Irreversible Electroporation of Hepatic Malignancy

Govindarajan Narayanan, MD¹ Tatiana Froud, MD¹ Rekhaben Suthar, MD¹ Katuska Barbery, MD¹

¹ Miller School of Medicine, University of Miami, Miami, Florida

Semin Intervent Radiol 2013;30:67–73

Address for correspondence Govindarajan Narayanan, MD, Radiology Department Vascular/Interventional Division, University of Miami, Miami, FL 33136 (e-mail: kbarbery@med.miami.edu).

Abstract

Keywords

- irreversible electroporation (IRE)
- NanoKnife
- hepatocellular carcinoma (HCC) ablation
- apoptosis

Hepatocellular carcinoma (HCC) is a worldwide problem of epidemic proportions, best treated in a multidisciplinary setting. Major advances have been made in all specialties that manage patients with HCC, with surgical options at one end of the spectrum and palliative chemotherapy on the other, and the vast majority of patients require the involvement and expertise of interventional oncology. Several ablative and transarterial technologies are currently available. Irreversible electroporation (IRE) is a new ablative technology that uses high-voltage, low-energy DC current to create nanopores in the cell membrane, disrupting the homeostasis mechanism and inducing cell death by initiating apoptosis. This article discusses the evolution of IRE as well as its safety and efficacy in the context of other ablative therapies in the treatment of hepatic malignancies.

Objectives: Upon completion of this article, the reader will be able to describe the technical basis of electroporation, as well as its role in the clinical management of patients undergoing ablative therapy.

Accreditation: This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of Tufts University School of Medicine (TUSM) and Thieme Medical Publishers, New York. TUSM is accredited by the ACCME to provide continuing medical education for physicians.

Credit: Tufts University School of Medicine designates this journal-based CME activity for a maximum of **1 AMA PRA Category 1 Credit™**. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Epidemiology of Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide and the third most common cause of cancer-related deaths, with >625,000 new patients affected each year.^{1,2} The risk factors have historically included alcoholic cirrhosis, hepatitis B virus infection, hepatitis C virus (HCV) infection, and hemochromatosis.³

Due to the HCV epidemic in the United States, HCC represents the fastest growing cause of cancer mortality overall, based on data from the Surveillance, Epidemiology, and End Results study.⁴ Less discussed factors, such as diabetes and nonalcoholic fatty liver disease (NAFLD), may also be important contributors to the development of HCC in the United States.⁵ A U.S. population-based study has established that diabetes is an independent risk factor for HCC, associated with a two- to threefold higher risk for disease.⁶ Both diabetes and obesity are also implicated in the development of nonalcoholic steatohepatitis, the severest form of NAFLD, believed to lead to HCC via progression of cirrhosis. This might further increase the incidence of HCC in developed countries, which are most affected by the obesity epidemic.

Staging of Hepatocellular Carcinoma

Staging of HCC is frequently based on the Barcelona Clinic Liver Cancer Staging classification (BCLC) or the Cancer of the Liver Italian program classification. The BCLC classification system classifies patients according to the size and number of tumors, liver function, and performance status, and it links each stage to the best treatment option according to the available evidence. It was developed in 1999 and has been revised twice: once in 2002 when transarterial chemoembolization was proven to be effective in intermediate-stage HCC, and in 2008 when sorafenib was

proven effective in advanced HCC.⁷ The Child-Turcotte-Pugh system is used to quantify the severity of cirrhosis through evaluation of five variables (ascites, albumin, bilirubin, international normalized ratio, and encephalopathy).

Treatment Options in HCC

In patients with HCC and an adequate hepatic reserve, surgical resection offers the best long-term survival. Resection is the treatment of choice in noncirrhotic patients with a solitary mass and without significant portal hypertension, but only 10 to 15% of patients qualify for this option. Major resections are not recommended even in compensated cirrhosis because the remnant liver may be insufficient to avoid liver failure and/or death.⁸

