Radio-frequency ablation-based studies on VX2 rabbit models for HCC treatment

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer worldwide with high morbidity, mortality and increasing incidence. It is of note that the main curative therapies for HCC are hepatic resection and transplantation although the majority of patients at the time of presentation are not eligible for resection or orthotopic liver transplantation (OLT) due to the underlying cirrhosis. Currently, a variety of loco-regional therapies, including radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), microwave coagulation therapy (MCT), transarterial chemoembolization (TACE) and others, have been developed as alternative treatment options for HCC. Among these techniques, RFA is currently the most widely used treatment, due to its several advantages, such as safety and efficacy. To date, the effectiveness of RFA for HCC is reduced by the presence of residual tumor as a consequence of insufficient treatment. In order to ameliorate the effects of RFA on HCC, several in vivo studies, have been performed on its application as single or in combination treatment with drugs or others loco-regional therapies, by using rabbit VX2 liver model. This represents an ideal model of liver cancers and is widely used for imaging and other experimental studies due to the rapid growth of these tumors and their similarity to human hepatocellular carcinoma. In order to elucidate the therapeutic potential of RFA with adjuvant treatments for HCC, we reviewed the latest findings on the RFA-based studies in rabbit VX2 hepatocarcinoma models.

Keywords: Hepatocellular carcinoma, RFA, Radiofrequency ablation, Vx2 tumors, Residual tumor

Background

HCC is still one of the most important diseases for health care systems due to its high mortality, morbidity, and increasing incidence worldwide [1, 2]. It is of note that the main curative therapies for HCC are hepatic resection and transplantation although the majority of patients at the time of presentation are not eligible for resection or OLT due to the underlying cirrhosis. Difficulties in surgical resection may be associated to site, size, and number of tumors, vascular and extra hepatic involvement as well as the general condition and liver function of the patients [3–6]. Only about 20% of HCC cases are classified as resectable [7]. Moreover, the liver is considered a site of metastasis from other solid cancers [8–12]. To date, a variety of loco-regional therapies, RFA, PEI, MCT, TACE and others, have been developed as alternative treatment options for HCC due to its benefits, such as safety, minimal invasiveness, and efficacy [7]. It has been demonstrated that the effectiveness of RFA for HCC was reduced by the presence of residual tumor and local recurrence after treatment, probably due to the location of tumor around the intrahepatic vasculature [13] or to the diameter of tumor. Several studies showed that, residual tumor progression after insufficient RFA could be associated to different reasons and molecular mechanisms [14–18] in particular to the inflammation process which is involved in tumor progression of different types of cancer [19, 20]. The inflammation is induced by thermal destruction of liver carcinoma after RFA at the target sites, leading to progression of HCC tumor [20]. In order to ameliorate the effects of RFA on HCC and to bypass the problem of residual tumor, several in vivo studies have been performed on its application as single or in combination treatment with drugs or other loco-regional therapies, by using rabbit VX2 liver carcinoma model [18, 21–24].
The rabbit VX2 tumor model is widely used in experimental oncology. It is classified as leporine anaplastic squamous cell carcinoma being characterized by rapid growth, hypervascularity and easy propagation in the skeletal muscle [25–28]. This model has been applied to various types of cancer [29–38], and recently it has been used in doxorubicin interventional chemotherapy of renal carcinoma [39]. The transplantation of the VX2 cells can be achieved either by injecting the tumor cell suspensions or by implanting solid tumor pieces (fresh or frozen) as previously described [40].

This represents an ideal model of liver cancers due to the rapid growth of VX2 tumors and their similarity to human hepatocellular carcinoma. Here we reviewed the latest findings on the RFA based studies in rabbit VX2 hepatocarcinoma models, with the aim of elucidating the therapeutic potential of RFA with adjuvant treatments for HCC.

