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Liver: Nonanatomical Resection

21

Fumihiko Fujita, Susumu Eguchi, Yoshitsugu Tajima, and Takashi Kanematsu

21.1 Introduction

The first laparoscopic procedure, a laparoscopic cholecystectomy (LC), was initially performed in Europe in the mid-1980s [1, 2]. More complex procedures, like laparoscopic liver surgery, have developed more slowly in the years thereafter. The first laparoscopic hepatectomy was initially performed for benign liver disease in 1991; however, this procedure has been slow to gain acceptance for malignant disease processes [3, 4]. Since 1995, several reports of laparoscopic hepatectomies for liver cancer have been published [5, 6]. Laparoscopic liver surgery had been limited initially to tumors located in peripheral segments of the liver [7]. Some authors report using this procedure for successful anatomical lobe resections or living donor hepatectomies for liver transplantation [8–11].

Hepatocellular carcinoma (HCC) and metastatic liver cancer, especially colorectal cancer, are the two most frequent liver malignancies. Both disease processes can be treated with laparoscopic partial hepatectomy depending on their location. Especially for HCC, anatomic resection techniques are recommended in order to prevent dissemination of cancer cells into the portal vein. These techniques should also be applied if laparoscopy is used to perform a hepatectomy [12, 13]. A Glissonean pedicle transection is recommended as well to prevent cancer cells from being disseminated during a hepatectomy. This technique is thought to improve the postoperative survival in patients with HCC [14]. On the other hand, patients with HCC commonly have a history of chronic hepatitis and liver cirrhosis with an incidence of 74.1% and 63.3%, respectively [15]. This contributes not seldom to severe liver cirrhosis or a poor liver reserve in that subset of patients; hence, a partial, nonanatomic hepatectomy may be more suitable for these patients than major liver resections. HCC can be a secondary cause of viral infections, including hepatitis B virus (HBV) or hepatitis C virus (HCV). Unfortunately, if present, these viral infections seem to increase the incidence of recurrence after surgical resection of the liver. Especially in such cases, laparoscopic procedures may be best suited in order to avoid an unnecessary exploratory laparotomy if only a biopsy or a nonanatomical liver resection is planned.

Metastatic disease to the liver from colorectal cancer is a well-accepted indication for liver resection and it has been demonstrated to improve overall patient survival [16]. Laparoscopic partial hepatectomies are accepted indications for the treatment of liver metastasis, but its successful completion very much depended on the location of the tumor. Mala et al. compared the short-term outcome of laparoscopic and conventional liver resections in patients with colorectal liver metastasis and concluded that the laparoscopic procedure was superior to the traditional open approach in terms of shorter hospital stay and reduced postoperative pain [17].

21.2 Non-surgical Therapies for Liver Malignancies

Optimal treatment strategies for patients with advanced and unresectable HCC are still under investigation [18, 19]. The prognosis of patients with

F. Fujita (✉), S. Eguchi, Y. Tajima, and T. Kanematsu
Department of Transplantation and Digestive Surgery, Nagasaki University Graduate School of Biomedical Science, 1-7-1 Sakamoto, Nagasaki, 852-8501, Japan
e-mail: ffujita@net.nagasaki-u.ac.jp

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unresectable disease, multiple intrahepatic metastases, and major portal vein thrombosis is much worse, and most of these patients die within several months [20, 21]. Given this dismal outcome, the development of effective chemotherapeutic agents or targeted molecular therapies is urgent and mandatory. Transarterial embolization (TAE) or transarterial chemoembolization (TACE) are the most used treatment options for patients with unresectable HCC. In addition, if cirrhotic patients have a poorly preserved liver function, hepatic arterial infusion chemotherapy (HAIC) is another modality used [19, 22, 23]. The chemotherapeutic agents used, either individually or in combination, are Cisplatin, 5-Fluorouracil (5-FU), Epirubicin, Doxorubicin, and Mitomycin-C [19]. Of these agents, 5-FU and Cisplatin are the most commonly applied to treat HCC [24]. Several molecular targeted therapies with drugs like Sorafenib are in trial and the results are awaited with much anticipation.

21.3 Minimally Invasive Approach to Liver Malignancies

21.3.1 Microwave Coagulation Therapy (MCT)

This technique has been widely applied for the treatment of malignant liver disease. A monopolar antenna is inserted into the area harboring cancer and induces tissue necrosis by coagulation [25]. Microwave coagulation therapy (MCT) is applicable for small HCC's with a maximum diameter of less than 2 cm. The big advantage of MCT is that it can be repeated several times over the course of the disease [26]. MCT is especially recommended for patients with poor hepatic reserve, and can be performed via either an open or laparoscopic approach [27, 28]. Sadamori et al. analyzed the serum levels of Interleukin-6, cytokine antagonists, and C-reactive protein, which reflect the severity of surgical stress, between patients following laparoscopic and open MCT, and they concluded that laparoscopic MCT could be recommended for patients with poor hepatic reserve when their indocyanine green retention rate at 15 min (ICG R15) is over 30% [26].

21.3.2 Radiofrequency Ablation (RFA)

Radiofrequency ablation (RFA) is often used percutaneously. As a guidance tool either ultrasound (US), computed tomography (CT), or magnetic resonance imaging (MRI) can be used [29]. RFA has specific characteristics which makes it suitable to treat liver malignancies. It is easy to apply, very effective, and can be repeated if necessary [30, 31]. In comparison to MCT, it coagulates the target point more widely. In general, RFA is indicated for tumors with a maximum diameter of less than 3 cm.

21.4 General Aspects in Laparoscopic Hepatectomies

21.4.1 Preoperative Considerations

The absence of coagulopathy and a sufficient hepatic reserve are important prerequisites. The amount of ascites, the serum level of total bilirubin, and the indocyanine green (ICG) clearance test results are important factors to determine the best surgical strategy. Portal vein pressure is also a useful measurement to evaluate the extent of liver cirrhosis and to determine the area of liver to be resected.

21.4.2 Indications for Laparoscopic Hepatectomy

Indications for laparoscopic hepatectomy should be the same as those for open hepatectomy. Tumor location is still one of the major drawbacks to successful completion of laparoscopic hepatectomy. In general, tumors located in lateral segments (Couinaud segments 2 and 3 and 6) and on the surface of the liver are more suitable for a laparoscopic partial hepatectomy because of their easy access [6, 11, 32]. On the other hand, tumors located in the posterior or superior portion of the right lobe are associated with poor visualization and control of bleeding might be difficult. For those tumors, Huang et al. recommended a hand-assisted laparoscopic approach [33].