Liver transplantation results have improved significantly following the implementation of the Milan criteria, which limited transplantation to patients who had a single tumor <5 cm in size or three or fewer tumors the largest of which is <3 cm. Following these criteria, transplant results have improved dramatically, with the latest data suggesting that patients who fit the criteria have a 4-year survival in excess of 80%, results better than surgical resection.⁹ Patients are placed on a waiting list based on their Model for End-Stage Liver Disease score. While on the waiting list for a transplant the probability of progression of HCC <3 cm is ~10%; larger tumors have approximately a 60% chance of progressing beyond Milan within a year of listing.¹⁰ Locoregional treatment choices that include ablative therapies and transarterial options such as transarterial chemoembolization, drug-eluting beads, and selective internal radiation therapy have become widely accepted both to maintain patients on the transplant list and to downstage patients who do not fit the listing criteria. Ablation therapy has gained momentum as a viable treatment alternative for patients, endorsed by the American Association for the Study of Liver Diseases as the best treatment option for nonsurgical patients.¹¹ There are several different ablative techniques including percutaneous ethanol injection, cryoablation, and microwave ablation. However, radiofrequency ablation (RFA) is the most widely used and studied ablative modality used for primary liver cancer.

RFA has been shown to provide improved local disease control for a broad range of tumor sizes.^{11,12} In spite of its widespread use and noted efficacy, RFA has some limitations. Its dependence on heating of the tissue to denature proteins means adjacent thermosensitive structures such as bile ducts, gallbladder, and hepatic capsule can be damaged resulting in complications, and large vessels within or close to the treatment zone may cause thermal sinks ("heat-sink" effect) that will prevent complete treatment of the target lesion.¹²⁻¹⁵ These limitations have generated interest in other methods of ablation that are potentially less damaging to nontarget tissues and not affected by heat-sink effects.

Evolution of Irreversible Electroporation

Electroporation increases the permeability of a cell membrane by exposing it to electrical pulses; the effects are either

reversible or irreversible, depending on the applied voltage. Its applications were described by Neumann et al nearly 3 decades ago when electrical fields were used to temporarily create pores in cell membrane to facilitate gene transfer into mouse lymphoma cells.¹⁶ The use of electroporation to increase the permeability of the cell membrane in tissue was introduced by Okino and Mohri in 1987¹⁷ and by Mir et al in 1991,¹⁸ who described that combining an impermeant anticancer drug with reversibly permeabilizing electrical pulses greatly enhanced the effectiveness of the treatment compared with either therapy alone. This form of cancer treatment is known as electrochemotherapy. Davalos et al further studied this concept using mathematical and in vitro models, and they demonstrated that irreversible electroporation (IRE) can ablate substantial volumes of tissue, comparable with those achieved with other ablation techniques, without causing any detrimental thermal effects and without the need of adjuvant drugs.¹⁹ This work laid the foundation for further investigation of IRE as a method to primarily kill cancer cells.

IRE technology is commercially available as the NanoKnife (AngioDynamics, Latham, NY) (► **Figs. 1A** and **1B**). Through the use of a high-voltage, low-energy direct current, electrons passing between electrodes pass through intervening cells and cause "nanopores" in the cell membrane. When repair of nanopores cannot match the frequency with which they are produced, and the number of nanopores reaches a critical number, damage to the cell membrane can no longer be reversed, cellular homeostasis is disrupted, and apoptotic cell death is triggered.¹⁹⁻²¹ Apoptosis is timed cell death, a controlled shutdown of the damaged cell after it has suffered a lethal insult. This manner of cellular death leaves no trace of the cell behind, and there is no significant inflammatory reaction as seen with other ablative techniques. Tissue destruction leads to the uncontrolled leakage of cell contents into the surrounding tissue, which does incite a prompt inflammatory reaction, cellular infiltration, and consequent permanent fibrosis and scarring.

Safety and Efficacy in Animal Models

The safety of IRE has been evaluated in several animal models. Lee et al published their study on the effectiveness of IRE in hepatic tissue and the radiologic-pathologic correlation of IRE-induced cell death in 16 Yorkshire pigs.²¹ No complications were seen in any of the 16 animals. Bile ducts and vessels integrity was completely preserved. The treatment areas were sharply demarcated, and areas of complete cell death stained positive for apoptotic markers (TUNEL, BCL-2 oncoprotein), suggesting involvement of the apoptotic process in the pathophysiology of cell death caused by IRE.

