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REVIEW

Bone metastasis of hepatocellular carcinoma: facts and hopes from clinical and translational perspectives

Zhao Huang[1,2,3,*], Jingyuan Wen[1,2,3,*], Yufei Wang[1,2,3,*], Shenqi Han[1,2,3], Zhen Li[4], Xuemei Hu[4], Dongling Zhu[5], Zhenxiong Wang[6], Junnan Liang[1,2,3], Huifang Liang[1,2,3], Xiao-ping Chen (✉)[1,2,3,7], Bixiang Zhang (✉)[1,2,3,7]

[1]Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; [2]Clinical Medical Research Center of Hepatic Surgery at Hubei Province, Wuhan 430030, China; [3]Hubei Key Laboratory of Hepato-Pancreatic-Biliary Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; [4]Department of Radiology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; [5]Department of Nuclear Medicine and PET, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; [6]Department of Radiology, Guangzhou First People's Hospital, School of Medicine, South China University of Technology, Guangzhou 510180, China; [7]Key Laboratory of Organ Transplantation, Ministry of Education; Key Laboratory of Organ Transplantation, National Health Commission; Key Laboratory of Organ Transplantation, Chinese Academy of Medical Sciences, Wuhan 430030, China

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Abstract **Patients with hepatocellular carcinoma (HCC) and bone metastasis (BM) suffer from greatly reduced life quality and a dismal prognosis. However, BM in HCC has long been overlooked possibly due to its relatively low prevalence in previous decades. To date, no consensus or guidelines have been reached or formulated for the prevention and management of HCC BM. Our narrative review manifests the increasing incidence of HCC BM to sound the alarm for additional attention. The risk factors, diagnosis, prognosis, and therapeutic approaches of HCC BM are detailed to provide a panoramic view of this disease to clinicians and specialists. We further delineate an informative cancer bone metastatic cascade based on evidence from recent studies and point out the main factors responsible for the tumor-associated disruption of bone homeostasis and the formation of skeletal cancer lesions. We also present the advances in the pathological and molecular mechanisms of HCC BM to shed light on translational opportunities. Dilemmas and challenges in the treatment and investigation of HCC BM are outlined and discussed to encourage further endeavors in the exploration of underlying pathogenic and molecular mechanisms, as well as the development of novel effective therapies for HCC patients with BM.**

Keywords HCC; bone; osteotropism; clinical; basic researches; advances

Introduction

Bone is one of the most frequent metastatic destinations for cancers [1], and the mechanisms underlying the preferences for bone have attracted long-lasting interest that goes back for more than 100 years to Stephen Paget's "seed and solid" hypothesis, which was proposed in 1889 [2]. Although skeleton involvement in breast, lung, and prostate cancers has received extensive attention, studies on the osseous metastasis of hepatocellular carcinoma (HCC) are quite limited.

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Correspondence: Xiao-ping Chen, chenxpchenxp@163.com;
Bixiang Zhang, bixiangzhang@163.com

*These authors contribute equally.

The incidence of HCC bone metastasis (BM) continues to increase with the prolongation of overall survival time (OS) due to the development of novel diagnostic and therapeutic methods [3]. In fact, bone has become the second common metastatic site for HCC; BM accounts for approximately 30% of extrahepatic metastases and is second only to lung metastases [4]. In addition to the primary tumor burden, patients with BM suffer from skeleton-related events (SREs), loss of mobility, reduced quality of life, increased medical costs, and shortened OS [1]. The versatility and imperceptibility of HCC BM confuse diagnosis, and some patients with HCC are diagnosed with BM only at postmortem examination. The early diagnosis of HCC BM reduces the risk of fracture and paralysis, thus improving the quality of life of patients [5]. Imaging tools, including bone scintigraphy

(BS), magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography/computed tomography (PET/CT), provide access for the identification and surveillance of bone lesions [6]. The therapeutic options for HCC BM remain mainly confined to palliative treatments, such as external beam radiotherapy (EBRT), bone-targeting agents (BTAs), biologically targeted therapy, and surgery. Given that these strategies are rarely curative for HCC BM, disease progression prevention and palliative symptom reduction are the main goals [1]. In terms of advances in basic studies, the underlying mechanisms, as well as the molecular markers for the diagnosis and prognosis of HCC BM, have been investigated as discussed in the following sections. At the same time, the enriched and extended cancer bone metastatic cascade gleaned from other osteotropic cancers opens new avenues for profound studies on osteotropism in HCC. In addition, with the intensive investigation of immunomodulatory approaches for tumor treatment, the involvement of the bone immune-microenvironment is attracting increasing attention [7] and hints at the possibility of using immunomodulators, including anti-PD-1 agents [8], for curing HCC BM. Nevertheless, the translation of these findings into clinical applications still has a long way to go.

The importance of HCC BM remains underappreciated by clinicians, specialists, and the literature. The absence of a full overview of HCC bone metastatic events necessitates an up-to-date systematic narration. This review aims to illustrate the current status and future hopes for the treatment of HCC BM by comprehensively summarizing its clinical features and diagnostic and treatment approaches, as well as advances in basic studies on this disease.

Prevalence and risk factors of HCC BM

BM from HCC was uncommon and was previously described only in rare case reports or autopsies [9,10]. From 1962 to 2015, HCC BM varied from 1.97% to 23.59% in the incidence and accounted for 8.33%–37.09% of extrahepatic metastases (Table 1). This discrepancy may be caused by the different diagnostic methods for HCC BM, diverse patient backgrounds, or statistical methods. Nevertheless, as evidenced by constitutive studies in the same centers [4,11] or the analyses of the same database [12,13], HCC BM has increased in prevalence over the past several decades (Table 1). Less than one third of patients present with BM at the time of initial HCC diagnosis, and others develop BM during disease progression [14]. A total of 85% of patients with HCC and BM present with skeletal lesions as the primary extrahepatic disease manifestation [15].

Multivariate logistic regression analyses have indicated that male sex, unmarried status, the high T/N stage of the primary tumor, the advanced histological grade of the primary tumor, high Child–Pugh score, lung metastases, intrahepatic metastases, and brain metastases are independent risk factors for BM diagnosis [14,15,22,23]. Additionally, HBV infection, insurance status, and high AFP levels are associated with the development of HCC BM [14,15].

Pathology and symptoms

HCC BM is mostly found in axial bones, such as vertebrae, the pelvis, and ribs [3,15,20]. The preferential skeletal localization sites of HCC metastases are found in areas of red marrow, which are rich in hematopoietic stem cells (HSCs), growth factors, and mineral substances and thus represent an active microenvironment for cellular growth [1]. Anatomically, concurrent portal hypertension and consequent collateral networks growing throughout the vertebral venous system in patients with HCC may be blamed for the premier propensity for BM to the axial skeleton [11,24]. More than half of the patients present with more than one skeletal lesion, and approximately 77.8% of BM cases are synchronous [14]. Histologically, osteolytic lesions of HCC BM account for 82.44% of all lesions, with 9.76% and 7.80% of lesions being osteoblastic or mixed, respectively [14]. Radiographically, HCC BM is destructive and expansive, with large hypervascular soft-tissue masses replacing the normal bone matrix [5,11].