21.5 Complications in Laparoscopic Liver Surgery

21.5.1 Intraoperative Bleeding

Intraoperative bleeding is one of the major complications associated with laparoscopic major hepatectomies or even smaller wedge resections. Several authors have reported to intermittently apply the Pringle maneuver for vascular control to reduce blood loss especially during the parenchymal part of the transection [34–36]. In the event of a hemorrhage from the parenchyma of the liver, gauze can be placed over the bleeding site for temporary packing. This packing can usually be removed after 10–15 min. If hemostasis cannot be achieved, a suture or clip can be applied, but the surgeon should consider converting to open surgery under those conditions.

21.5.2 Gas Embolism

In order to obtain good visualization, establishing a pneumoperitoneum using carbon dioxide (CO_2) is recommended especially because of the solubility of CO_2. However, laparoscopic liver surgery using CO_2 carries a high risk of inducing gas embolism [37–39]. Although an accidental gas embolism is rare [40, 41], some authors recommend a gasless laparoscopic technique while resecting the hepatic parenchyma [5, 6, 42]. In addition, the elevated intra-abdominal pressure caused by CO_2 insufflation bears not only the risk of air embolism but also significantly decreases portal blood velocity [39]. Careful monitoring for a gas embolism and meticulous dissection of the liver are crucial preventive measures.

21.5.3 Trocar Site Metastasis

The possibility of port-site recurrence remains one of the main controversies in the use of laparoscopic surgery for malignancies [43–46]. Clinical evidence demonstrated the incidence of wound recurrences to be similar between laparoscopic and conventional procedures [35, 44, 47]. Lang et al. also concluded that laparoscopy does not increase the risk of either port-site or peritoneal metastases in patients with HCC [48]. Vittimberga et al. reported that the immune response is better preserved after laparoscopic surgery than compared with an open procedure. This would result in less port site recurrences [49].

21.6 Surgical Technique

21.6.1 Operating Room Setup and Patient Positioning

The patient is placed in supine position, with split-leg technique. The surgeon positions himself between the legs with one assistant on each side of the patient. Two monitors are placed at the head of the table and as close as possible to the surgeon (Fig. 21.1). For lesions in segment 6, the patient is placed in the left lateral decubitus position in order to expose the lateral aspect of the right lobe of the liver.

21.6.2 Trocar Placement

Four, sometimes five trocars are generally used. A set of two 5-mm and three 12-mm trocars, placing the camera trocar slightly supraumbilical, is used for our preferred setup (Fig. 21.2). Pneumoperitoneum using CO_2 is established and abdominal pressure monitored and maintained below 8 mmHg at all times to reduce the risk of gas embolism. An abdominal wall lift technique is sometimes used in order to reduce the risk of gas embolism (Fig. 21.3). We prefer a laparoscope with a flexible tip.

21.6.3 Diagnostic Laparoscopy and Determination of the Dissection Line

The liver is examined under direct visualization in conjunction with intraoperative ultrasound to confirm the number and size of the lesions to resect. It is important

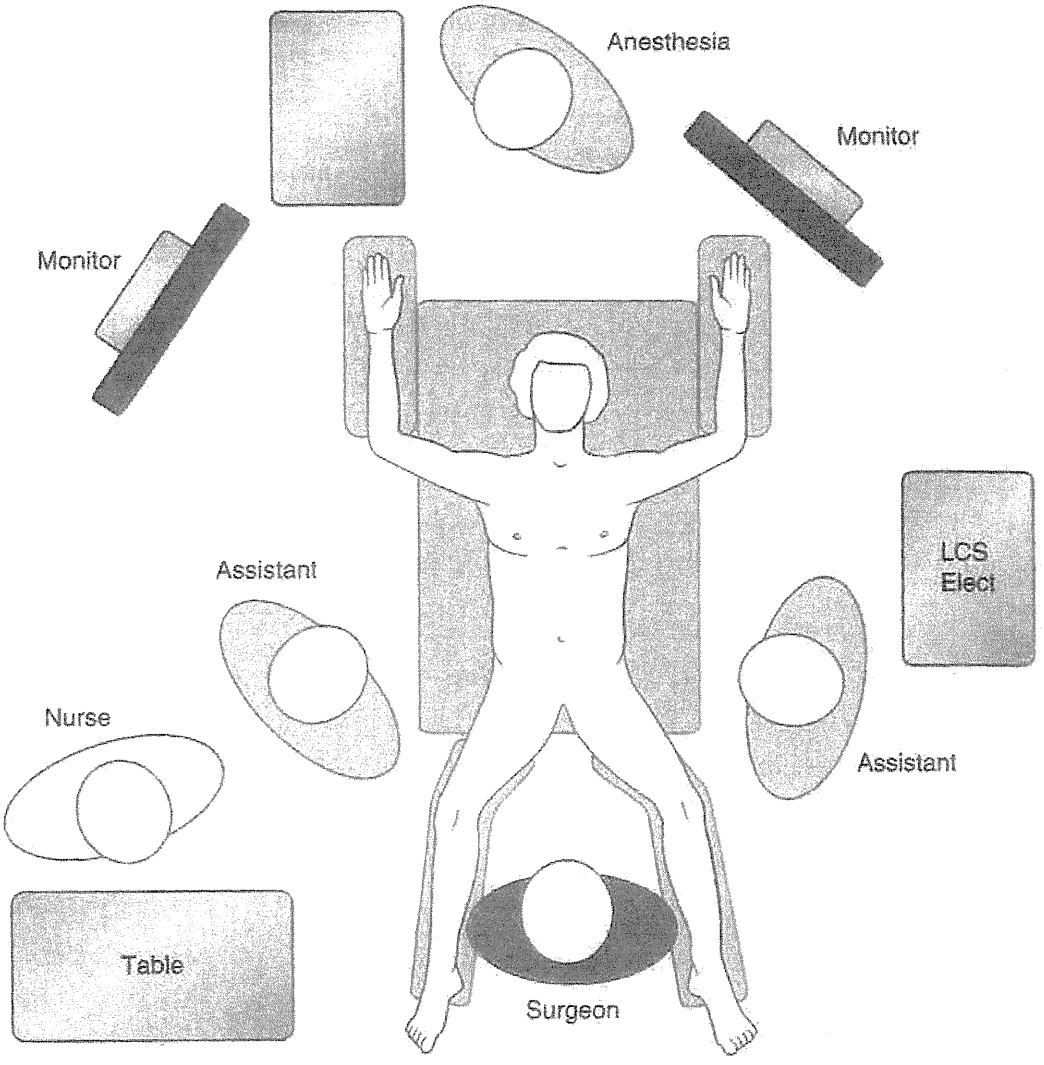

Fig. 21.1 Patient positioning and operating room setup. *Su* surgeon, *As* assistant, *Ns* nurse, *Anes* anesthesiologist. (Drawing by Hippmann GbR, Schwarzenbruck, Germany)