Rubinsky et al demonstrated that IRE ablation in porcine liver can completely ablate target tissue up to the margin of blood vessels without vascular insult.²⁰ The vessel-preserving effect of IRE is believed to be due to the fact that the vessel wall contains a higher proportion of collagenous connective tissue and elastic fiber, and it lacks a normal cellular membrane. This belief was supported by the finding of mild inflammatory changes that were seen in vessels in the IRE-treated zone. This

Figure 1 (A) NanoKnife Tissue Ablation System. (B) NanoKnife Ablation Monopolar Probe.

finding could be seen due to the fact that the innermost layer of the vessels lacks collagenous and elastic fibrous tissue. Another explanation for preservation of the blood vessels following IRE is the presence of gap junctions in the smooth muscle cells of the blood vessels that may facilitate the passage of the electrical pulses of IRE without damaging the cell membrane, thereby preserving the integrity of the smooth muscle cell membrane.²¹

Safety and Efficacy in Human Subjects

The first human experience of IRE was published by Thomson et al,²² which described a single-center prospective nonran-

domized cohort study performed to investigate the safety of IRE. Thirty-eight volunteers with advanced malignancy of the liver, kidney, or lung (69 separate tumors) unresponsive to standard treatment underwent IRE under general anesthesia. No mortalities occurred at 30 days. Transient ventricular arrhythmia occurred in four patients; electrocardiographically (EKG) synchronized delivery was used subsequently in the remaining 30 patients. The authors concluded that IRE appeared to be safe for human clinical use, provided EKG-synchronized delivery is used. This led to the use of the AccuSync 72 (►Figs. 2 and 3), a gating device with a five-lead system and a printer being used as a standard with the NanoKnife machine. When energy is applied, the EKG trigger monitor automatically detects the rising slope of the R wave and sends a signal to the NanoKnife generator, which delivers an energy pulse after a 50 ms (0.05 second) delay. The energy pulse is delivered during (or just before) the ventricular refractory period.

Figure 2 AccuSync 72 ECG Trigger Monitor.

Figure 3 Sync device (e.g., AccuSync 72) senses the rising slope of the R wave and sends a signal to the NanoKnife. The NanoKnife waits 50 ms (0.05 s) and delivers one energy pulse. The energy pulse is delivered during (or just before) the ventricular refractory period.

Multidisciplinary Approach

The treatment options for HCC patients are best discussed in a multidisciplinary format with the key services such as hepatology, oncology, transplant, interventional oncology (IO)/radiology, and pathology participating and sharing their expertise. When referred to interventional oncology for treatment, these patients are initially evaluated in the IO clinic. Patient evaluation includes a history and physical examination, and an assessment of the patient's performance status using the Eastern Cooperative Oncology Group (ECOG) score of 0 to 4, reviews of the laboratory values and imaging studies, and discussion about treatment options along with alternatives. A detailed cardiac history is crucial for IRE because patients with a known history of cardiac arrhythmias are not ideal candidates due to the risk of inducing dangerous rhythms during the procedure. IRE is approved by the Food and Drug Administration in the United States under a 510(k) for surgical ablation of soft tissue; use of the technology in the liver is considered off label and should be discussed with the patient. Consultation with anesthesia is required because all IRE procedures are performed under general anesthesia. We typically arrange this on the same day as the IO consult. If the patient's imaging studies are more than a month old, they are repeated to confirm stability because progression of disease may render these patients ineligible for this treatment option.

Treatment Planning

A treatment procedure with the NanoKnife device requires the use of monopolar or bipolar probes. Monopolar probes are currently available in 15- and 20-cm lengths. When using the monopolar probes, a minimum of two probes are required to create an appropriate treatment zone. A maximum of six probes can be used for a single treatment, and the treatment zone at any given time is the tissue between two probes. The NanoKnife generator has a treatment planning algorithm software that enables the user to evaluate different combinations with the number of needles required to create an adequate treatment zone. The only limitation is that the machine does not actually know precisely where the lesion is in relation to the needle placements, a factor that the operator must determine. The ideal spacing between two monopolar electrodes should be between 1.5 and 2 cm. A lever embedded within the probe is used to expose the active tip between 1 and 3 cm. The exposure length of the active tip is determined by the depth of the lesion and by the type of the tissue treated, and treatments in the liver can be safely performed with an exposure between 1.5 and 3 cm. If the depth of the tumor is greater than the exposure (i.e., 4 cm), when the needle exposure is 2 cm then a pullback of the probes followed by a second treatment will be required to cover the depth of 4 cm while obtaining an appropriate treatment margin. Bipolar electrodes have the two poles on the same needle separated by an insulated region, and they can ablate larger areas up to $2.0 \times 2.0 \times 2.5$ cm.