In addition to the primary tumor burden, patients with BM mostly complain about bone pain [25], which is more likely caused by bone lesions (fractures, increased pressure on the endosteum, periosteum distortion, nerve root compression, and muscle spasms) rather than the tumor burden [26]. Patients also commonly develop SREs, such as pathological fractures, the need for radiotherapy (RT) to relieve bone pain or reduce structural damage within the bone, bone surgery to prevent or repair a fracture, spinal cord compression, and hypercalcemia, which often lead to impaired mobility, decreased quality of life, and an overwhelming medical burden [1]. More than half of the patients suffer from at least one SRE after the diagnosis of BM [14]. In patients with HCC, the RT of the affected bone is the leading SRE, followed by pathologic fracture, spinal cord compression, bone surgery, and hypercalcemia for the SREs throughout disease progression and for the first emerged SREs [14]. The percentages of patients with two and three SREs are 18.90% and 2.84%, respectively, whereby the median number of SREs is 2 [14]. The rates of occurrence of the first SRE within 1, 3, and 6 months after the diagnosis of BM are 30% (95% CI 22%–38%), 43% (95% CI 35%–52%), and 50% (95% CI 41%–59%), respectively. Patients who have an SRE are more susceptible to developing a second SRE after a median time of 0.9 months than those without [15]. The median

Table 1 Reported incidences of HCC BM in various patient cohorts

Period	Center	Diagnostic approaches	Incidence of BM	Rate of BM in EhMs
1962–1981 [16]	Single	Autopsy	16.10% (14/87)	N.A.
1969–1978 [9]	Single	Autopsy	5.33% (12/225)	8.33% (12/144)
1969–1983 [4]	**Single**	**Autopsy**	**N.A.**	**20%**
1978–1987 [11]	**Single**	**BS, radiography, CT, and/or MRI**	**4.46% (12/269)**	**N.A.**
1983–1987 [10]	Single	Plain films, bone scans, or CT	5.06% (20/395)	N.A.
1988–1997 [11]	**Single**	**BS, radiography, CT, and/or MRI**	**12.87% (52/404)**	**N.A.**
1988–2012 [4]	**Single**	**Autopsy**	**N.A.**	**32.1%**
1990–2005 [17]	Single	CT, MRI, BS, and/or PET with FDG	5.63% (56/995)	37.09% (56/151)
1990–2006 [18]	Single	Bone X-ray, BS, and CT or MRI	3.65% (87/2386)	25.44% (87/342)
1992–1997 [19]	Single	CT	10.17% (41/403)	27.70% (41/148)
2002–2014 [15]	Single	At least one imaging modality	23.59% (151/640)	32.90% (151/459)
2005–2015 [20]	Single	Symptoms, and CT or MRI	1.97% (20/1017)	N.A.
2006–2009 [21]	Single	PET/CT	N.A.	19.07% (49/257)
2009–2016 [5]	Single	Radiological images or pathological findings	9.71% (76/783)	N.A.
2010–2013 [12]	**Multiple**	**Unknown**	**N.A.**	**22.93% (1008/4396)**
2010–2014 [22]	Multiple	Unknown	4.29% (1567/36 507)	N.A.
2010–2015 [13]	**Multiple**	**Unknown**	**N.A.**	**32.47% (1015/3126)**

Note: Bolded information indicates constitutive analyses from the same institutions or database.Abbreviations: BM, bone metastasis; EhMs, extrahepatic metastases; N.A., not available; CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography; FDG, fluorodeoxyglucose; BS, bone scintigraphy.

times to the first, second, and third SREs after confirmed diagnosis of BM are 3, 6, and 9 months, respectively [14].

Imaging-based diagnostic approaches

The clinical symptoms and laboratory findings of patients with BM and HCC before the initiation of bone destruction or nerve depression are usually similar to those of patients with HCC but without BM [10,27]. Therefore, osseous involvement is frequently diagnosed when patients complain about pain in bone areas, bone fractures, or discomfort in the abdomen or undergo imaging examination during routine follow-up [27,28]. Imaging approaches are helpful for the early detection of BM and contribute to reduced rates of paralysis and bone fracture [6]. A meta-analysis conducted by Yang *et al*. concluded that different imaging techniques displayed distinct sensitivity and specificity [29]. For per-patient-based analysis, MRI (90.6%) and ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) (89.7%) exhibited higher sensitivity than BS (86.0%) or CT (72.9%), whereas ^{18}F-FDG PET (96.8%) together with CT (95.4%) and MRI (94.8%) were superior to BS (81.4%) in terms of specificity. For per-lesion based analysis, CT (77.1%), and BS (75.1%) showed inferior sensitivity than ^{18}F-FDG PET (86.9%) or MRI (90.4%), whereas ^{18}F-FDG PET (97.0%), MRI (96.0%), BS (93.6%), and CT (83.2%) showed decreased specificity.

Plain X-ray

Regardless of its relatively low sensitivity, plain X-ray is still recommended as one of the standard imaging methods for the diagnosis and evaluation of BM [1,6]. Rather than the cancer cells themselves, skeletal pathological changes are preconditions for the diagnosis of BM via X-ray given that they manifest as morphological changes in bone induced by cancer cells within the bone marrow (Fig. 1A). However, the sensitivity of X-ray for the BM of breast cancer in the skull, spine, and pelvis is limited to 44%–50% [30], and even metastases measuring up to 1 cm in the spongiosa of a vertebral body may be overlooked on plain X-ray [6]. Nevertheless, X-ray is of great importance, especially for detecting fractures and evaluating potential risks [6], because it is easily accessible and inexpensive.

BS

BS, also referred to as bone scanning, is based on the accumulation of labeled phosphonates in bone areas with reactive hypermetabolism (Fig. 1B). This technique exhibits great sensitivity for osteosclerotic or mixed metastases, such as those in prostate cancer and breast cancer [6]. However, BS has inadequate sensitivity for dominantly osteolytic cancers, such as renal cell carcinoma, lymphoma, and HCC [11,31,32]. Furthermore, BS often fails to distinguish the flare phenomenon, which is caused

by bone matrix regeneration and actually indicates successful treatment, from disease progression. Combination with single-photon emission computed tomography (SPECT) or SPECT/CT markedly increases the sensitivity and specificity of BS [33].

MRI

HCC BM commonly presents as hypervascular focal nodular masses with moderately intense enhancement on hepatic arterial dominant phase images and concomitantly moderately high signal intensity on fat-suppressed T1- and T2-weighted images. A study on HCC BM using the standard abdominal MRI protocol showed that BMs are best visualized at the arterial phase, and 56.4% show an arterial peak of enhancement; thus, early washout and arterial ring enhancement may be the specific signs of BM [34]. MRI can detect small BMs even when they are confined to the bone marrow (Fig. 1C) [35,36]. Most HCC BMs are located in the thoracic and lumbar spine, which are commonly included in the field-of-view of standard abdominal MRI protocols. This situation emphasizes that during routine follow-up scanning, radiologists should pay attention to the early diagnosis of BM in patients with HCC and high risks of bone involvement.