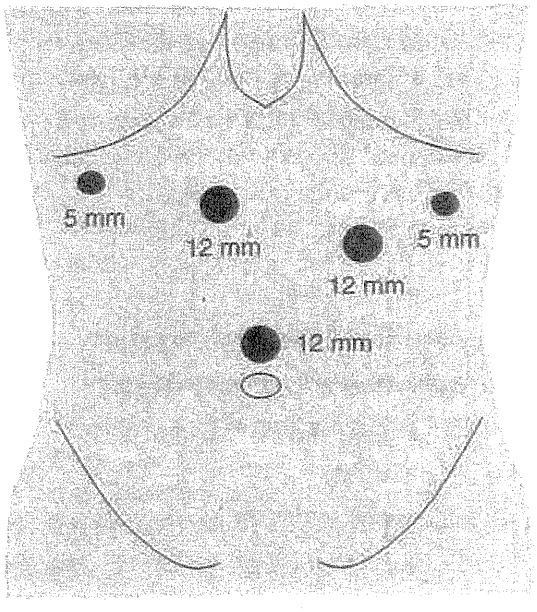

Fig. 21.2 Trocar placement

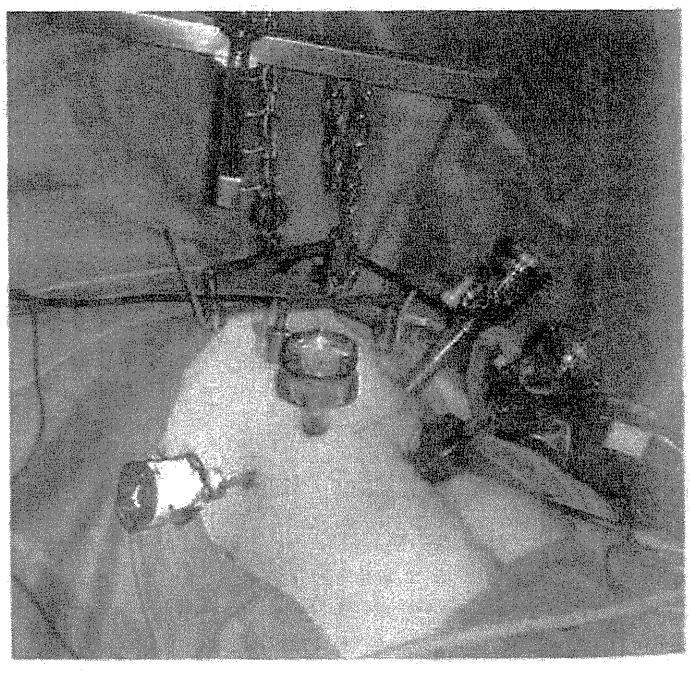

Fig. 21.3 Abdominal wall-lift technique is an alternative to pneumoperitoneum when dissecting the parenchyma of the liver. It is thought to reduce the risk of CO_2 gas embolism

to define their relationship to the intrahepatic vascular structures. In cases where a left lateral segmentectomy or wedge resection is planned, we determine and mark the transecting line on the surface of the liver using intraoperative ultrasound prior to starting the dissection (Fig. 21.4).

21.6.4 Dissection – The Operative Steps

As an initial step, we divide the falciform ligament, and the dissection is then carried on, down to the level of the inferior vena cava. After that, a small hole is made in the coronary ligament, which is located on the extended transecting line. The left triangular ligaments are usually preserved (Fig. 21.5). A penrose drain is inserted into the abdominal cavity and one side of it fixed to the abdominal wall. The other side is passed through the hole in the coronary ligament and positioned behind the posterior surface of the lateral segment of the liver (Fig. 21.6). During the hepatic parenchymal dissection, this penrose drain plays an important role in lifting up the liver and exposing the dissection plane (Figs. 21.7 and 21.8). The preserved

Fig. 21.4 Laparoscopic ultrasound is the key imaging modality to locate the target lesions and to define the dissection line

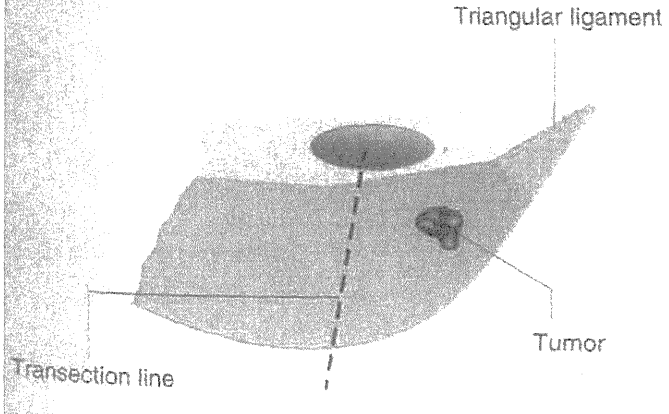

Fig. 21.5 The transection line is determined using intraoperative ultrasound. A hole is made in the coronary ligament that is located on the extended transecting line. This will help for further exposure. (Drawing by Hippmann GbR, Schwarzenbruck, Germany)

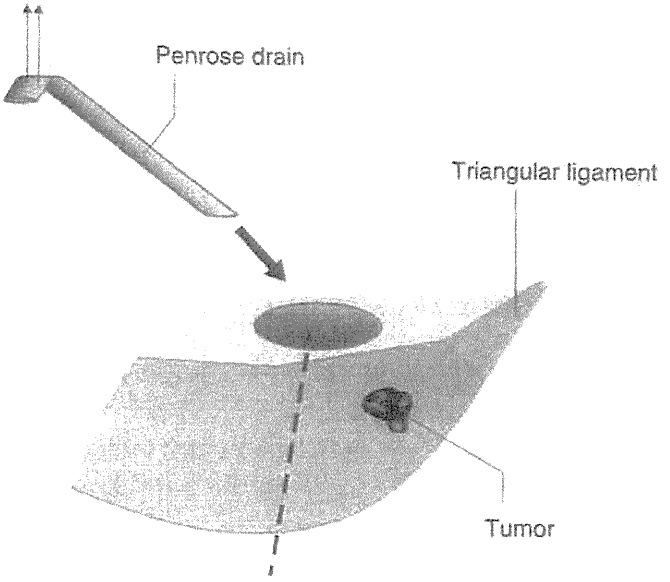

Fig. 21.6 A penrose drain is passed through the divided coronary ligament. One side is fixed to the abdominal wall. The triangular ligament is usually preserved, and helps to prevent the penrose drain from slipping out. (Drawing by Hippmann GbR, Schwarzenbruck, Germany)

