Relationship between the imaging features and pathologic alteration in hepatoma of rats

Jia-He Yang, Tian-Geng You, Nan Li, Qi-Jun Qian, Ping Wang, Zhen-Lin Yan, Meng-Chao Wu

MATERIALS AND METHODS

Imaging Instruments

MRI equipment was produced by Siemens AG (1.0 T). Its SE rank, 128*128 matrix, T1-weighted sequence (T1WI) were TR/TE 350-480/15 ms; T2-weighted sequence (T2WI) was TR/TE 2 200/90 ms. Slice thickness was 2 mm and scan space was 0.5 mm. Both of coronal and sagittal scanning was fulfilled. Hepatic artery was scanned by DSA (Toshiba DFP-0.3A) with perfusing iodized oil (Lipiodol). Instruments and drugs were sterilized routinely.

Animals and Hepatoma cell

Male Wister rats (200-250 g wt) were obtained from Animal Center of Chinese Academy of Sciences. Animals were maintained on a standard diet. Cellular strains of hepatoma CBRH3 were provided kindly by Prof. Xie Hong (Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science).

Implanted liver cancer model in rat

Hepatoma CBRH3 cells were injected into abdominal cavity of rat. Rats were sacrificed and tumors were removed from abdominal cavity 7-9 days late. The tumors were cut into pieces of 0.05-0.75 cm and then was inoculated into rat liver for one or more locations respectively. The tumor was grown up to diameter in 0.6-1 cm after 7-10 days of inoculation.

Induced liver cancer model in rat

Male Wister rats (age for 8 weeks) took orally 1:10 000 diethylnitrosamine for 80 days to induce liver cancer. The micro-nodules of hepatoma were developed 14 weeks late and 0.2-1 cm of tumor masses were observed 16-18 weeks late. There were poly-cysts in rat liver. This model was used to study for poly-nodules of hepatoma. The success rate of induced cancer was 100 %.[9,10]

Experimental procedure

Both of 10 implanted tumor rats (age of 9 days) and 10 induced tumor rats (age of 16-18 weeks) was divided to experimental group and control group, randomly. While rats were scanned by MRI, they were anesthetized by injecting ketamine into muscle. In addition, the hepatic artery and gastroduodenal artery were isolated carefully and micro-catheter was inserted into these artery for injection of iodine oil before DSA[11].

RESULTS

A stable model of rat hepatoma was established by either implanted tumor with cancer piece or inducing tumor with diethylnitrosamine.
Imaging features of the implanted tumors in MRI and DSA and the pathology: The 10 implanted models showed 18 nodules of tumor in MRI and DSA. 11 nodules of them were round shape and 7 of them were ellipse. The margin of tumors was smooth. Implanted cancer showed homogeneous hypointensity on T1WI (Figure 1) and homogeneous hyperintensity on T2WI. The rat liver showed normal signal and normal organ shape.

In DSA, the tumors showed no stain for blood vessels in the center of tumor (vacancy of vessel) and abnormal ring-like staining of vessel around tumor. The liver tissues were normal. Necrosis and colliquational cavity were shown on the core of tumors in all of 18 tumor mass and the yellow-gray tissue could be seen in cross section of tumors. The cancer cells were small or mediate in size with great nucleus. The parenchyma of liver was normal (Figure 2).

The scanning pictures of MRI and DSA and the pathological alteration in induced cancer: Liver cirrhosis with different degree and multiple nodules of cancer were observed in 10 tumor cases. Eight among 10 tumors showed homogeneous slight hypo-intensity on T1WI (Figure 3) and inhomogeneous slight hyper-intensity on T2WI with different shapes, but some of them were with normal signal or more contrast enhancement (Figure 4). Two among 10 tumor livers were shown inhomogeneous slight low signal on T1WI, but equal signal was found in interval. Hypointensity was observed on T2WI.