**Generation of VX2 model for liver tumor treatment: an overview of loco regional therapy-based studies**

Several studies demonstrated that intrahepatic implantation of solid tumor fragments is more successfully that the injection of a cell suspension [41–43], although a sonography implantation of liver tumors achieved a good success rate [44]. The protocol for liver implantation has been detailed described by Parvivian et al. [40]. It is of note that the preferred implantation site for VX2 tumors is the left lateral hepatic lobe due to a more favorable angle of the feeding artery for later angiographic catheterization. Our group and other researchers demonstrated that the tumors developed after 2–4 weeks form tissue implantation with nodules of 2-3 cm [38] (Fig. 1).

It also has been reported that the application of ultrasonography, is useful to following the solid tumor growth and to detect the presence of necrosis [44]. The use of non-necrotic tumors allows optimizing the evaluation of tumor response to loco-regional therapy experiments. It is largely provided that loco-regional therapies, including RFA and TACE, play a major role in the clinical management of hepatocellular carcinoma [45]. Regarding pre-clinical studies, many evidences reported the safety and the efficacy of different loco-regional treatments in rabbit VX2 liver tumor model, especially in combination with adjuvant substances. Gholamrezaneshad et al, evaluated the pharmacokinetic profile (PK) and embolization effect of 70–150-μm doxorubicin eluting beads (DEBs) following intra-arterial injection, in the rabbit liver VX2 tumor model [46]. In VX2 model of liver metastases, it has been reported that HepaSphere and DEBS microspheres loaded with irinotecan, caused significant necrosis of tumor nodules [47]. Xia et al, showed that intra-arterial interleukin-12 gene delivery combined with chemoembolization, had a potent anti-tumor effect in a VX2 rabbit HCC [48]. In another study performed on the rabbit VX2 liver tumor model, was demonstrated that the application of Electroporation-Mediated Transcather Arterial Chemoembolization (E-TACE) increased liver tumor chemotherapeutic uptake following targeted transcatheter infusion [49]. In addition, Deng et al, showed that TACE with arterial administration of Endostar (an antiangiogenic agent) inhibited the angiogenesis biomarkers associated with TACE in a rabbit model bearing VX2 liver tumor [50]. Potentiated anti-tumor effects on liver cancer, were also observed in a similar study with chloroquine and TACE. The authors showed that chloroquine, which is a traditional drug used for treatment of malaria [51], promoted the anticancer effect of TACE in a rabbit VX2 liver tumor model. Other studies proved that single-bolus regional chemotherapy with doxorubicin, had limited anti-tumor effects when compared with TACE in a rabbit VX2 liver tumor model [52]. Very recently, it has been reported that the elaborate integration of TACE with nanoparticle-enhanced High-intensity focused ultrasound (HIFU) cancer surgery, applied in VX2 liver tumor model, could efficiently enhance the HCC cancer treatment outcome, representing a new and efficient therapeutic protocol/modality for clinic cancer treatment [53]. Another research demonstrated that Transarterial oily chemoembolization (TOCE), one of the most effective approaches for

**Fig. 1 VX2 tumor liver development. a** Picture reveals resected hepatic tumor after 4 week from VX2 tumor pieces implant, **b** Tumor bisected shows necrotic core (asterisk) and peripheral viable tumor (arrows)
the treatment of patients with HCC, who are not suitable for surgical therapy [54], combined with Lidamycin (LDM) [55], shows potent therapeutic efficacy in VX2 rabbit liver tumor model. Synergistic effects of RFA and toll like receptor 9 (TRL9) stimulation result in a potentiated antitumor T cell response and cytotoxicity in the VX2 hepatoma tumor [23].

Taken together all these different data, summarized in Table 1, suggest that these combined treatments may have potential synergistic effects on liver cancer.

