chip implantation method might have the benefit of preventing peritoneal seeding in the early stage. Lastly, tumor monitoring was only conducted using MRI. Other non-invasive monitoring techniques, such as ultrasonography and positron emission tomography, might be necessary to apply on the 13762-MAT-B-III hepatoma model in diverse experiments.

In conclusion, the 13762-MAT-B-III hepatoma model can be useful in many interventional oncology studies by providing consistent and rapidly growing tumors.

Acknowledgements

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References


Table I. Transarterial embolization responses of 13762-MAT-B-III hepatomas.

<table>
<thead>
<tr>
<th></th>
<th>Treated tumors</th>
<th>Untreated tumors</th>
<th>p-Values</th>
</tr>
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<tbody>
<tr>
<td>Tumor size (cm)†</td>
<td>9.4±0.6</td>
<td>12.8±1.8</td>
<td>0.040</td>
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<td>Apoptosis ratio (%)‡</td>
<td>95.5±2.1</td>
<td>53.7±14.4</td>
<td>0.046</td>
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†Data are mean±standard deviation.