CT, PET, and PET/CT

CT can be used for the detection of osteolytic and osteoblastic lesions, as well as the assessment of the stabilities of bony structures (Fig. 1D) [6]. A CT scan has low sensitivity for tumors, except for very extensive tumors, restricted to the marrow space [6].

The combination of ^{18}F-FDG PET with CT (PET/CT) can detect up to 80% of HCC skeletal metastases (Fig. 1E) [21,37] and is thus superior to BS, CT, and MRI [38–41]. Additionally, ^{11}Cacetate PET is more sensitive than ^{18}F-FDG PET in patient-based and in lesion-based analyses [21,42]. The combined use of ^{11}Cacetate and ^{18}F-FDG as a dual tracer is due to the preference of ^{11}Cacetate for well-differentiated tumors that complements the action of ^{18}F-FDG in identifying the location of HCC [3]. In the evaluation of HCC metastasis, dual-tracer PET/CT displays higher sensitivity than single tracers [21,42]. The old bone tracer ^{18}F sodium fluoride (^{18}F-NaF) was recently rejuvenated with the help of PET. The accuracy of ^{18}F-NaF PET/CT in the diagnosis of HCC BM is up to 95.7%, and the presence of ^{18}F-NaF PET/CT-positive bone lesions predicts short survival in patients with HCC [43].

Prognosis and therapeutic approaches

The median OS time after the diagnosis of BM is dismal and ranges from 3.0 months to 11.7 months (Table 2). The location, rather than the number, of bone lesions is the main factor associated with OS. Lesions in the spine are correlated with decreases in OS after the diagnosis of HCC and after the development of the first SRE [14,15]. Most patients die due to their intrahepatic HCC lesions instead of skeletal complications, indicating the importance of controlling intrahepatic tumors in patients with HCC whenever possible [11,17,44]. Aggressive treatments, including surgery for metastatic lesions, may produce a satisfactory local response and prolong OS [45]. Uka *et al*. recommended that most patients with HCC and extrahepatic metastases should mainly undergo treatment for the primary HCC tumor, although the treatment of extrahepatic metastases may improve survival in selected patients with HCC who have good hepatic reserve and low intrahepatic tumor stage (T0–T2) and are free of portal venous invasion [17]. To date, no guidelines or consensus has been established regarding the treatment strategy for HCC BM. Experiences from the guidelines for the management of BM in other cancers imply that treatment selection for patients with HCC and BM (Fig. 2) should be based on the expected survival, symptoms, and condition of the patient, as well as the number and localization of bone lesions [46,47].

Prognosis

Patients with HCC and BM have poor prognosis [13,15,22]. Survival estimates made by clinicians purely on the basis of experience are usually inaccurate [59]. Given the priority of expected OS in selecting therapeutic approaches to prevent overtreatment or undertreatment, numerous endeavors have been made to identify prognostic factors (Table 2) or to develop prognostic models for patients with HCC and BM.

A prognostic nomogram encompassing RT, chemotherapy, and lung metastasis exhibited good accuracy for predicting OS, and its areas under the curve for 6-, 9-, and 12-month OS in the validation cohort were 0.698, 0.770, and 0.823, respectively [23]. A graded prognostic assessment for patients with HCC with spinal metastasis (HCC–SM GPA) consisting of Eastern Cooperative Oncology Group performance status, controlled primary HCC, and extrahepatic metastases other than bone, was used to stratify HCC–SM patients into low risk (GPA = 0), intermediate risk (GPA = 1 to 2), and high risk (GPA = 3 to 4) groups and successfully predicted survival outcomes in the validation cohort [57]. A prediction model for other cancer BM also provided available options. The Katagiri scoring system, which includes the primary lesion, visceral or cerebral metastases, abnormal laboratory data, poor performance status, previous chemotherapy, and multiple skeletal metastases, is suitable for OS estimation in patients with BM regardless of the location of skeletal involvement even after surgery and RT [60]. The models