Typically, 90 high-voltage (1500 to 3000 V) direct current (25 to 45 A) electrical pulses are delivered between paired unipolar electrodes or a single bipolar electrode. With the revision of the treatment guidelines, the number of pulses for

Figure 4 Kaplan-Meier curves demonstrating the median progression-free survival of 11.6 months for hepatocellular carcinoma patients (95% confidence interval, 10.2 to 12.9). Abbreviations: CT, computed tomography; CR, complete response.

a successful treatment between a pair of monopolar electrodes has been reduced from 90 to 70. The voltage setting is determined by the distance between each pair of electrodes with the intent to generate at least 1000 V between the electrodes. The generator is programmed to stop delivery and recharge if the current flow exceeded 48 A.

The electrodes are placed percutaneously under imaging (computed tomography [CT] or ultrasound) guidance, with the maximum separation between the electrodes 2.2 cm; no tissue separation maneuvers are used in our institution to protect structures adjacent to the IRE electrodes.

Percutaneous IRE Ablation Procedure Technique

During treatment, a neuromuscular blockade is necessary to counteract the high electrical voltage generated during treatment. The paralyzing agents include cisatracurium besylate (Nimbex, Abbott Laboratories, North Chicago, IL) or rocuronium bromide (Hospira, Inc., Lake Forest, IL), depending on the preference of the anesthesia provider and the patient's liver function. The patient's electrocardiograph tracing, heart rate, oxygen saturation, respiratory rate, blood pressure, bispectral index, end tidal carbon dioxide, temperature, and urine output is continuously monitored by an anesthesiologist. The number of electrodes used in treatment and the number of treatment sessions are both based on tumor size.

Following completion of the IRE treatment, we perform a postprocedure CT scan the same day or the following day to evaluate for any immediate complications and to evaluate if the lesion has been entirely treated. Contrast is administered if renal function permits. Successful treatment is signified by a lack of contrast enhancement in the ablation zone and complete coverage of the lesion by the ablation zone. Following ablation, patients are transferred to radiology recovery and, if stable and/or not scheduled to receive additional therapies, discharged home the following day. Postprocedure pain is controlled with Dilaudid administered through a patient-controlled analgesia pump.

Figure 5 Irreversible electroporation (IRE) procedure on a patient with biopsy-proven hepatocellular carcinoma. (A) Contrast-enhanced axial computed tomography (CT) scan demonstrating caudate lobe lesion prior to IRE procedure (arrow). (B) Axial CT scan during IRE procedure demonstrating two monopolar probes in liver lesion. (C–E) Magnetic resonance imaging at 6, 11, and 14 months post-IRE procedure demonstrating no residual or recurrent tumor (arrow).

We compared postprocedure pain following ablation of HCC treated with IRE and RFA.²³ In this report, the postprocedure pain in 21 patients who underwent IRE of 29 intrahepatic lesions (mean size: 2.20 cm) in 28 IRE sessions was compared with 22 patients who underwent RFA of 27 lesions (mean size: 3.38 cm) in 25 RFA sessions. Pain was determined by patient-disclosed scores with an 11-point numerical rating scale and 24-hour cumulative hydromorphone use from a patient-controlled analgesia pump. Statistical significance was evaluated by the Fisher exact test, the chi-square test, and the Student *t* test. There was no significant difference in the cumulative hydromorphone dose (1.54 mg [IRE] versus 1.24 mg [RFA]; $p = 0.52$) and in the mean pain score (1.96 [IRE] versus 2.25 [RFA]; $p = 0.70$). In 9 of 28 IRE sessions (32.14%) and 11 of 25 RFA sessions (44.0%), patients reported no pain, which led to the conclusion that IRE is comparable with RFA in the amount of pain that patients experience and

the amount of pain medication self-administered. Both modalities appear to be well tolerated by patients.

Follow-up contrast-enhanced CT scans and/or magnetic resonance imaging are generally obtained 1, 3, and 6 months, and 1 year after treatment. Apoptosis following IRE has been described in animal studies and is distinct from the fibrosis and scarring that results from thermal ablation. On a macroscopic level, the progressive response of the ablation zone with complete resolution in many patients has obvious advantages in determining complete tumor necrosis or local progression. Not all IRE zones disappear completely, which may be secondary to a proportion of the treated cells suffering such severe destruction of the cellular membrane that cell contents leak out before apoptosis can take place; this may cause an inflammatory reaction that results in the typical fibrosis and scarring seen with other techniques.