Radio-frequency ablation of VX2 rabbits model for HCC treatment: experimental studies

RFA is considered one of the standard procedures for local tumor treatment such as prostate [56, 57], kidney [56], bone [58, 59], brain [60, 61], lung [62, 63] and liver tumors [45, 64–67]. RFA is commonly used for the treatment of early HCC, although recently the technique has been improved to bypass the problem of burden for patients and operators [68]. RFA is classified as thermal technique since induces cell tumor destruction by heating tumor tissue to high temperatures [69, 70]. It is important to underline that the effectiveness of RFA for HCC is reduced by the presence of residual tumor as a consequence of insufficient treatment. It is very difficult to avoid the residual tumor due to several causes such as the liver’s anatomy, the mechanisms of RFA, the pathological characteristics of HCC and the inflammation. It has been demonstrated that despite it is possible to set the target temperature around 105–115 °C during RFA, only the tissues surrounding the electrodes can reach that temperature due to “heat sink” effect of blood large vessels near the tumor [71].

Table 1 Effects of Loco-regional treatments on tumor growth in VX2 rabbit model of liver cancer

| Animal model | Treatment | Effects on tumor | Reference |
|--------------|-----------|------------------|-----------|
| Rabbit VX2 liver tumor model | TACE, DEBs | Tumor growth reduction | [46] |
| Rabbit VX2 liver metastasis model | HepaSphere and DC Bead microspheres loaded with | Enhanced Tumor necrosis | [47] |
| Rabbit VX2 HCC model | IL12, TACE | Tumor growth reduction | [48] |
| Rabbit VX2 HCC model | Endostar, TACE | Tumor growth reduction | [50] |
| Rabbit VX2 liver model | Chloroquine, TACE | Tumor growth reduction | [51] |
| Rabbit VX2 liver model | TACE, HIFU | Tumor growth reduction | [53] |
| Rabbit VX2 liver tumor model | LDM, TOCE | Tumor growth reduction | [54] |
| Rabbit VX2 HCC model | TRL9, RFA | Tumor growth reduction | [23] |

**Table 2** Effects of RFA on the progression of residual HCC in VX2 rabbit model of liver cancer

| Animal model | Treatment | Effects on the progression of residual HCC | References |
|--------------|-----------|------------------------------------------|------------|
| Rabbit VX2 HCC model | RFA at different temperatures (55,70 and 85 °C) | Low temperatures induce rapid progression of residual HCC | [18] |
| Rabbit VX2 HCC model | Aspirin, RFA | Reduced progression of residual HCC associated to reduced inflammation | [21] |

Many evidences have demonstrated a rapid progression of residual HCC after RFA [72, 73]. In addition, this tumor becoming very aggressive, switches to sarcoma [74, 75] thus leading to bad prognosis for patients.

In order to ameliorate the effects of RFA on HCC and to clarify the underlying mechanisms of rapid progression of residual tumor after insufficient RFA, several in vivo studies, have been performed using rabbit VX2 hepatocarcinoma model. The study conducted by Ke et al., was designed to prove whether low temperature of RFA at the target sites, and could facilitate progression of residual hepatic VX2 carcinoma trying to dissect the underlying mechanisms. The VX2 nodules were transplanted into the liver rather than derived from the liver itself, in order to reduce the feeding artery and the heat sink effect. The residual VX2 hepatoma model in rabbits was established by using RFA at different temperatures (55, 70 and 85 °C). Actually, it is of note that different molecular factors are involved in HCC progression and metastasis, such as IL-6, PCNA, MMP-9, VEGF, HGF [76–78]. The authors demonstrated that residual hepatic VX2carcinoma facilitated its progression through inducing over expression of several molecular factors, such as PCNA, MMP-9, VEGF, HGF and IL-6 [18].

In another research, was proved that the inflammation induced by RFA at the target sites, facilitated the progression of residual HCC tumor [21]. The authors generated the orthotropic VX2 rabbit HCC model with two hepatic tumors in different lobes and treated the animals with different doses of aspirin used as anti-inflammatory drug. Results from this study demonstrated that aspirin inhibited the inflammatory reaction of animals after RFA, suggesting that aspirin could be potentially used as an adjuvant therapy with RFA for treating HCC. Table 2 summarizes the effects of RFA on the progression of residual HCC in VX2 rabbit model of liver cancer.