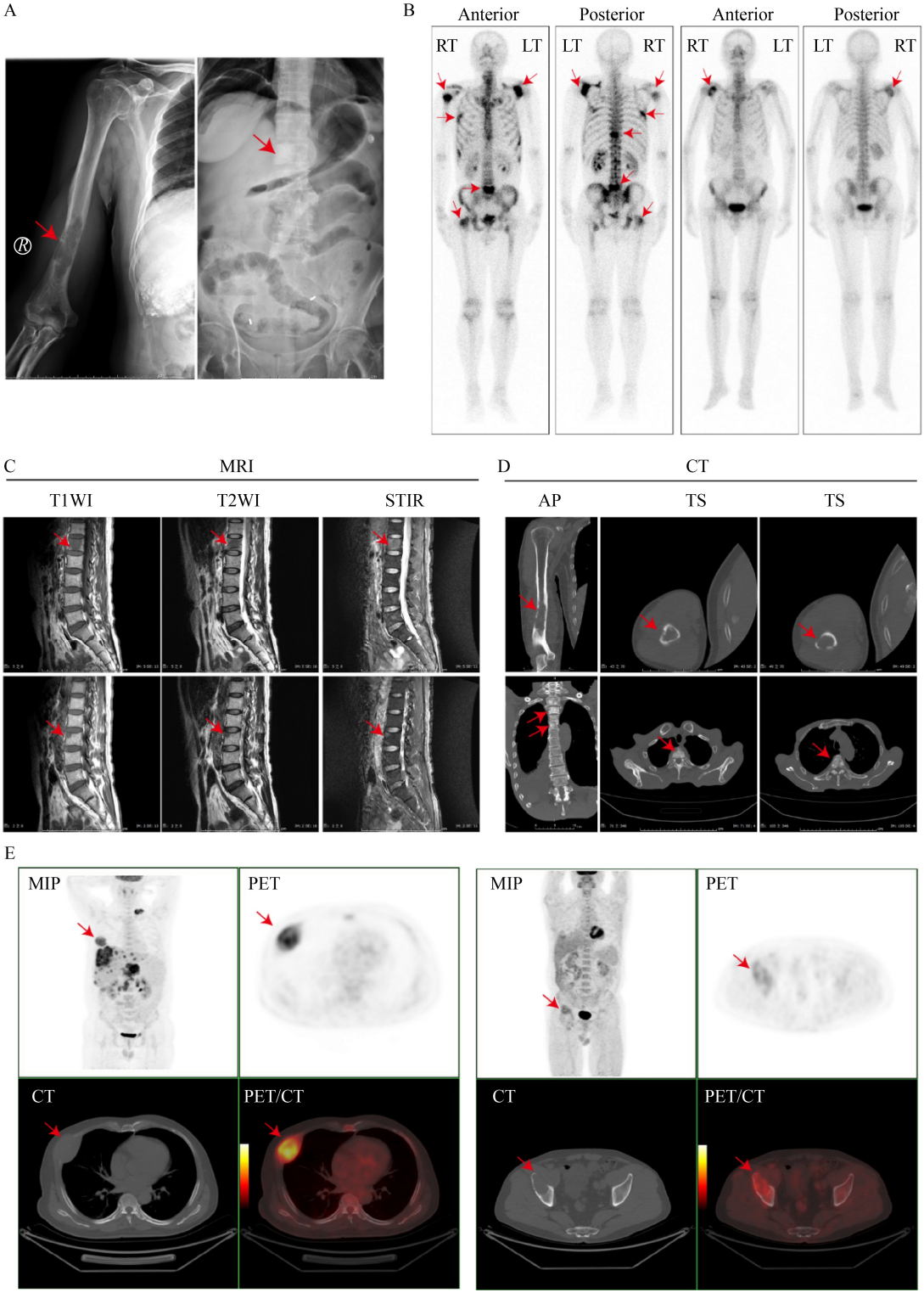

Fig. 1 Imaging techniques for the diagnosis of HCC BM. (A) X-ray images of metastatic HCC lesions in the right humerus (left image) and in the L1 vertebral body (right image). (B) Anterior and posterior positions of BS for a patient with multiple BM (left panel) and a patient with a bone lesion in the right scapula (right panel). (C) MRI of skeletal HCC lesions in the L1 vertebral body (upper row) and L3 vertebral body (lower row). (D) CT scans of BM in the right humerus (upper row) and the spine (lower row). (E) Whole-body ^{18}F-FDG PET/CT images of BM in the fifth right rib (left panel) and right ilium (right panel). Arrows point to metastatic HCC bone lesions. Abbreviations: CT, computed tomography; BS, bone scintigraphy; MRI, magnetic resonance imaging; PET, positron emission tomography; BM, bone metastasis; LT, left; RT, right; AP, anteroposterior; TS, transverse section; T1WI, T1 weighted image; T2WI, T2 weighted image; STIR, short time inversion recovery; MIP, maximum intensity projection.

Table 2 Prognosis and risk factors for HCC patients with BM

Period	Treatment	Prognosis	Independent risk factors
1978–1987 [11]	RT, surgery, ethanol injection, and supportive care	Median OS: 170 days	–
1981–1997 [48]	Palliative RT	Median OS from the diagnosis of spinal metastasis: 3 months	Responssive RT (complete response, partial response) and good performance status (score < 2)
1988–1997 [11]	RT, surgery, ethanol injection, and supportive care	Median OS: 227 days	–
1991–2000 [25]	RT	OS rates at 1 and 2 years of 50% and 20%, respectively, with a median OS of 12 months from the time of HCC diagnosis; OS rates from the occurrence of BM at 1 and 2 years of 15% and 4%, respectively, with the median OS of 5 months	Tumor stage within the liver and the presence of metastases to organs
1992–2012 [49]	RT	Median OS from diagnosis of spinal metastases: 4.5 months; 1- and 2-year OS rates of 18.1% and 6.3%, respectively	Performance status (ECOG), presence of uncontrolled primary HCC, and presence of extrahepatic metastases
1993–2013 [14]	Sorafenib, RT, BP and surgery	Median OS from the diagnosis of BM: 7 months	HCC etiology, performance status (ECOG), BM localized to the spine and not receiving any BP treatment
1997–2007 [44]	RT	Median OS after the diagnosis of BM: 7.4 months; 1-year and 2-year OS rates of 32.4% and 13.2%, respectively	Low KPS, high AFP levels, uncontrolled intrahepatic tumor, and receiving treatment within the past 5 years
2000–2011 [50]	RT	Median OS: 7.0 months; OS rates at 1 and 2 years of 13.8% and 6.9%, respectively	–
2000–2018 [45]	Surgery, RT, chemotherapy, and bone-modifying agents	Median OS from the initiation of treatment: 7.4 ± 8.2 months (range 0.3–36 months) for all; 10.46 ± 8.05 months for surgical groups, and 5.19 ± 7.72 months for the conservative treatment groups	Patient's general condition, the serum albumin level, and bone-modifying agent treatment
2002–2009 [51]	Irradiation/zoledronic acid	Median OS from the initial date of therapy: 6.0 months (95% CI 0.0–12.7 months) for patients treated with zoledronic acid, and 4.2 months (95% CI 1.2–7.2 months) for patients treated with non-zoledronic acid; cumulative OS rates at 3 months of 74% and 44% and at 6 months of 79% and 37%	–
2002–2011 [52]	SRS, cRT	Median OS: 3 months in the cRT group and 7 months in the SRS group	Child–Pugh class and KPS
2002–2014 [15]	Radiation, surgical resection, BPs, and sorafenib	Median OS after the diagnosis of any type of metastasis: 5.6 months (95% CI 4.6–6.9)	AFP levels, Child–Pugh score, and SREs
2005–2011 [53]	EBRT	Median OS after the first EBRT: 3.8 months	–
2006–2013 [54]	Surgery, EBRT	Median OS: 261 days (range 22–1359 days) after the diagnosis of metastasis, and 180 days (range 19–1351 days) after the initial operation	Tomita scoring system
2009–2014 [55]	EBRT	Median OS for the entire cohort: 8.0 months; 1-year and 2-year survival rates of 35.1% and 10.8%, respectively in patients receiving conventional fraction EBRT, and of 38.7% and 15.1%, respectively, in patients receiving hypofraction RT	KPS, TB, and intrahepatic tumor control
2009–2016 [5]	Sorafenib, sunitinib or lenvatinib RT, zoledronic acid and denosumab	Median OS after the diagnosis of BM: 11.7 months (range 0.2–94.5 months)	Age over 75 years, HCV, and Child–Pugh class B/C
2010–2014 [22]	Surgery and other N/A	Median OS from the time of diagnosis of HCC: 3.00 months (95% CI 2.77–3.24 months)	Unmarried, uninsured, high primary tumor stage, high regional lymph node (N1), lung metastases, poor tumor differentiated grade, and elevated AFP, without surgery
2010–2014 [56]	Zoledronic acid, palliative RT, curettage, and wide resection	Median OS after BM diagnosis: 11 months (range 4–52); 1- and 2-year survival rates of 44.2% and 11.6%, respectively	Progression beyond the University of California San Francisco criteria and the treatment of the primary tumors
2011–2016 [57]	RT	Median OS after RT: 13.6, 4.8, and 2.6 months for the low-, intermediate-, and high-risk groups, respectively	ECOG performance status, controlled primary HCC, and extrahepatic metastases other than bone

(*Continued*)

Period	Treatment	Prognosis	Independent risk factors
2014–2017 [58]	RT and other N/A	Median OS from the start of the RT for BM: 6.5 months; 1- and 2-year survival rates after diagnosis of BM of 35.5% and 13.5%, respectively	Child–Pugh class A group, increase in AFP beyond 30 ng/mL, and HCC size of more than 5 cm

Abbreviations: RT, radiotherapy; OS, overall survival time; BM, bone metastases; ECOG, Eastern Cooperative Oncology Group; KPS, Karnofsky performance status; TB, total bilirubin; SRS, stereotactic radiosurgery; cRT, conventional radiation therapy; BP, bisphosphonate.