In DSA the distribution of internal hepato-vascularity was disorder and the angio-clumps were different in sizes in tumor (Figure 5). Pathology: Tumor liver showed poly-nodules, tumor diffusion and inequality of size. The tumor was light yellow. In microscopy examination, distribution of tumor and liver tissue was in interval. The cancer tissue showed plenty and dilation of vessel. The cell plasma of cancer was not abundant. The arrangement of tumor cell was nest and bunch. Liver tissue was easy for proliferation of nodule with acid-like change and water-alteration. The dilation of lymphatic tube and the proliferation of bile duct were shown between hepato-lobule. Hepato-lobule was divided by proliferation of fibre. The artery and vein were not distinguished because of dilation of vessel (Figure 6).
DISCUSSION

Characterization of image technique of rat
The available MR image photos were taken because technique is as follow. (1) Technique of eye surface loop: To avoid disturbing factors for image definition that focus on the location of rat liver, this technique was used. (2) Folium technique keeps away the cabin effect on conceal of small tumor nodules. (3) False shadow caused by move of body act and breath was prevented by suitable anesthesia for rats. On DSA image, deep anesthesia and careful anatomy of hepatic artery with catheterer were necessary. But it is difficult to puncture femoral artery. In order to find available condition of exposure time, we try to test more times[12].

The relation of image and pathology in implanted cancer
The component of tumor is of long longitudinal relaxation time (T1) and long transverse relaxation time (T2) since the implanted cancer show low signal on T1WI and equal signal on T2WI in MRI. Equal signal intensity implies that there are no tissue component in the core of tumor apart from necrosis, liquefaction and cystic change. Therefore, the situation of blood supply was not described due to no-contrast enhancement of Gadolium-DTPA[13,14].

On DSA, the imaging of blood vessel around tumor was found such as folium annularity, whereas central liquefaction and necrosis were existed in tumor. Thus, cancer cell on the border of tumor grows actively because of blood supply in plenty and cancer cell in center of tumor does not grow because of insufficient blood supply. There is no liver cirrhosis because signal intensity of liver tissue in MRI and distribution of blood vessel in DSA are normal compared to normal liver parenchyma[19].

Necrosis at core of tumor and the proliferated nodule of tumor cell were found around blood sinus and dilation of blood vessel after 9 days of implanted cancer[16,17]. The change in MRI and DSA was accord to pathology.

The relation of image and pathology in induced cancer
Recent study has suggested that a random clonal origin of hepatocyte carcinoma from mature hepatocytes is seen in the diethylnitrosamine model of hepatocarcinogenesis[18].

Induced cancer is of two different signal intensities including 1. slight low signal intensity on T1WI and slight high signal intensity on T2WI in 8 cases and 2. inhomogeneous low signal on T1WI and low signal on T2WI with regular appearance in 2 cases. We hypothesize that both signals is caused by the difference in hepatoma component and hepatoma cellular arrange. Amount of fibrocyte, apart from tumor cells, is found in these 2 cases which result in extended longitudinal relaxation time (T1) and contracted transverse relaxation time (T2) with low signal on T1WI and low signal on T2WI. Irregular liver shape and liver nodule proliferation in deferent degree with liver cyst could be manifested on MRI because of liver cirrhosis and degeneration resulting from taking carcinogen for long time. To differentiate with cirrhosis nodules and tumor nodules, some investigators reported that the early-enhancing single nodule with cirrhosis is high predictive to hepatocyte cancer in patient. This phenomenon is also seen in our data of photo and microscopy examination[20,21]. On DAS, the tumor stain is displayed by reason of disorder of blood vessel and angio-clamp[22].

Comparison imaging of implanted, induced and human hepatoma

Primary liver cancer Based on the liver cirrhosis, primary liver cancer display the slight low signal intensity on T1WI and equal or slight high signal intensity following extended echo time without raise of signal in MRT[23]. In DSA, the plenty amateur vessel is observed[24].