**Conclusions**

Altogether these data suggest that inflammation and low temperature of RFA at the target sites could be important reasons for rapid progression of residual hepatic VX2
carcinoma. Future studies will be needed to validate the therapeutic potential of drugs or other loco-regional techniques as an adjuvant therapy with RFA for treating HCC.

**Abbreviations**

(E-TACE), Electroporation-Mediated Transcatheter Arterial Chemoembolization; (HIFU), high-intensity focused ultrasound; DBIs, doxorubicin eluting beads; HCC, hepatocellular carcinoma; HGF, Hepatocyte growth factor; IL-6, Interleukin-6; LDM, lidamycin; MCT, microwave coagulation therapy; MDR, multidrug resistance; MMP-9, matrix metalloproteinase-9; OLT, orthotopic liver transplantation; PCNA, Proliferating cell nuclear antigen; PEI, percutaneous ethanol injection; PK, pharmacokinetic profile; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; TOCE, transarterial oily chemoembolization; TR9, toll like receptor 9; VEGF, Vascular endothelial growth factor

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**Authors’ contributions**

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**Competing interests**

The authors declare that they have no competing interests.

**Ethical approval**

Not applicable.

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**References**

1. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011;365(12):1118–27.
2. Shariff MI, Cox IL, Gornall AI, Khan SA, Gedroyc W, Taylor-Robinson SD. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis and therapeutics. Expert Rev Gastroenterol Hepatol. 2009;3(4):533–67.
3. Cho YK, Kim JK, Kim WT, Chung JW. Hepatic resection versus radiofrequency ablation for very early stage hepatocellular carcinoma: a Markov model analysis. Hepatology. 2010;51(4):1284–90.
4. Roust C, Gores GJ. Locoregional management of hepatocellular carcinoma. Surgical and ablation therapies. Clin Liver Dis. 2001;5(1):161–73.
5. Lee WS, Yun SH, Chun HK, Lee WR, Kim SJ, Choi SH, et al. Clinical outcomes of hepatic resection and radiofrequency ablation in patients with solitary colorectal liver metastasis. J Clin Gastroenterol. 2008;42(8):945–9.
6. Muller S, Ruers T, Jamart J, Michel L, Marchal G, Ni Y. Radiofrequency ablation versus resection for resectable colorectal liver metastes: time for a randomized trial? An update. Dig Surg. 2008;25(6):445–60.
7. Lau WY, Lai EC. The current role of radiofrequency ablation in the management of hepatocellular carcinoma: a systematic review. Ann Surg. 2009;249(1):20–5.
8. Zavadsky KE, Lee YT. Liver metastases from colorectal carcinoma: incidence, resectability, and survival results. Ann Surg. 1994;220(6):929–33.
9. McCaughan GW, Koorey DJ, Strasser SI. Hepatocellular carcinoma: current approaches to diagnosis and management. Intern Med J. 2002;32(8):394–400.
10. Liu LX, Zhang WH, Jiang HC. Current treatment for liver metastases from colorectal cancer. World J Gastroenterol. 2003;9(2):193–200.
11. Tsim NC, Frampton AE, Habib NA, Jiao LR. Surgical treatment for liver cancer. World J Gastroenterol. 2010;16(8):297–33.
12. Michalski CW, Erkan M, Huser N, Muller MW, Hartel M, Friess H, et al. Resection of primary pancreatic cancer and liver metastasis: a systematic review. Dig Surg. 2008;25(5):473–80.
13. Chen X, Liu HP, Li M, Qiao L. Advances in non-surgical management of primary liver cancer. World J Gastroenterol. 2014;20(44):16630–8.
14. Kong J, Kong L, Kong J, Ke S, Gao J, Ding X, et al. After insufficient radiofrequency ablation, tumor-associated endothelial cells exhibit enhanced angiogenesis and promote invasiveness of residual hepatocellular carcinoma. J Transl Med. 2012;10:239.
15. Kong J, Kong J, Pan B, Ke S, Dong S, Li X, et al. Insufficient radiofrequency ablation promotes angiogenesis of residual hepatocellular carcinoma via HIF-1alpha/VEGFA. PLoS One. 2012;7(5):e37266.
16. Dong S, Kong J, Kong F, Gao J, Ke S, et al. Insufficient radiofrequency ablation promotes epithelial-mesenchymal transition of hepatocellular carcinoma cells through Akt and ERK signaling pathways. J Transl Med. 2013;11:273.