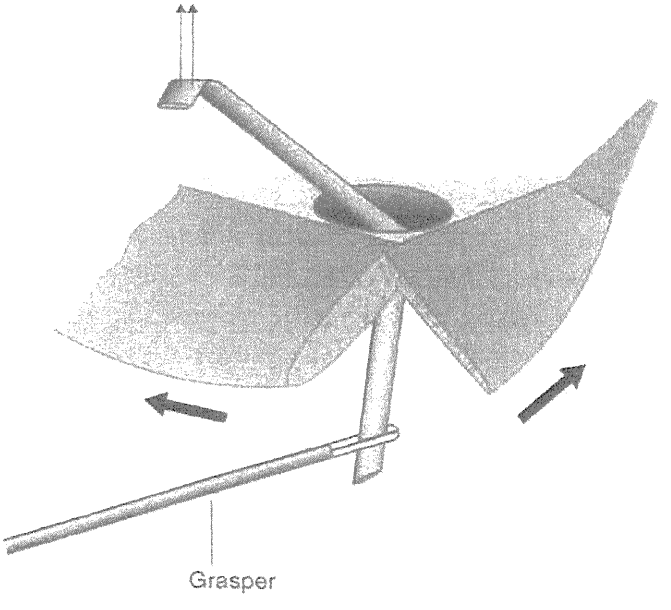

Fig. 21.7 The penrose drain is controled with a grasper. The transecting plane opens up nicely and we obtain great exposure. (Drawing by Hippmann GbR, Schwarzenbruck, Germany)

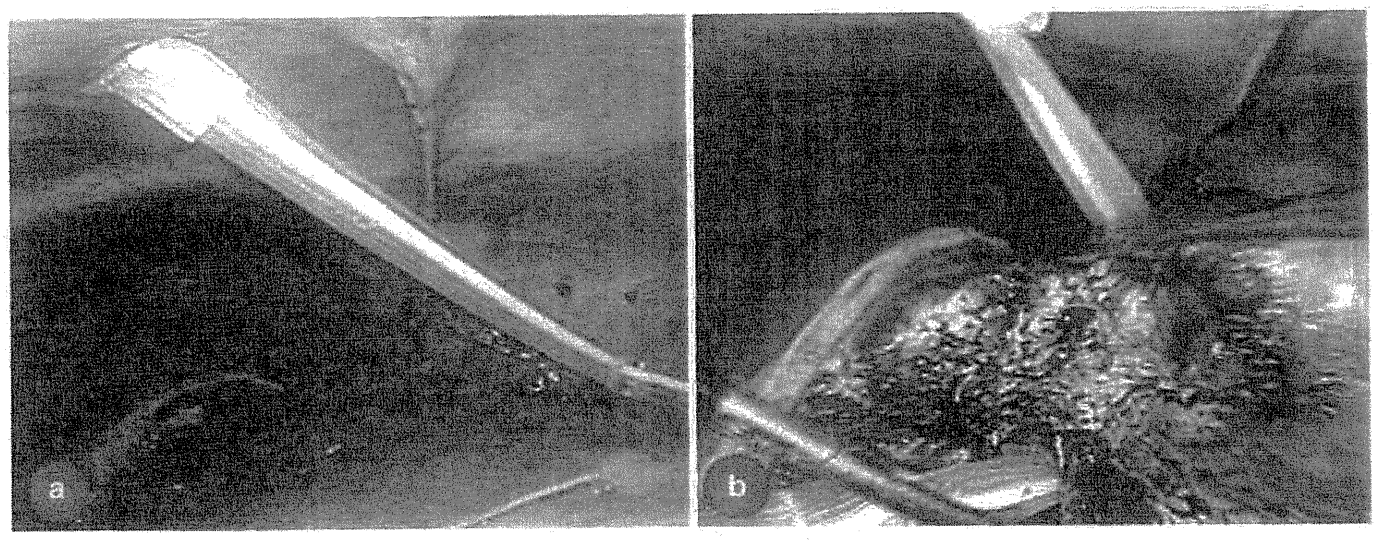

Fig. 21.8 (a) One side of the penrose drain is fixed to the abdominal wall. (b) The penrose drain allows good exposure of the transection plane, contributing to less intraoperative blood loss and safe dissection

left triangular ligaments are useful in preventing the penrose drain from slipping out.

We regularly use the harmonic scalpel (Ultracision; Ethicon Endo-Surgery) to perform the hepatic transection. This is a surgical device utilizing ultrasonic energy to cut and coagulate tissues. This device is sufficient to seal and divide vascular and biliary structures up to 3 mm in diameter. Other larger structures should only be divided after initial clipping. The TissueLink® device, an instrument using monopolar energy, is also used to dissect the parenchyma of the liver. This device provides excellent coagulation and limits bleeding to a great extent [40]. The surface of this radiofrequency device is covered by a continuous flow of saline, to keep the tissue temperature at or below 100°C without producing any char [50]. An important step at this point of the operation is to maintain constant contact with the liver tissue while dissecting the parenchyma. Smaller vessels can be divided safely using the TissueLink device only. Because the TissueLink device has a characteristic mode of action during parenchymal dissection, the vascular and biliary structures are preserved and they are sealed by shrinking the natural collagen in tissue [51] (Fig. 21.9). This sealing is effective for structures up to 3 mm in diameter. Larger structures should be secured with clips before division as we would do when using the harmonic scalpel. Portal pedicles and major hepatic veins are divided by applying a linear stapler using a vascular white load. When dividing the major hepatic veins, make sure that these structures are circumferentially freed off parenchyma in order to safely apply the stapler (Fig. 21.10).

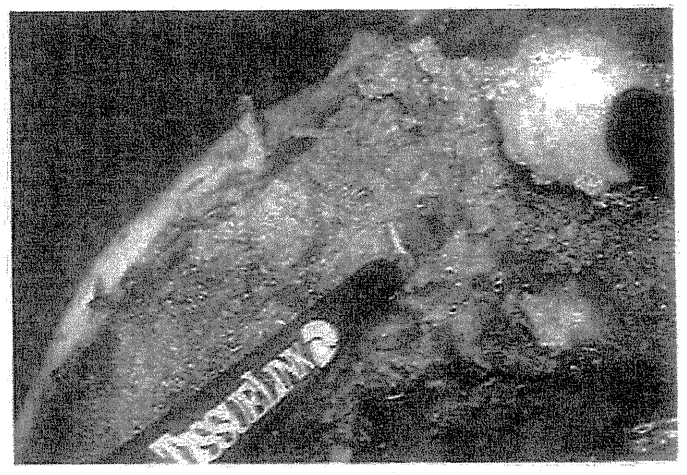

Fig. 21.9 TissueLink™ is used to dissect the parenchyma of the liver. The blunt force applied greatly reduces intraoperative blood loss