Figure 6 Patient with hepatocellular carcinoma (HCC), status post radiofrequency ablation (RFA) of prior HCC lesion. (A) Computed tomography (CT) imaging 2 years post RFA procedure demonstrating new HCC focus (black arrow) next to recanalized umbilical vein. White arrow shows prior RFA site. (B) CT imaging 3 months after irreversible electroporation (IRE) procedure of second lesion demonstrating the lesion treated with IRE, which is barely visible (black arrow). White arrow shows prior RFA site.

Due to the onset of apoptosis following IRE, the healing mechanism following treatment is different when compared with the fibrosis and scarring observed following thermal ablation. In one report using a pig model, death occurred at 24 hours, 3 days, 7 days, and 14 days following IRE.²⁰ This study showed that by day 7, there was regeneration in most ablation zones, with large veins demonstrating necrotic endothelium but luminal patency; by day 14, it was difficult to identify the ablation zones.²⁰ This finding was validated in humans by Kingham et al,²⁴ where the initial ablation zone 1 month postprocedure had an area of 9.0 cm² (range: 1.3 to 32.2 cm²). This was reduced by more than two thirds by 3 months postprocedure when the area was, on average, 2.3 cm (range: 0 to 17.6 cm²). In this study, several of the ablation zones visible on the 1-month scan were not visible on the 3-month scans.

Results of Percutaneous IRE Ablation

We recently reported our experience with IRE for HCC and metastatic colorectal cancer between January 2010 and August 2011.²⁵ The primary end point was progression-free survival (PFS). Responses were assessed using the modified Response Evaluation Criteria in Solid Tumors. Thirty-three patients with unresectable HCC were treated with IRE, and they demonstrated an 11.6-month PFS for the HCC patients (95% confidence interval, 10.2 to 12.9 months) (►Fig. 4). Three patients went on to have liver transplantation. There was no treatment-related mortality, although one patient (3%) died in hospice 25 days posttreatment. Complications included pneumothorax ($n = 2$ [6%]), pleural effusion ($n = 2$ [6%]), atrial flutter/fibrillation ($n = 2$ [6%]). The results of this early experience demonstrated that IRE is safe and effective in

the treatment of HCC, and it compares well with early RFA data.

In the initial human experience of Thomson et al, the most common complications included cardiac arrhythmias, brachial plexus injury, pneumothorax, and pain. This report clearly established the safety in human use. A separate study on ablation of perivascular malignancies in the liver using IRE was published recently, and the safety and short-term outcomes were evaluated.²⁴ In this report, 28 patients had 65 tumors treated via an open approach or percutaneously. Median tumor size was 1 cm (range: 0.5 to 5 cm). Twenty-five tumors were 1 cm from a major hepatic vein; 16 were 1 cm from a major portal pedicle. Complications included one intraoperative arrhythmia and one postoperative portal vein thrombosis. Overall morbidity was 3%, and there were no treatment-associated mortalities. At median follow-up of 6 months, there was one patient with persistent disease (3.6%), and three tumors recurred locally (4.6%).

Conclusion

RFA is still the most widely used ablative modality and has shown an excellent ability to destroy tumor. Limitations exist with RFA such as the heat-sink effect and proximity to critical structures such as colon, stomach, gallbladder, and major bile ducts.^{26,27} Although there are new thermal technologies such as microwave ablation, which may potentially generate a larger ablation zone in a shorter time, they still have the limitations associated with thermal technologies. The lack of heat-sink effect and the ability to treat zones near vessels, bile ducts, and critical structures makes IRE a valuable complement to these ablative tools.

IRE comes with its own share of limitations. Human experiences are still limited, whereas thermal ablative techniques such as RFA have been time-tested for nearly 3 decades (►Figs. 5 and 6). The procedure has a learning curve because multiple needle placements are required within a prescribed distance, which can be challenging, and parallel placement of the probes may be hindered by issues such as intervening ribs. Track ablation performed with RFA and microwave cannot currently be performed with IRE. The actual timing of imaging follow-up and the best modality for follow-up are still being determined. IRE also poses a unique challenge to the current imaging response criteria because unlike other ablation modalities, there is a marked decrease in the size of a successfully treated lesion. IRE may represent an area where volumetric assessment may have a role in the evaluation of treatment response. As the technology matures and gains more widespread acceptance, IRE should be compared with the current standards in ablation as well as surgical resection to further validate the promise that it has shown so far.