17. Zhang N, Wang L, Chai ZT, Zhu ZM, Zhu XD, Ma DN, et al. Incomplete radiofrequency ablation enhances invasiveness and metastasis of residual cancer of hepatocellular carcinoma cell HCCLM3 via activating beta-catenin signaling. PLoS One. 2014;9(12):e115949.
18. Ke S, Ding XM, Kong J, Gao J, Wang SH, Cheng Y, et al. Low temperature of radiofrequency ablation at the target sites can facilitate rapid progression of residual hepatic VX2 carcinoma. J Transl Med. 2010;8:73.
19. Wu Y, Zhou BP. Inflammation: a driving force speeds cancer metastasis. Cell Cycle. 2009;8(20):3267–73.
20. Schleimer RP. Inflammation: Basic principles and clinical correlates edited by John Gallin, Ita Goldstein and Ralph Snyderman, Raven Press, 1987. $219.00 (xvii + 995 pages) ISBN 0 88167 344 7. Immunol Today. 1988(10):327.
21. Jiang T, Zhang X, Ding J, Duan B, Lu S. Inflammation and cancer: inhibiting the progression of residual hepatic VX2 carcinoma by anti-inflammatory drug after incomplete radiofrequency ablation. Int J Clin Exp Pathol. 2015;8(11):13945–56.
22. Weinberg BD, Blanco E, Lemppa SF, Anderson JM, Exner AA, Gao J. Combined radiofrequency ablation and doxorubicin-eluting polymer implants for liver cancer treatment. J Biomed Mater Res A. 2007;81(1):265–13.
23. Behn B, Di Fazio P, Mitchel H, Neureuter D, Nemmering R, Hahn EG, et al. Additive antitumour response to the rabbit VX2 hepatoma by combined radio frequency ablation and toll like receptor 9 stimulation. Gut. 2016;65(1):134–43.
24. Fan L, He Z, Ma K, Huang X, Zhou D, Feng X, et al. Hepatic VX2 tumor in rabbits: treated with radio frequency ablation and evaluated with enhanced CT. Zhonghua Gan Zang Bing Za Zhi. 2002;10(5):362–5.
25. Pour P, Beard JD. The progression to carcinoma of virus-induced rabbit papillomas (Shope). J Exp Med. 1935;62(4):523–48.
26. Kidd JG, Poulos A. A transplantable rabbit carcinoma originating in a virus-induced papilloma and containing the virus in masked or altered form. J Exp Med. 1940;71(6):813–38.
27. Galasko CS, Muckle DS. Intracerebral neoplasma of the VX2 carcinoma. Br J Cancer. 1974;29(9):559–65.
28. Maruyama H, Matsutani S, Saisho H, Kaniyama N, Mine Y, Hisata T, et al. Sonographic shift of hypercellular liver tumor on blood pool harmonic images with definity: time-related changes of contrast-enhanced appearance in rabbit VX2 tumor under extra-low acoustic power. Eur J Radiol. 2005;56(1):60–5.
29. van Es RJ, Dullens HF, van der Bilt A, Koole R, Slootweg PJ. Evaluation of the VX2 rabbit auricle carcinoma as a model for head and neck cancer in humans. J Craniomaxillofac Surg. 2000;28(5):300–7.
30. Lee JM, Kim SW, Chung GH, Lee SY, Han YM, Kim CS. Open radiofrequency thermal ablation of renal VX2 tumors in a rabbit model using a cooled-tip electrode: feasibility, safety, and effectiveness. Eur Radiol. 2003;13(6):1324–32.
31. Frank JA, Gitron M, Dwyer AJ, Cohen PJ, Knop RH, Diggis R, et al. A reproducible model of metastatic brain and ocular tumor by hematogenous inoculation of the VX2 tumor in rabbits. J Neurosurg. 1987;67(1):106–9.
32. Anayama T, Nakajima T, Dunne M, Zheng J, Allen C, Driscoll B, et al. A novel minimally invasive technique to create a rabbit VX2 lung tumor model for nano-sized image contrast and interventional studies. PLoS One. 2013;8(6):e67355.
33. Yang WH, Liebert M, Price RE, Crommeens DM, Lin JS, Grossman HB. Extravalvular cysotorus approach for VX2 bladder tumor in rabbits. Urol Res. 2001;29(5):345–9.
34. Rhee TK, Young JY, Larson AC, Haines 3rd GK, Sato KT, Salem R, et al. Effect of transcatheter arterial embolization on levels of hypoxia-inducible factor-1alpha in rabbit VX2 liver tumors. J Vasc Interv Radiol. 2007;18(1):639–45.
35. Burgener FA. Peripheral hepatic artery embolization in rabbits with VX2 carcinomas of the liver. Cancer. 1980;46(1):56–63.
36. Hoye RC, Thomas LB, Rogge GK, Ketcham A. Effects of neodymium laser on normal liver and VX2 carcinoma transplanted into the liver of experimental animals. J Natl Cancer Inst. 1968;41(5):1071–82.