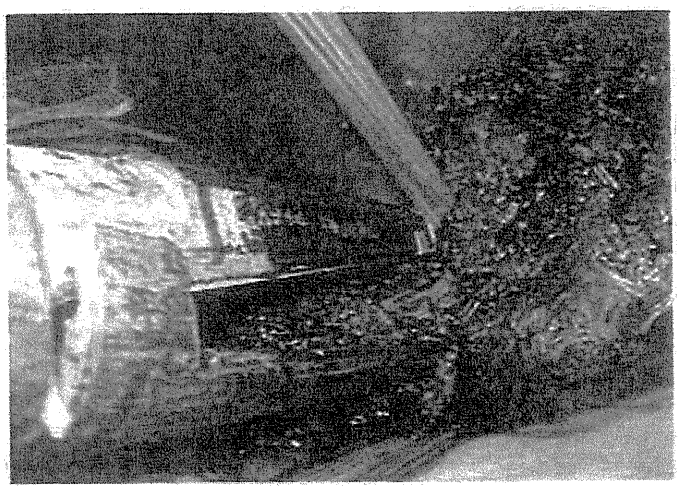

Fig. 21.10 The left hepatic vein is divided with a linear stapler. Make sure that the entire vein is freed off the parenchyma. Blind stapler application may cause major bleeding

21.6.5 Retrieval of the Specimen

The resected specimen is placed in an impermeable retrieval bag and externalized without fragmentation through a separate incision made in the suprapubic region. After retrieval of the specimen, we close this incision immediately and reestablish the pneumoperitoneum. The surgical field is now irrigated and checked for any bleeding or bile leakage. Residual fluid is removed by suction.

21.7 Hand-assisted Technique (HALS)

Hand-assisted laparoscopic surgery (HALS) can overcome some disadvantages associated with a total laparoscopic approach. In 2000, Fong et al. reported preliminary results using HALS for liver resections [36]. Recently, more authors reported the advantages of a HALS approach to the liver [10, 33, 34, 52, 53]. The benefits of HALS are mainly in facilitating manual retraction, in assessing safe resection margins using tactile feedback, and safe parenchymal dissection [10, 34]. The assisting hand can be used for blunt dissection, and to place stapling devices more precisely [34]. Cushieri et al. reported shorter operative times using HALS [52]. The most important advantage seems to be superior control of bleeding, because the fingers can be used to grasp the bleeding vessels immediately. HALS may be converted to an open procedure more easily, whenever it becomes necessary. In this case, we are able to extend the incision for the hand port to a full laparotomy incision much faster. Although HALS usually requires a larger incision of 6–8 cm when compared to a totally laparoscopic procedure, this wide incision will be used to deliver the specimen.

21.8 Future Trends

Will laparoscopic liver surgery become the "gold standard" for all types of liver procedures?

Laparoscopic techniques will probably take the place of open techniques with regard to wedge resections and segmentectomies for focal lesions. Most hepatobiliary surgeons already accept laparoscopic partial hepatectomies for benign liver tumors in various locations. However, for malignant disorders, many surgeons may choose a laparoscopic approach only for tumors located in peripheral segments. In cases, where the extent of the resection is bigger than just a wedge or one segment, an open approach may be chosen in many surgical departments because of safety concerns or out of technical reasons. Recent advancements in technology have made laparoscopic liver surgery safer. New instruments such as TissueLink™ or harmonic scalpel can reduce the amount of intraoperative blood loss. Laparoscopic liver surgery for major hepatectomies may be easily accepted by many surgeons in the near future as technology evolves and surgeons acquire advanced skills through specialized training. Laparoscopic procedures for malignant lesions should only be performed by surgical experts and the same oncological rules should apply when compared to an open resection.

Quick Reference Guide

1. Patient positioning and port placement are dependend on tumor location.
2. Maintain intra-abdominal pressure as low as possible while still achieving adequate visualization. A pressure of less than 8 mmHg should be used to reduce the risk of gas embolism.
3. Laparoscopic ultrasonography is useful to confirm the location of the tumor and to determine the dissection line.
4. Stitches placed on both sides of the dissection line can help to provide good counter traction while dissecting the parenchyma of the liver.
5. Appropriate devices should be selected to dissect the parenchyma of the liver. This can greatly reduce intraoperative blood loss.
6. The harmonic scalpel is used to incise the capsule of the liver and to seal vessels or intrahepatic bile ducts up to 3 mm in diameter. The TissueLink® device is used to dissect the parenchyma of the liver
7. Vessels or intrahepatic bile ducts more than 3 mm in diameter should be clipped before they are divided.
8. If bleeding occurs from the cut edge of the liver, immediate packing with gauze should be the

first choice. Thereafter, clips or stitches should be applied.

9. It is necessary to obtain great visualization when approaching the hepatic vein. If a linear cutting stapler is used to divide the hepatic vein, the surrounding parenchyma should be dissected carefully before firing the device.
10. Upon completion of the operation, confirm that there is no bleeding or bile leakage at the cut surface.

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REVIEW ARTICLE

Role of tumor-associated macrophages in the progression of hepatocellular carcinoma

Ken Shirabe · Yohei Mano · Jun Muto · Rumi Matono · Takashi Motomura · Takeo Toshima · Kazuki Takeishi · Hidekaki Uchiyama · Tomoharu Yoshizumi · Akinobu Taketomi · Masaru Morita · Shunichi Tsujitani · Yoshihisa Sakaguchi · Yoshihiko Maehara

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Abstract Recent studies have shown that the tumor microenvironment plays an important role in cancer progression. Tumor-associated macrophages (TAMs), in particular, have been found to be associated with tumor progression. Macrophages have multiple biological roles, including antigen presentation, target cell cytotoxicity, removal of foreign bodies, tissue remodeling, regulation of inflammation, induction of immunity, thrombosis, and endocytosis. Recent immunological studies have identified two distinct states of polarized macrophage activation: the classically activated (M1) and the alternatively activated (M2) macrophage phenotypes. Bacterial moieties such as lipopolysaccharides and the Th1 cytokine interferon-γ polarize macrophages toward the M1 phenotype. The M2 polarization was discovered as a response to the Th2 cytokine interleukin-4. In general, M2 macrophages exert immunoregulatory activity, participate in polarized Th2 responses, and aid tumor progression. TAMs have recently been found to play an important role in hepatocellular carcinoma (HCC) progression. Based on the properties of TAMs, obtained from pathological examination of resected specimens, we have identified new therapeutic approaches, involving the targeting of TAMs with adjuvant therapy after hepatic resection for HCC. This review discusses the roles of TAM in HCC progression and the possibility of new therapies targeting TAMs.