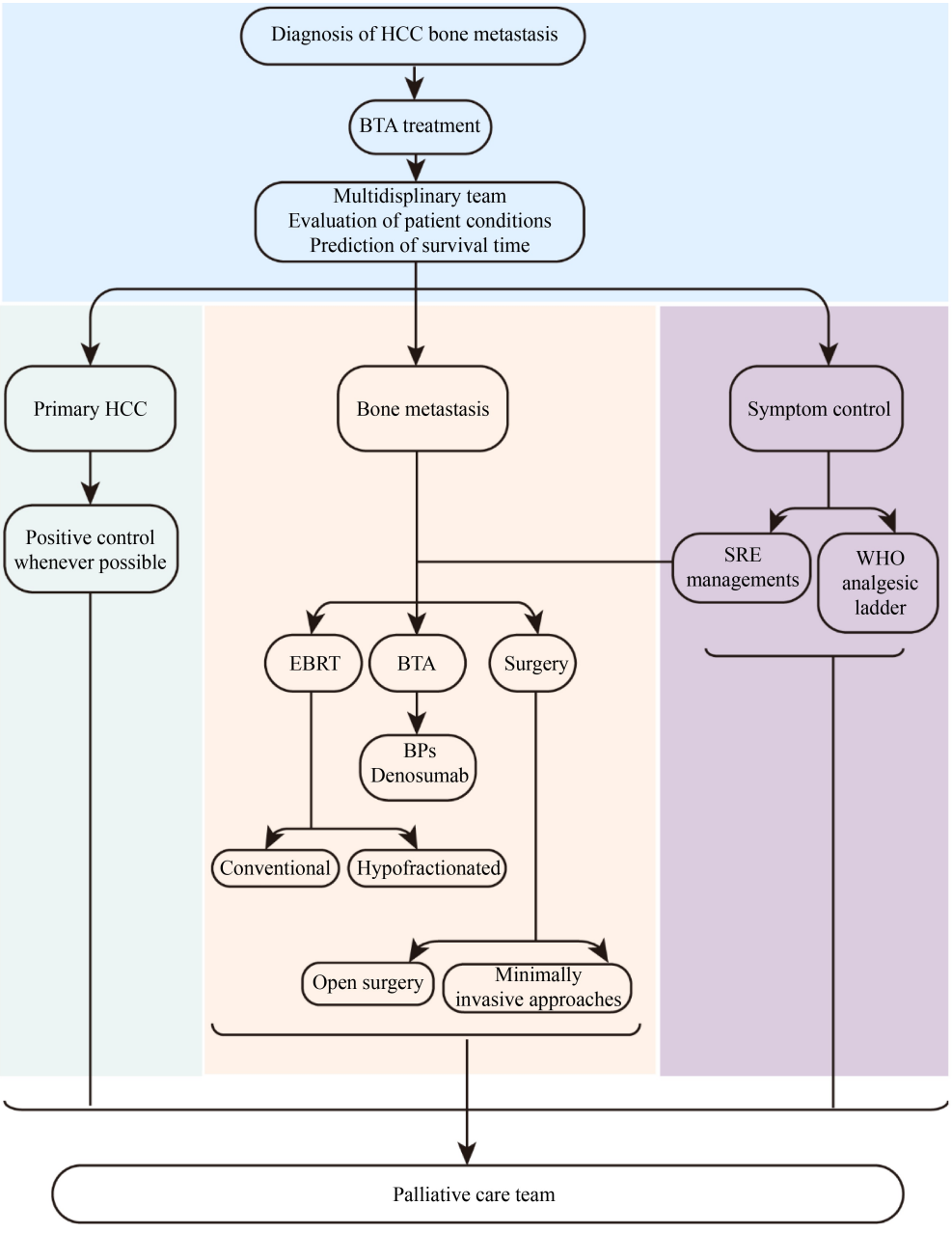

Fig. 2 Outline of the multidisciplinary treatment options for patients with HCC and BM. Therapeutic strategies for HCC BM should be determined in accordance with the systematic evaluation of each patient's general condition by a multidisciplinary team. BTAs are recommended to be started at the definite diagnosis of BM. Treatments for primary HCC, BM, and systematic symptoms are the three approaches for controlling disease progression and alleviating cancer-induced bone pain. Abbreviations: BM, bone metastases; BTAs, bone-targeting agents; EBRT, external beam radiotherapy; BPs, bisphosphonates; SRE, skeleton-related events.

proposed by Bartels *et al*. [61], van der Linden *et al*. [62], or Bollen *et al.* [63], recommended by the Dutch national guidelines may also aid the choice of treatment.

RT

In analogy to BM in malignancies, such as breast and lung cancer, EBRT is frequently prescribed as palliative treatment for skeletal involvement in patients with HCC [55,64]. Although RT alone does not restore bone structure or stability, it is prescribed for relieving bone pain, preventing the worsening of the structural damage caused by BM, and reducing the need for subsequent surgery by controlling metastatic spread and preventing prosthesis displacement after surgery [1]. In patients with HCC and BM, EBRT results in an overall pain improvement in 73%–99.5% of cases and helps 17.0%–44.0% of patients achieve complete pain relief, thus reducing analgesic requirements [64].

Different radiation schedules have been proposed for common use in patients with BM [65]. Previous works showed that although no dose–response relationship existed for the overall response (OR), complete response (CR), and pain response rates of the palliative treatment of BM, the retreatment rate was higher in patients with expansile soft tissue receiving a lower dose of EBRT, whereas a higher radiological response rate and longer time to progression were achieved in high-dose RT groups [44,53,55,66]. Conventional RT led to a long duration of pain relief, whereas hypofractionated RT achieved early pain relief [55]. Similarly, a cohort study on 28 patients with HCC and BM reported that patients undergoing conventional high-dose multiple fractions (MFs) achieved pain relief for 5.0 months, which was significantly longer than the 1.8 and 3.0 months of pain relief experienced by those undergoing 8 Gy single fraction or moderate-dose MF therapy, respectively, but found no difference in the OR rates [50]. These results suggested that a high-dose MF schedule benefits patients with long predicted OS due to its long period of pain relief, whereas hypofractionated RT should be considered as an alternative for patients with short predicted survival times [55].

A prospective phase II study on 60 patients with gastrointestinal cancers (HCC, n = 25) and BM from 2014 to 2016 reported that RT in combination with zoledronic acid decreased the bone pain score from 6.7 to 2.8 at 1 month and to 2.1 at 3 months with the overall pain response rates of 95% and 96%. At the same time, the combination treatment improved the mean quality of life score from 66 to 56 and 55 at 1 and 3 months, respectively [67]. The addition of transcatheter arterial embolization (TAE) managed to shorten the mean time interval of pain relief from 15 days to 4.8 days and to reduce the recurrence rate of bone pain from 88% to 20% [68].