37. Eifler AC, Lewandowski RJ, Virmiani S, Chung JC, Wang D, Tang RL, et al. Development of a VX2 pancreatic cancer model in rabbits: a pilot study. J Vasc Interv Radiol. 2009;20(8):1075–82.

38. Wang D, Bangash AK, Rhee TK, Woloschak GE, Paunesku T, Salem R, et al. Liver tumors: monitoring embolization in rabbits with VX2 tumors—tumors to intrarenal first-pass perfusion MR imaging. Radiology. 2007;245(1):130–9.

39. Zhao S, Yu H, Du N. Experimental study of doxorubicin interventional chemotherapy in the treatment of rabbit VX2 renal transplantation carcinoma. Int J Clin Exp Med. 2015;8(7):10793–9.

40. Parvianin A, Casadaban LC, Gaca RC. Development, growth, propagation, and angiographic utilization of the rabbit VX2 model of a rabbit: a pictorial primer and ‘how to’ guide. Diag Interv Radiol. 2014;20(4):335–40.

41. Virmiani S, Harris KR, Szolk–Kowalska B, Paunesku T, Woloschak GE, Lee FT, et al. Comparison of two different methods for inoculating VX2 tumors in rabbit livers and hind limbs. J Vasc Interv Radiol. 2008;19(6):931–6.

42. Chen JH, Lin YC, Huang YS, Chen TJ, Lin YW, Han KW. Induction of VX2 carcinoma in rabbit liver: comparison of two inoculation methods. Lab Anim. 2004;38(1):79–84.

43. Sun JH, Zhang YL, Nie CH, Yu XB, Xie HY, Zhou L, et al. Considerations for two inoculation methods of rabbit hepatic tumors: Pathology and image features. Exp Ther Med. 2012;3(3):386–90.

44. Luo W, Zhou X, Zheng X, He G, Hu Y, Li Q, et al. Role of sonography for implantation and sequential evaluation of a VX2 rabbit tumor model. J Ultrason Med. 2010;29(1):51–60.