Keywords Hepatocellular carcinoma · Tumor-associated macrophages · Hepatic resection · Cancer progression

K. Shirabe (✉) · Y. Mano · J. Muto · R. Matono · T. Motomura · T. Toshima · K. Takeishi · H. Uchiyama · T. Yoshizumi · A. Taketomi · M. Morita · S. Tsujitani · Y. Maehara
Department of Surgery and Science,
Graduate School of Medical Sciences, Kyushu University,
3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
e-mail: kshirabe@surg2.med.kyushu-u.ac.jp

Y. Sakaguchi
Department of Gastroenterological Surgery,
National Kyushu Cancer Center, Fukuoka, Japan

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and accounts for almost 700,000 deaths annually [1]. Although surgical procedures for HCC, such as hepatic resection and liver transplantation, have progressed and the outcomes have improved, HCC is still characterized by frequent recurrence [2–4], even after liver transplantation [5]. The recent introduction of the molecular targeting agent, sorafenib, was reported to improve survival rates in patients with nonresectable or advanced HCC [6]. Sorafenib is a multikinase inhibitor, found by that study to prolong survival significantly, although still by less than 3 months [6]. Clearly, new therapeutic strategies are needed to improve the survival of patients with HCC.

For more than 100 years, pathologists have reported that solid tumors consist of malignant tumor cells and stromal cells [7]. Recent studies have revealed that the stromal cells in solid tumors are a dynamic, flexible asset to tumor progression [7]. In particular, tumor-associated macrophages (TAM) are an important component of the tumor microenvironment and can promote tumor progression. Macrophages are a major component of the leukocytes that infiltrate most tumors [8], and numerous studies have shown that a high frequency of infiltrating TAMs is associated with poor prognosis [9–12]. Macrophages have multiple biological roles, which include antigen

presentation, target cell cytotoxicity, removal of foreign bodies, tissue remodeling, regulation of inflammation, induction of immunity, thrombosis, and endocytosis. Recent immunological studies [13, 14] have identified two distinct states of polarized macrophage activation, namely, the classically activated (M1) and the alternatively activated (M2) macrophage phenotypes (Fig. 1). Bacterial moieties, such as lipopolysaccharides and the Th1 cytokine interferon (IFN)-γ, polarize macrophages toward the M1 phenotype, whereas the M2 polarization was discovered as a response to the Th2 cytokine IL-4. In general, M2 macrophages exert immunoregulatory activity, participate in polarized Th2 responses, help with parasite clearance, suppress inflammation, and promote tissue remodeling, particularly tumor progression. Although the mechanisms by which TAMs promote tumor progression are poorly understood, recent research has shed new light on their roles.

An analysis of TAMs, which interact with cancer cells, must be based on surgical specimens; hence, pathological examination of the resected specimen is particularly important for the analysis of TAMs. Therefore, surgeons will provide pivotal information to open this field and may help to develop new adjuvant therapies that target TAMs. There are many unanswered questions about TAMs: how are TAMs recruited into the tumor? How are the antitumor immune effects of TAMs impaired? What roles do TAMs play in the metastasis, angiogenesis, and endothelial–mesenchymal transition (EMT) of HCC? What is the prognostic value of TAMs in patients with surgically resected HCC? Can we develop new therapeutic strategies for HCC that specifically target TAMs?

This review discusses the recent research on TAMs in cancer, particularly in HCC, and evaluates the new therapeutic strategies in this field.

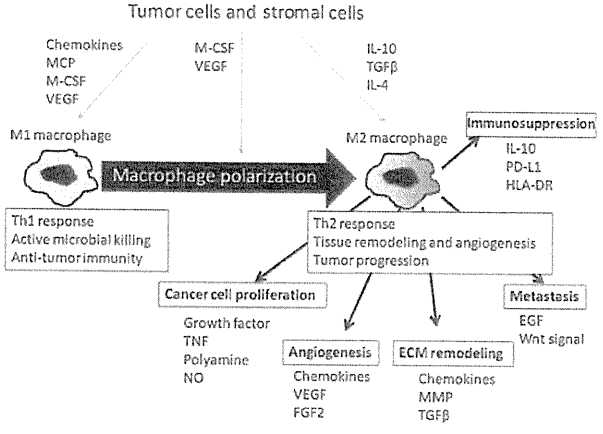

Fig. 1 Proposed functions of tumor-associated macrophages in tumor progression. Two distinct states of polarized activation for macrophages have been recognized: the classically activated (*M1*) macrophage phenotype and the alternatively activated (*M2*) macrophage phenotype. M2 macrophages are involved in cancer proliferation, angiogenesis, extracellular matrix remodeling, metastasis, and immunosuppression, producing a microenvironment that benefits cancer progression. *IL* interleukin, *MCP* macrophage chemoattractant protein, *M-CSF* macrophage colony-stimulating factor, *VEGF* vascular endothelial growth factor, *TGFβ* tumor growth factor-β, *NO* nitric oxide, *MMP* matrix metalloproteinase, *PD-L1* programmed cell death-ligand 1, *ECM* extracellular matrix, *TNF* tumor necrosis factor, *EGF* epidermal growth factor, *FGF* fibroblast growth factor

TAM recruitment

Macrophages are released from the bone marrow as immature monocytes. After circulating in the bloodstream, they migrate into tissues and undergo final differentiation into resident macrophages, including Kupffer cells in the liver. TAMs are derived from monocytes, which are recruited largely by CCL2 [chemokine (C–C motif) ligand 2; formerly known as monocyte chemotactic protein (MCP)] and chemokines [15]. CCL2 is mainly expressed by tumor cells, but also by endothelial cells, fibroblasts, and macrophages in human tumors [16]. Some studies suggest that it is highly expressed in a wide range of tumors, including glioma [17], meningioma [18], ovarian carcinoma [19], and squamous cell carcinoma of the uterine cervix [20].

Other tumor-derived signals have been reported to recruit TAMs into tumors, including macrophage-colony stimulating factor (M-CSF or CSF-1), macrophage inflammatory protein 1α (MIP-1α), vascular endothelial growth factor (VEGF), CCL3, CCL4, CCL5, CCL8, and angiopoietin-2 [21]. Zhu et al. [22] demonstrated that high expression of M-CSF in peritumoral liver tissue is correlated with macrophage density and poor survival following curative resection for HCC. Similarly, Jia et al. [23] reported that the MCSF-1 receptor expression in peritumoral tissue was associated with intrahepatic metastasis and poor prognosis following hepatectomy. Shen et al. [24] detected high levels of IL-10 and transforming growth factor (TGF)-β, and low levels of IFN-γ in the HCC microenvironment, representing an immunosuppressive state. Takai et al. [25, 26] showed that the expression of glypican-3 on the cell membrane of HCC cells was involved in TAM recruitment. Although the mechanisms involved in TAM recruitment are not yet fully understood, the microenvironment and cytokine network in HCC seem to promote TAM recruitment and M2 polarization.

TAMs and immunity

Macrophages are essential for host defense. In humans, macrophages play a crucial role in the innate and adaptive

immune responses to pathogens and are critical mediators of inflammatory processes.