References

- 1 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55(2):74–108
- 2 Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol* 2008;48(Suppl 1):S20–S37

- 3 Bosch FX, Ribes J, Díaz M, Cléries R. Primary liver cancer: world-wide incidence and trends. *Gastroenterology* 2004;127(5, Suppl 1): S5–S16
- 4 Seeff LB. Introduction: the burden of hepatocellular carcinoma. *Gastroenterology* 2004;127(5, Suppl 1):S1–S4
- 5 Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. *Gastroenterology* 2004;127(5):1372–1380
- 6 Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut* 2005;54(4):533–539
- 7 Llovet JM, Ricci S, Mazzaferro V, et al; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359(4):378–390
- 8 Rodriguez de Lope C, Tremosini S, Forner A, et al. Management of HCC. *J Hepatol* 2012;56(6):1317–1323
- 9 Figueras J, Jaurrieta E, Valls C, et al. Resection or transplantation for hepatocellular carcinoma in cirrhotic patients: outcomes based on indicated treatment strategy. *J Am Coll Surg* 2000;190(5):580–587
- 10 Yao FY, Bass NM, Nikolai B, et al. Liver transplantation for hepatocellular carcinoma: analysis of survival according to the intention-to-treat principle and dropout from the waiting list. *Liver Transpl* 2002;8(10):873–883
- 11 Bruix J, Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology* 2005;42(5):1208–1236
- 12 Cho YK, Kim JK, Kim MY, Rhim H, Han JK. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. *Hepatology* 2009;49(2):453–459
- 13 Teratani T, Yoshida H, Shiina S, et al. Radiofrequency ablation for hepatocellular carcinoma in so-called high-risk locations. *Hepatology* 2006;43(5):1101–1108
- 14 Mahvi DM, Lee FT Jr. Radiofrequency ablation of hepatic malignancies: is heat better than cold? *Ann Surg* 1999;230(1):9–11
- 15 Nahum Goldberg S, Dupuy DE. Image-guided radiofrequency tumor ablation: challenges and opportunities—part I. *J Vasc Interv Radiol* 2001;12(9):1021–1032
- 16 Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider PH. Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J* 1982;1:841–845
- 17 Okino M, Mohri H. Effects of a high-voltage electrical impulse and an anticancer drug on in vivo growing tumors. *Jpn J Cancer Res* 1987;78(12):1319–1321
- 18 Mir LM, Orlowski S, Belehradek J Jr, Paoletti C. Electrochemotherapy potentiation of antitumour effect of bleomycin by local electric pulses. *Eur J Cancer* 1991;27(1):68–72
- 19 Davalos RV, Mir IL, Rubinsky B. Tissue ablation with irreversible electroporation. *Ann Biomed Eng* 2005;33(2):223–231
- 20 Rubinsky B, Onik G, Mikus P. Irreversible electroporation: a new ablation modality—clinical implications. *Technol Cancer Res Treat* 2007;6(1):37–48
- 21 Lee EW, Chen C, Prieto VE, Dry SM, Loh CT, Kee ST. Advanced hepatic ablation technique for creating complete cell death: irreversible electroporation. *Radiology* 2010;255(2):426–433
- 22 Thomson KR, Cheung W, Ellis SJ, et al. Investigation of the safety of irreversible electroporation in humans. *J Vasc Interv Radiol* 2011;22(5):611–621
- 23 Narayanan G, Froud T, Lo K, Barbery KJ, Perez-Rojas E, Yrizarry J. Pain analysis in patients with hepatocellular carcinoma: irreversible electroporation versus radiofrequency ablation—initial observations. *Cardiovasc Intervent Radiol* 2012; June 30 (Epub ahead of print)
- 24 Kingham TP, Karkar AM, D'Angelica MI, et al. Ablation of perivascular hepatic malignant tumors with irreversible electroporation. *J Am Coll Surg* 2012;215(3):379–387
- 25 Narayanan G, Hosein P, Arora G, Barbery KJ, Yrizarry J. Percutaneous irreversible electroporation (ire) in the treatment of HCC and metastatic colorectal cancer (MCRC) to the liver. *J Vasc Interv Radiol* 2012;23(12):1613–1621
- 26 El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004;127(5, Suppl 1):S27–S34
- 27 El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med* 2003;139(10):817–823