Liver metastases MRI shows the low signal intensity on T1WI, high signal on T2WI and annular enhancement by Gadolium. In DSA, annular staining is displayed without background of cirrhosis[15]. Compared with manifestation of MRI and DSA, studies confirm that implanted hepatoma lead that the signal intensity is high following the extended echo time and annular staining around tumor is occurred without liver cirrhosis[25].

In induced cancer, liver cirrhosis with slight high signal or low signal due to extended echo time without raise of signal is observed in MRI. The abundant malignant vessel stain is indicated that is similar to human primary liver cancer[26,27]. In conclusion, the imaging features of implanted cancers were similar to human liver metastases in manifestation of MRI and DSA. Therefor, it could serve as an experimental model of human liver metastatic tumor. The imaging feature of induced cancers, whereas, were similar to that of human primary liver cancer. It could be use as an experimental model of human primary liver cancer.

REFERENCES

1. Foley LM, Towner RA, Painter DM. In vivo image-guided H-magnetic resonance spectroscopy of the serial development of hepatocarcinogenesis in an experimental animal model. Biochim Biophys Acta 2001; 15: 230-236
2. Eliat PA, Lechaux D, Gervais A, Rioux-Leduc N, Franconfi C, Lemaire L, Dazorl D, Catros-Quemener V, de Certaines JD. Is magnetic resonance imaging texture analysis a useful tool for cell therapy in vivo monitoring? Anticancer Res 2001; 21: 3857-3860
3. Scappaticci FA, Morris AJ. Mechanisms and future directions for angiogenesis-based cancer therapies. J Clin Oncol 2002; 20: 3906-3927
4. Kawai K, Tani K, Yamaohita N, Tornikawa S, Eriugica M, Fumije M, Okumura K, Kazikoe T, Clift S, Ando D, Mulligan R, Yamauchi A, Noguchi M, Asano S, Akaza H. Advanced renal cell carcinoma treated with granulocyte-macrophage colony-stimulating factor gene therapy: A clinical course of the first Japanese experience. Int J Radiol 2002; 9: 462-466
5. Parkes AT, Spears V. British cancer research meeting, 30 June-3 July 2002, glasgow. Breast Cancer Res 2002; 4: 202-204
6. Zhang L, Shuy L, Huang X, Roth JA, Fang B. Mechanisms involved in development of resistance to adenosinemediated proapoptotic gene therapy in DLD1 human colon cancer cell line. Gene Ther 2002; 9: 1262-1270
7. Gillies SD, Lan Y, Bruhnkhorst B, Wong WK, Li Y, Lo KM. Bi-functional cytokine fusion proteins for gene therapy and anti-body-targeted treatment of cancer. Cancer Immunol Immunother 2002; 51: 449-460
8. Zhao W, Kobayashi M, Ding W, Yuan L, Seth P, Cournan S, Wang J, Okada F, Hosokawa M. Suppression of in vivo tumorigenicity of rat hepatoma cell line KDH-8 cells by soluble TGF-beta receptor type II. Cancer Immunol Immunother 2002; 51: 381-388
9. Balansky RM, Ganchev G, D’Agostini F, De Flora S. Effects of N-acetylcysteine in an esophageal carcinogenesis model in rats treated with diethylnitrosamine and diethyldithiocarbamate. Int J Cancer 2002; 98: 493-497
10. Rao MS, Kashirreddy P. Effect of castration on dehydroepiandrosterone-induced hepatocarcinogenesis in male rats. Anticancer Res 2002; 22: 3400-3411
11. Thorlacius H, Larmark M, Randell M, Hultberg B, Jeppsson B. Isolated liver perfusion permits administration of high doses of chemotherapeutic agents. Comparison with hepatic artery infusion. Eur Surg Res 2003; 33: 342-347
12. Zhang H, Liu Y, Cao W, Liu Y, Fan A. Establishment of modified model of VX-2 carcinoma in rabbit liver and DSA imaging features of the tumor. Zhonghua Ganzangbing Zazhi 2002; 10: 149
13. Krupski G, Amann D, Catalaehrmann G, Herbst H, Hanschel MG, Nicolas V, Rogiers X, Bucheler E. Native magnetic resonance tomography imaging of the Morris hepatoma (MH-7777A) in the rat. Rofo Fortschr Geb Rontgenstr Neuen Bildgeb Verfahr 2001; 173: 639-642
14. **Lauenstein TC**, Goehde SC, Herborn CU, Treder W, Ruehm SG, Debatin JF, Barkhausen J. Three-dimensional volumetric interpolated breath-hold MR imaging for whole-body tumor staging in less than 15 minutes: a feasibility study. *Am J Roentgenol* 2002; **179**: 445-449