Macrophages can assume a range of different phenotypes based on the environmental stimuli. The extremes of this range obtained in vitro are represented by the M1 phenotype, which is associated with active microbial killing, and the M2 phenotype, which is associated with tissue remodeling and angiogenesis. When monocytes in the tumors are exposed to tumor-derived antiinflammatory molecules, such as IL-4, IL-10, TGF-β1, and prostaglandin E_2, they develop into polarized or M2 macrophages [13].

The M2 phenotype appears to be the dominant macrophage phenotype in tumors because TAMs show similar molecular and functional profiles. These profiles are characterized by low expression of differentiation-associated macrophage antigens, such as carboxypeptidase M and CD51, high constitutive expression of arginase I, IL-1 decoy, IL-1ra, IL-6, and IL-10, and low expression of tumor necrosis factor (TNF) and IL-12. M2 macrophages also express chemokines, including CCL17 and CCL22 [8, 27, 28]. The polarization of M2 macrophages has not been clearly demonstrated in HCC because, in most studies, the anti-CD 68 antibody has been used for the immunohistochemical evaluation of macrophages in HCC. Takai et al. [25] used two antibodies to differentiate between resident macrophages and activated macrophages, with a pan-macrophage marker (AM-3K) to label resident macrophages, whereas PG-M1, which reacts with the human CD68 antigen, was used to label activated macrophages. Unfortunately, to our knowledge, no further functional analysis of macrophages has been performed.

The serum levels of TGF-β1 are elevated in patients with HCC, which may explain the phenomenon that the M2 phenotype macrophages, as well as TAMs, secrete down-regulatory cytokines such as TGF-β1 [29, 30]. M2 macrophages can induce the differentiation of regulatory T cells. For example, Zhou et al. [31] reported that the intratumoral prevalence of $FOXP3^+$ regulatory T cells in HCC was associated with a high density of macrophages. Macrophages exposed to tumor culture supernatants form hepatoma-derived cell lines with an increased frequency of $FOXP3^+$ regulatory T cells in vitro, and this increase is partially blocked by anti-IL-10 antibodies. Wu et al. [32] and Kuang et al. [33] demonstrated that activated macrophages in the peritumoral stroma and Kupffer cells express programmed cell death (PD) (B7-H1)-ligand 1 (L1) and suppress tumor-specific T-cell immunity. These experimental findings may explain how TAMs suppress antitumor immunity in HCC. A recent study by Ju et al. [34] showed a positive correlation between the density of peritumoral activated hepatic stellate cells (HSC) and macrophage or regulatory T cells, and that the presence of HSCs, macrophages, and regulatory T cells was associated with aggressive clinicopathological features. Taken together, these findings suggest that TAMs, regulatory T cells, and HSCs might provide an immunosuppressive environment that ultimately aids HCC recurrence.

Recent studies have revealed the important roles of TAMs and memory Th 17 cells in tumor progression. In ovarian carcinoma, $CD8^+$ T cell-derived IL-17 mediates the recruitment of myeloid cells into tumors and enhances tumor growth [35]. Zhang et al. [36] showed that intratumoral IL-17-producing cells were correlated with a poor prognosis in HCC, whereas Kuang et al. [37, 38] reported an interaction between TAMs and memory Th 17 cells in HCC and found that TAMs promote memory Th 17 cell expansion.

Role of TAMs in the metastasis of HCC

Many reports state that TAMs promote distant metastasis of cancer cells. According to Gorelik et al. [39], macrophages increased the number of lung tumor nodules following an intravenous injection of murine tumor cells. Rolny et al. [40] reported also that inhibiting TAM infiltration into tumors using antibodies to neutralize monocyte chemoattractants impaired metastases.

TAMs and cytokines such as IL-1, TNF, and IL-6 are believed to have prometastatic properties, and recent studies provide new evidence to support this. For example, the IL-6 levels in patients with liver cancer are much higher than those in healthy adults [41]. Liu et al. [42] reported that IL-6 exerted antiapoptotic activity via the STAT3 signaling pathway in human HCC cell lines. These phenomena may be related to TAMs, which can produce IL-6, and further studies in this field are necessary.

In a mammary carcinoma model, gene expression profiling of macrophages supporting cancer invasion revealed activation of the Wnt signaling pathway, which may play a key role in the prometastatic activity of TAMs [43]. Activation of the Wnt signaling pathway in cancer cells may promote cell proliferation and cell detachment, a step that is essential for cancer cells to metastasize to the distant organ.

The next stage in cancer metastasis is extravasation of the cancer cells. In vitro and in vivo experiments have revealed that extravasation of tumor cells into the hematogenous or lymphatic circulation requires an interaction with TAMs. Condeelis et al. [44] reported that epidermal growth factor (EGF), released by the TAMs, and CSF-1, released by tumor cells, act on the reciprocal cell types to stimulate tumor cell migration. Using in vivo multiphoton microscopy, they showed that metastasis occurs more frequently when the cancer cells are in the vicinity of TAMs and can communicate in this manner.

Role of TAMs in the angiogenesis of HCC

In human tumors, TAMs accumulate in poorly vascularized, necrotic areas. These areas become hypoxic, which in turn triggers a pro-angiogenic program in TAMs [45]. Several clinical studies have shown a significant correlation between a high density of TAMs in human tumors and microvessel density. Peng et al. [46] reported that the TAM count was significantly correlated with microvessel density in HCC. Human TAMs also express various pro-angiogenic factors in tumors, including VEGF [47] and thymidine phosphorylase [48].

Angiogenesis is also facilitated by TAM-derived proteases because extracellular proteolysis is necessary for new vessel formation. Macrophages can secrete proteinases to release a number of pro-angiogenic molecules bound to heparin sulfate in proteoglycans, and fragment fibrin and collagen, which induces angiogenesis. The most prominent proteinases that promote tumor-directed angiogenesis include matrix metalloproteinase (MMP 1, 2, 3, 9, and 12), plasmin, and urokinase-type plasminogen activator (uPA) and its receptor. TAMs were reported to be a major source of MMP-9 and synthesize uPA in various cancers [49, 50].

Role of TAMs in the EMT in HCC

The concept of the EMT, which was originally developed in embryology, has recently been extended to cancer progression and metastasis. In vitro and in vivo studies, as well as clinical samples, support the important role of EMT in cancer progression [49]. Analysis of the EMT has been aided by the development of EMT-associated markers, including epithelial-specific markers (E-cadherin and Claudin-1), mesenchymal-specific markers (vimentin, N-cadherin, and fibronectin), and transcriptional markers (SNAIL, SLUG, and Twist). Using these markers, recent studies have shown that crosstalk between cancer cells and the tumor microenvironment facilitates the EMT in cancer cells [51].