BTAs

BTAs mainly include bisphosphonates (BPs) and monoclonal antibodies. BPs are well known for reducing the frequency of SREs and bone pain and as a documented effective agent against malignant hypercalcemia in various cancers [1]. Three generations of BPs have been developed, with third-generation BPs, which include zoledronic acid, ibandronate, neridronate, and risedronate, exhibiting the strongest potency [69]. Zoledronic acid is currently the strongest nitrogen-containing BP and is used to treat cancer-induced osteolysis and thereby prevent skeletal complications associated with BM [70]. Zoledronic acid significantly delays the time to pain progression and shortens the time to radiographic progression in patients receiving non-RT for HCC with BM [51]. A case series consisting of 17 patients with BM from 2006 to 2008 reported that the visual analog scale decreased from 7.1 (±0.24) to 5.3 (±0.20) after the administration of zoledronic acid for at least three times; such an effect allowed a reduction in the use of analgesic drugs [71]. In addition to effects targeting osteoclasts, zoledronic acid suppresses HCC cell growth by inhibiting the translocation of Ras and RhoA, further terminating the mevalonate pathway and inducing the apoptosis of HCC cells [70].

Denosumab, an anti-RANKL antibody, is widely used to relieve bone pain and delay disease progression [1]. It has also been used for patients with HCC and BM [5,15,45]. In breast cancer and castration-resistant prostate cancer, denosumab has been proven to have superior efficacy over zoledronic acid as evidenced by the significant delay in the first and subsequent on-study SRE and the skeletal morbidity rate in the denosumab group relative to those in the zoledronic acid group [72–74]. Moreover, Body *et al*. demonstrated that the suppression of bone resorption markers by denosumab is independent of prior BP treatment and of its biochemical efficacy. In patients with a poor biochemical response to BPs, denosumab displays superior performance with the significant suppression of bone resorption markers [75]. At the same time, denosumab was associated with low renal toxicity and few acute-phase reactions in a double-blind study for advanced breast cancer [72]. In terms of side effects, no significant difference in the rate of osteonecrosis of the jaw was found between denosumab and zoledronic acid [72,73].

Medical criteria for when BTA treatment should be started and stopped, especially for patients with HCC, are lacking. On the basis of experience with breast cancer, BTA treatment is recommended when skeletal involvement is diagnosed (Fig. 2) and should be continued until the patient's general performance status shows a substantial decline [76]. On the other hand, stopping or reducing the frequency of BTA treatment should be considered when

the prognosis worsens and SRE-related biochemical markers of bone resorption decrease because this situation indicates that the osteotropic disease is not aggressive or is under control by the anticancer treatment [77].

To date, clinical trials on the BP zoledronic acid and the monoclonal antibody denosumab have excluded a large proportion of patients with advanced HCC [15]. Therefore, the benefits and side effects of these drugs in patients with HCC and BM still need further validation.

Sorafenib

In patients with advanced HCC, the multitarget receptor tyrosine kinase inhibitor sorafenib exhibits antitumor activity and is known to modestly improve survival over best supportive care [78]. Du *et al*. reported the case of a 74-year-old patient with HCC that had metastasized to the vertebrae and who responded to a reduced dose of sorafenib and subsequently demonstrated no signs of disease progression since starting treatment almost 5 years ago [79]. Sorafenib was also reported to protect against SREs [15,80].

Surgery

Although surgery for primary liver tumors significantly improves the survival of patients with BM, this result can potentially be explained by the biased selection of patients [22]. Surgery for bone metastatic lesions is controversial and is not predictive of prolonged survival according to some reports [13,23,81,82]. The median survival time of the patients with HCC and BM who underwent surgery is similar to that of patients who received only supportive care [11]. However, surgery relieves bone pain and reduces the incidence of SREs, thus improving the patients' activities of daily living and life quality [45,81]. Even if a patient's neurological function is compromised, surgery has the potential to reverse damage [81]. Surgery can maintain or recover ambulatory ability until recurrence or other spinal cord compression occurs, and a univariate analysis of the postoperative Frankel score has shown that patients who are ambulatory after surgery have better survival than those who become paraplegic [81].

The surgical indications and management specific to HCC BM are not different from those of other tumors [81]. The aim of surgery for BM is to stabilize pathological fractures, manage spinal cord compression, functionality and mobility, prevent impending fractures, and alleviate pain [1]. Patients with the life expectancy of at least 3 months are recommended to undergo minimally invasive procedures, whereas those with a life expectancy of 6 months can opt for open surgical procedures [46]. For spinal metastases, percutaneous techniques, such as vertebroplasty and kyphoplasty, are performed to stabilize the vertebrae, reduce microfracture-induced pain, and prevent the potential collapse of the vertebral body. These techniques do not reduce tumor size and should not be used in patients suffering from pain or neurological deficit caused by nerve root or spinal cord compression. Radiofrequency ablation is comparable with vertebroplasty, and surgeons often combine these two techniques to improve local relief [83,84]. Dorsal spinal decompression and stabilization are the standard surgical techniques for lumbar and thoracic metastases. The ventral/combined approach of decompression and ventral stable-angle plate osteosynthesis is commonly used for cervical lesions. A titanium mesh filled with bone cement can be applied to replace impaired vertebral bodies [81]. For long-bone metastases, intramedullary nailing with locking screws via minimally invasive techniques allows immediate full weight bearing, and a long-stem cemented endoprosthesis or a modular tumor endoprosthesis are alternatives for orthopedists [1]. Notably, preoperative low bone mineral density is an independent risk factor for cancer-specific mortality after hepatectomy for HCC [85].

TAE

Several studies have reported the beneficial effect of TAE in relieving bone pain or as a preoperative preparation for bone surgery [68,86–88]. Uemura *et al.* showed that the mean time interval for initial pain relief was 4.7 days, which was shorter than the mean time interval in patients who received EBRT. At the same time, the combination of TAE and EBRT decreased the pain recurrence ratio from 75% to 20% [68].

Multidisciplinary teams and other palliative approaches

The specialists involved in a multidisciplinary team for the treatment of HCC BM should include the originally treating specialist, a radiation oncologist, a medical oncologist, and a radiologist. A neurologist should be present in the case of nerve root or spinal cord compression, and a neurosurgeon and/or orthopedic surgeon should participate in the case of possible operation indications [47].

Corticosteroids can be started in the case of symptomatic spinal cord compression to reduce swelling and edema around the spinal cord [46,47]. Analgesic drugs are usually unavoidable for pain management. The usage of analgesics, namely, non-narcotics, weak narcotics, and narcotics, should follow the three steps from the World Health Organization (WHO) guidelines all in association with adjuvant drugs [44,89].

Advances in basic studies

New findings on the osteotropism of various cancers

precede the explorations of HCC BM and continue to provide the broad insights necessary for investigating the mystery and inspiring the studies on HCC BM. Thus, introducing the consensus and advances in cancer BM is critical.

To our knowledge, some prometastatic characteristics, such as the dynamic adhesive and migratory capabilities intrinsic to tumor cells, are common to the metastasis of most cancers, whereas some pathological steps and processes are specific to skeletal preference. For BM, the tumor-associated uncoupling of bone remodeling and the immunosuppressive microenvironment in the bone marrow are the essential prerequisites for the survival and thriving of cancer cells. These prerequisites will be our key points in the following discussion.

Cancer BM cascade

BM is a multistep process, and emerging findings have enriched our knowledge of the metastatic cascade. Osteotropism is a positive selection process at the primary site and in the bone, rather than passive adaption in the bone. Briefly, it encompasses premetastatic niche preparation, local invasion and intravasation, survival and spread in circulation, extravasation and adaption in the bone, micro- and macro-metastatic lesion formation, reciprocal communication with primary cancer, and further metastasis (Fig. 3) [90,91].