45. Bimonte S, Barbieri A, Palaira R, Leongtoni M, Albino V, Piccirillo M, et al. An overview of loco-regional treatments in patients and mouse models for hepatocellular carcinoma. Infect Agent Cancer. 2015;10:

46. Ghobiemranezehad A, Mirpour S, Geschwind JH, Rao P, Loffroy R, Pellerin O, et al. Evaluation of 70-150 μm doxorubicin-eluting beads for transarterial chemoembolization in the rabbit liver VX2 tumor model. Eur Radiol. 2016 [Epub ahead of print].

47. Namur J, Pascale F, Groff P, Zoli M, Bianchi G, Francesconi R, et al. Increased contrast-enhanced sonography in the follow-up algorithm of hepatocellular carcinoma treated with radiofrequency ablation: single tumor center experience. Acta Radiol. 2015;56(2):135–42.

48. Izzo F, Barnett Jr CC, Curley SA. Radiofrequency ablation of primary and metastatic malignant liver tumors. Adv Surg. 2001;35:225–50.

49. Curley SA, Izzo F. Radiofrequency ablation of primary and metastatic liver tumors. Surg Technol Int. 2002;10:99–106.

50. Nplusi O, Ionomi A, Waktani A, Kariyama K. Modified radiofrequency ablation for the treatment of hepatocellular carcinoma. Hepatol Res. 2016 [Epub ahead of print].

51. McGahan JP, Brock JM, Telsk H, Gu WZ, Schneider P, Browning PD. Hepatic ablation with use of radio-frequency electrosurgery in the animal model. J Vasc Interv Radiol. 1992;3(2):291–7.

52. Curley SA. Radiofrequency ablation of malignant liver tumors. Oncologist. 2001;6(1):14–23.

53. Rossi S, Garbagnati F, Lencioni R, Allgaier HP, Marchiolo A, Fornari F, et al. Percutaneous radio-frequency thermal ablation of nonresectable hepatocellular carcinoma after occlusion of tumor blood supply. Radiology. 2000;217(1):119–26.

54. Seki T, Tamai T, Ikeda K, Inamura M, Nishimura A, Yamashiki N, et al. Rapid progression of hepatocellular carcinoma after transcatheter arterial chemembolization and percutaneous radiofrequency ablation in the primary tumor region. Eur J Gastroenterol Hepatol. 2001;13(3):291–4.

55. Ruzzeneante A, Manzoni GD, Mofetta M, Pacher S, Genco B, Donatocci M, et al. Rapid progression of hepatocellular carcinoma after Radiofrequency Ablation. World J Gastroenterol. 2004;10(8):137–40.

56. Kasugai H, Osaki Y, Kudo M, Seki T, Osaka Liver Cancer Study G. Severe complications of radiofrequency ablation therapy for hepatocellular carcinoma: an analysis of 3,891 ablations in 2,614 patients. Oncology. 2007; 72 Suppl 1:72–5.

57. Obata K, Matsumoto N, Okamoto M, Kobayashi M, Ikeda H, Takahashi H, et al. Insufficient radiofrequency ablation therapy may induce further malignant transformation of hepatocellular carcinoma. Hepatol Int. 2002;6(1):116–23.

58. Strozuc C, Dragnea A, Ivanov B, Pechianu C, Herlea V, Sgarlato O, et al. Expression of p53, Bcl-2, VEGF, Ki67 and PCNA and prognostic significance in hepatocellular carcinoma. J Gastrointestin Liver Dis. 2006;17(4):411–7.

59. Ballardini G, Gropp F, Zoli M, Bianchi G, Girota F, Francesconi R, et al. Increased risk of hepatocellular carcinoma development in patients with cirrhosis and with high hepatocellular proliferation. J Hepatol. 1994;20(2):218–22.

60. Yang P, Yuan W, He J, Wang J, Yu L, Jin X, et al. Overexpression of EphA2, MWP-9, and MVD-CD34 in hepatocellular carcinoma: Implications for tumor progression and prognosis. Hepatol Res. 2009;39(12):1169–77.