The tumor microenvironment is composed of an extracellular matrix, fibroblasts, myofibroblasts, endothelial cells, immune cells (including TAMs), and soluble factors. The tumor microenvironment is strongly related to the EMT of cancer cells. In addition to cancer cells themselves, cancer-associated fibroblasts may facilitate the EMT of cancer cells. TGF-β is an essential cytokine for the EMT [52], whereas inflammatory cytokines, such as IL-6, are important for the EMT in HCC [53]. TAMs are known to produce these cytokines, particularly the M2 macrophages that produce IL-6 and TGF-β. Recently, Werno et al. [54] demonstrated that HIF-1α-deficient macrophages exhibited the M2 phenotype and could stimulate the differentiation of stem cells toward CD31-positive cells. This finding suggests that the M2 macrophages may facilitate the EMT in cancer. Therefore, the relationship between the TAMs and the EMT in HCC warrants further research.

Prognostic value of TAMs in patients with surgically resected HCC

The prognostic value of the high density of TAMs in HCC is summarized in Table 1. All the previous related studies evaluated the prognostic value of TAMs in patients who underwent hepatic resection for HCC [22, 31, 33, 55, 56]. Most of these studies [22, 31, 36, 55] found that a high density of TAMs was associated with poor prognosis, suggesting that TAMs were related to the immunosuppressive state. However, Li et al. [56] reported that the presence of TAMs and memory T cells was associated with a good prognosis.

New therapeutic strategies for HCC that target TAMs

Three major features of TAMs are potentially amenable to therapeutic interventions: inhibition of their recruitment and/or M2 polarization, inhibition of angiogenic and tissue remodeling activities, and inhibition of their immunosuppressive effects and restoration of antitumor cytotoxicity.

Pharmacological drugs, such as clodronate-encapsulated liposomes or aminobisphosphonates, which knock down macrophages in vivo, have reduced angiogenesis and tumor progression in several experimental tumor models. Recently, Zhang et al. [57] showed that TAMs played an important role in tumor progression during sorafenib therapy. Clodronate-encapsulated liposomes and zoledronic acid, which deplete the macrophage population, are promising drugs that enhance the antitumor effects of sorafenib when used in combination.

Another more sophisticated strategy is to prevent the M2 polarization of TAMs or reorientate M2 TAMs to M1 TAMs. The phenotype of TAMs in most cancers is reversible. In one experimental study, the combination of CpG immunostimulatory oligonucleotide plus an anti-IL-10 receptor antibody switched infiltrating macrophages from the M2 to the M1 phenotype and triggered an innate response that debulked large tumors [58]. Moreover, TAMs lacking STAT6, the major intracellular mediator of IL-4 and IL-13, display an M1 phenotype. As a result, these mice rejected spontaneous mammary carcinoma by exhibiting adaptive immunity to cancer. Similarly, the inhibition of STAT3 activity, which is required for IL-10 activity and gene transcription, restored the expression of pro-inflammatory mediators, including IL-12 and TNF-α, and tumor inhibition.

Table 1 Tumor-associated macrophages and prognosis after hepatic resection for hepatocellular carcinoma

Study	Other factors	Location	Prognosis
Zhu et al. [22].	MCSF	Peritumoral	DFS, OS (poor)
Kwang et al. [33].	PD-L1	Peritumoral	DFS, OS (poor)
Zhou et al. [31].	T_{reg}	Intratumoral	CD68 is correlated with T_{reg}, T_{reg}: DFS, OS (poor)
Ju et al. [34].	HSC, T_{reg}	Peritumoral	DFS, OS (poor)
Ding et al. [55].		Intratumoral, marginal	DFS, OS (poor)
Li et al. [56].	T_M	Intratumoral	DFS, OS (good)

MCSF macrophage colony-stimulating factor, T_{reg} regulatory T cell, *HSC* hepatic stellate cell, *TM* $CD45RO^+$ memory T cell, *DFS* disease-free survival, *OS* overall survival

A recent study also showed that a DNA vaccine against the M2-associated molecule legumain, a member of the asparaginyl endopeptidase family and overexpressed by TAMs, induced a robust $CD8^+$ T-cell response against TAMs. This vaccine also suppressed angiogenesis, tumor growth and metastasis [59]. Several studies revealed the inhibition of macrophage infiltration into tumors after attenuating the effects of VEGF-A with bevacizumab or other neutralizing antibodies. In xenograft-bearing mice, the number of TAMs declined following treatment with anti-VEGF-A therapy [60]. These studies suggest that suppressing TAM accumulation in tumors may enhance the activity of anti-angiogenic therapies by preventing TAMs from secreting additional pro-angiogenic factors.

Kakinoki et al. [61] and Tsuchiyama et al. [62] recently described the biphasic antitumor effects of CCL2/MCP-1 using suicide gene therapy against HCC. They generated low and high expression models using adenoviral vectors. In the low expression model of CCL2/MCP-1, the proportion of M1 macrophages was high and marked antitumor effects were observed. In contrast, in the high expression model, VEGF-A expression and number of $CD31^+$ microvessels were increased. Clearly, further studies are needed to clarify the possible benefits of gene therapy.

Another therapeutic strategy for TAMs is to block the PD-1/PD-L1 pathway [33, 63]. Kuang et al. [33] found that tumor supernatant-treated macrophages suppressed tumor-specific T cells and an anti-PD-L1 antibody improved macrophage-mediated T-cell activation in HCC in vivo. A phase I clinical study of the anti-PD-1 antibody has been conducted in patients with cancer [64]. Taken together, anti-PD-1 antibody therapy may offer another therapeutic strategy for HCC.

Conclusion

Recent studies of TAMs have shown that TAMs play important roles in various stages in the growth and metastasis of cancer cells, including tumor angiogenesis, suppression of antitumor immunity, extravasation of cancer cells, and possibly the EMT of cancer cells. In HCC, recent studies have shown that the accumulation of TAMs is an important cause of cancer cell progression.

The quantification of TAMs in HCC involves immunohistochemical techniques that can be performed on formalin-fixed paraffin-embedded tissues obtained from surgically resected specimens. Determining the density of TAMs in the tumor may help to predict the recurrence of HCC and to identify patients who may benefit from future immunotherapies targeting this pathway. In the near future, pathologists may play critical roles in the diagnostic assessment of TAM infiltration, thereby providing prognostic information and guidance in selecting an appropriate immunotherapeutic strategy for each patient.

A phase I/II clinical trial of an anti-PD-1 antibody for cancer patients is underway in the United States of America [64]. New concepts in adjuvant immunotherapy, such as targeting TAMs following hepatectomy or liver transplantation, may also be introduced to improve the outcome of patients with HCC in the near future.

Conflict of interest The authors have no conflict of interest.

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