Before tumor cells arrive at the bone, the bioactive substances secreted by primary cancer cells, such as cytokines and extracellular vesicles (EVs), instruct the bone marrow to prepare niches for tumor cell settlement [92]. The differentiated status and spatial localization of bone stromal cells in these niches provide advantages for the homing, adhesion, and colonization of the arriving metastatic cancer cells [93–95]. For detachment from the primary site, primary cancer cells disrupt homeostasis by communicating with the surrounding stromal cells and degrading the extracellular matrix to achieve local invasion. Epithelial-to-mesenchymal transition (EMT) confers tumor cells with increased mobility, plasticity, self-renewal ability, and apoptosis resistance [96]. Invasive tumor cells enter the bloodstream for further spreading; this process is accompanied by angiogenesis. Circulating tumor cells (CTCs) suffer stress due to attack from the immune system and anoikis [97]. In fact, less than 1% of CTCs finally survive in the circulation and reach the distant metastatic destination [98–101]. The size of the vasculature and the adhesive molecules of cancer cells largely determine their trapping in distant organs. For extravasation, given that the vasculature in the bone is highly fenestrated, bone metastatic tumor cells are more likely to penetrate the microvascular wall rather than rupture blood vessels by initiating intraluminal growth and forming an embolus [90]. The trapped CTCs then undergo mesenchymal-to-epithelial transition (MET) to settle and adhere to the bone marrow [96]. Bone-settled tumor cells either remain dormant or immediately interact with bone stromal cells to create a favorable environment for the formation of metastatic foci [90]. In addition to osteoclasts and osteoblasts, which are widely known for their involvement in a vicious cycle, other bone stromal cells, including osteocytes, bone marrow endothelial cells, hematopoietic cells, adipose stem cells, nerve cells, platelets, and myeloid and immune cells have been found to participate in cultivating “fertile soil” for metastatic “seeds” [94,95]. Importantly, although BM is the final step in bone metastatic cascades, the bone microenvironment can facilitate cancer cells in the bone to further metastasize and establish multiorgan secondary metastases [102]. Moreover, signaling from bone stromal cells to primary cancer cells can affect the progression of the primary tumor [85,103–105]. In fact, the content of bone metastatic cascades continues to expand, and beyond the primary tumor and bone, other cells, organs, and systems may also be involved in cancer osteotropism. Cancer-associated fibroblasts (CAFs) in triple-negative breast cancer select bone metastatic seeds by skewing heterogeneous cancer cell populations toward a predominance of clones that thrive on the CAF-derived factors CXCL12 and IGF1, which are richly expressed in the bone microenvironment [106].

Uncoupled bone remodeling

Excessive bone remodeling processes lead to the release of growth factors and ionized calcium from the mineralized bone matrix. This effect promotes the growth of the metastatic tumor cells and the further secretion of osteolytic and osteoblastic factors, thus resulting in a vicious cycle. Tumor cells trigger this cycle by disrupting the balance between osteoclasts and osteoblasts through endocrine and paracrine factors (Fig. 4).

Osteoclasts are polarized, multinucleated myeloid lineage cells that have differentiated from their mononuclear macrophage/monocyte lineage hematopoietic precursors. The activation of nuclear factor-κB, NFATc1, JUN N-terminal kinase, mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways promotes the formation and maturation of preosteoclasts [93,94]. Receptor activator for nuclear factor-κB ligand (RANKL) and macrophage colony stimulating factor are two important factors for the formation, activation, and function of osteoclasts that act by binding with their respective receptors, namely, RANK and colony stimulating factor 1 receptor, on preosteoclasts. Osteoprotegerin (OPG) is the endogenous decoy receptor that competes with RANK for RANKL and thereby inhibits osteoclastogenesis [93,94]. Some RANKL substitutes have been found to induce osteoclast