15. **Turler A**, Schaefer H, Schaefer N, Wagner M, Maintz D, Qiao JC, Hoelscher AH. Experimental low-level direct current therapy in liver metastases: influence of polarity and current dose. *Bioelectromagnetics* 2000; **21**: 395-401

16. **Kuppen PJ**, Gorter A, Hagenaars M, Jonges LE, Giezeman-Smits KM, Nagelkerke JF, Fleuren G, van de Velde CJ. Role of NK cells in adoptive immunotherapy of metastatic colorectal cancer in a syngeneic rat model. *Immunol Rev* 2001; **184**: 236-243

17. **Nakamoto T**, Inagawa H, Nishizawa T, Honda T, Kanou J, Nagasue N, Soma G. Antitumor effects of isolated hypoxic hepatic perfusion (IHHP) with high-dose TNF against colonic liver metastases in a rat model. *Anticancer Res* 2002; **22**: 2455-2459

18. **Bralet MP**, Pichard V, Ferry N. Demonstration of direct lineage between hepatocytes and hepatocellular carcinoma in diethylnitrosamine-treated rats. *Hepatology* 2002; **36**: 623-630

19. **Guo WJ**, Li J, Ling WL, Bai YR, Zhang WZ, Cheng YF, Gu WH, Zhuang JY. Influence of hepatic arterial blockage on blood perfusion and VEGF, MMP-1 expression of implanted liver cancer in rats. *World J Gastroenterol* 2002; **8**: 476-479

20. **Dean CE Jr**, Benjamin SA, Chubb LS, Tessari JD, Keefe TJ. Non-additive hepatic tumor promoting effects by a mixture of two structurally different polychlorinated biphenyls in female rat livers. *Toxicol Sci* 2002; **66**: 54-61

21. **Stoker J**, Romijn MG, de Man RA, Brouwer JT, WEVERLING GJ, van MUISWINKEL JM, ZONDERVAN PE, LAMERIS JS, IJZERMANS JN. Prospective comparative study of spiral computer tomography and magnetic resonance imaging for detection of hepatocellular carcinoma. *Gut* 2002; **51**: 105-107

22. **Carroll NM**, Alexander HR Jr. Isolation perfusion of the liver. *Cancer J* 2002; **8**: 181-193

23. **Jeong YY**, Mitchell DG, Kamishima T. Small (<20 mm) enhancing hepatic nodules seen on arterial phase MR imaging of the cirrhotic liver: clinical implications. *AJR Am J Roentgenol* 2002; **178**: 1327-1334

24. **Vogi TJ**, Balzer JO, Mack MG, Bett G, Oppelt A. Hybrid MR interventional imaging system: combined MR and angiography suites with single interactive table. Feasibility study in vascular liver tumor procedures. *Eur Radiol* 2002; **12**: 1394-1400

25. **Teng GJ**, He SC, Guo JH, Cai XL, Gao GR. Preoperative transcatheter hepatic arterial embolization for hepatic malignancy. *Invest radiol.* 1993; **28**: 235-241

26. **An Y**, Bie P, Dong J. Hepatic artery and portal vein dual perfusion chemotherapy in combination with injection of lipiodol-ethanol in treatment of advanced primary hepatocellular carcinoma. *Zhonghua Wai Ke Za Zhi* 2001; **39**: 593-595

Edited by Pang LH