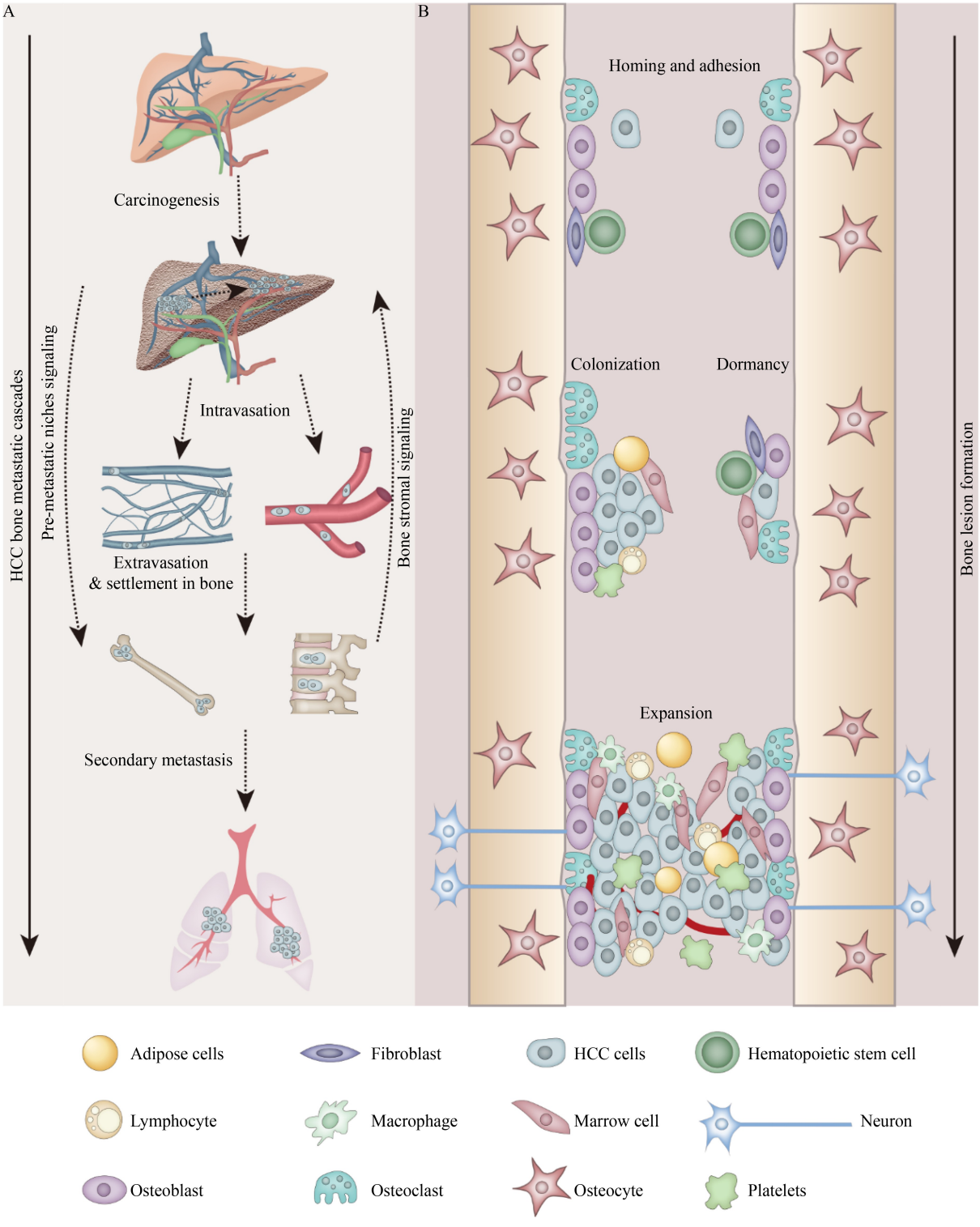

Fig. 3 Hypothetical bone metastatic cascade of HCC. On the basis of the academic advances in cancer BM, an integrated metastatic process was proposed for HCC bone lesion formation. (A) The malignant transformation of liver cells and microenvironment predispose HCC cells to metastasis by augmenting their mobility and inducing angiogenesis. HCC cells exploit the arterial bloodstream and vertebral venous system to spread to the bone cavity. The dysregulation and immunosuppressive status of the bone microenvironment benefit the settlement, proliferation, and further metastasis of disseminated tumor cells in the bone. Additionally, signals that induce the osteotropism of cancer cells are predelivered to the bone from the primary tumor site and prepare metastatic niches. Conversely, the bone microenvironment modulates the behavior of tumor cells at the primary site via the secretion of various bioactive substances. (B) The formation of a metastatic lesion in bone. The disseminated tumor cells first home to the bone cavity and adhere to bone stromal cells to settle inside the bone. Bone metastatic tumor cells either remain dormant or directly start to colonize and form micro-metastatic lesions. By interacting with various bone stromal cells, the migrated tumor cells finally thrive inside the bone and expand into a macrometastatic lesion.

formation in a RANKL-independent manner. Nontumor necrosis factor (TNF) superfamily growth factors, such as transforming growth factor β (TGFβ), interleukin-6, nerve growth factor (NGF), insulin-like growth factor (IGF) I, and IGF II induce osteoclast differentiation and activation. TNF superfamily growth factors, such as TNFα and TNF superfamily member 14; a proliferation inducing ligand (APRIL); and a B-cell activating factor (BAFF) belonging to the tumor necrosis factor family also generate significant numbers of tartrate-resistant acid-phosphatase-positive multinucleated cells, which can resorb bone. Notably, these noncanonical factors induce osteoclastogenesis in normal and pathological bone resorption [107]. PTHrP stimulates RANKL expression and inhibits OPG expression in osteoblasts, leading to malignant osteolysis [94]. Macrophage-stimulating protein binds to the RON receptor tyrosine kinase in osteoclasts, which is absent from osteoblasts, activating a RANK-independent, Src phosphorylation-dependent pathway to further stimulate osteoclast survival and activity [108].

Osteoblasts originate from mesenchymal stem cells (MSCs). Signals from bone morphogenetic proteins (BMPs), WNTs, TGFβ, endothelin 1 (ET1), insulin-like growth factors (IGFs), platelet-derived growth factor, urinary plasminogen activator, and fibroblast growth factors recruit the precursors of osteoblasts and accelerate their maturation [94]. During destructive bone remodeling, tumor cells suppress the formation and activity of osteoblasts by secreting activin A, the BMP inhibitor noggin, dickkopf-1, and sclerostin, thus aggravating the uncoupling between osteolytic and osteoblastic activities [95]. Transcriptional factors, including RUNX2, osterix, and activating transcription factor 4, drive the formation of osteoblasts and are used as markers for osteoblastogenesis.

Immunosuppressive bone microenvironment for tumor cells

During BM, tumor cells, local immune cells, and bone stromal cells intimately communicate with each other and perpetuate the vicious cycle (Fig. 4) [109]. Although the bone is a major organ of the immune system, it appears to be an immunologically privileged environment for tumor cells for reasons that remain insufficiently understood. Several cell types and molecular signals, including the components of the innate and adaptive immune system, are responsible for the immunologically privileged status of cancer cells in bones. Natural killer (NK) cells, macrophages, regulatory T cells (Tregs), immature myeloid cells (IMCs), dendritic cells (DCs), and osteoblasts are well recognized pathological players and are of particular interest [7,110]. NK cells perform tumor-killing functions by inducing apoptosis through granule-mediated-exocytosis or Fas–Fas ligand interactions [111]. In bones, cancer cells express core2 β-1,6-N-acetylglucosaminyltransferase to escape NK cell-mediated apoptosis by disrupting the ligand-receptor-mediated (NKR/NKR-L and TRAIL/DR4) immune response [112]. Macrophages are mononuclear myeloid lineage cells that can be polarized into the M1-like subtype (proinflammatory tumor-suppressing macrophages) and the M2-like subtype (anti-inflammatory tumor-associated macrophages (TAMs)). Increased numbers of TAMs are found in prostate cancer with bone metastatic lesions, and macrophage depletion inhibits tumor growth inside the bone [113,114]. Moreover, chemokine (C–C motif) ligand 2 (CCL2)-expressing breast cancer cells recruit $CCR2^+$ macrophages to prepare metastatic niches in lungs and bones [115]. These findings demonstrate that TAMs are closely associated with BM. Tregs are well known as $CD4^+$ T cells that contribute to immune suppression, and bone marrow Tregs are significantly more abundant in bone metastatic prostate cancer than in other cancers [116]. In addition to anti-inflammatory cytokines, such as IL-10, IL-35, and TGFβ [110], RANK/RANKL signaling on DCs increases the number of Tregs [117–119]. At the same time, CXCL12/C-X-C motif chemokine receptor (CXCR4) signaling promotes the trafficking of Tregs to the bone marrow [120]. $CD4^+$ helper T cells (Th17 cells) are another important subset of $CD4^+$ cells in cancer BM, and they also contribute to enhancing the activation of preosteoclasts and promoting osteolysis [121], Th17 cells can differentiate into Tregs under TGFβ stimulation during the immune response [122]. During the development of HSCs, a proportion of myeloid cells remains immature and executes an immunosuppressive function that is similar to the effect of Tregs [123]. IMCs, also referred to as myeloid-derived suppressor cells (MDSCs), are morphologically categorized into polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (M-MDSCs). PMN-MDSCs and M-MDSCs interfere with cytotoxic $CD8^+$ T cells by generating reactive oxygen species and inducible NO synthase, and thereby promote bone metastatic seeding [124]. DC cells act as the key promoters of a functional cytotoxic T cell immune response via their antigen-presenting cell (APC) function. On the other hand, plasmacytoid DCs (pDCs) can recruit Tregs and MDSCs to promote tumor progression and metastasis [125], whereas tumor-infiltrating DCs can secrete TGFβ, nitric oxide, IL-10, VEGF, and arginase I to suppress the cytotoxic capacity of $CD8^+$ cells [126]. The increased abundance of pDCs was found in the bone marrow of mice inoculated with breast cancer cells, and the deficiency of pDCs remarkably inhibited BM [127]. A group of osteoclasts prefers aerobic glycolysis, resulting in the accumulation of lactate. The lactate-rich environment benefits the growth of cancer cells, thus facilitating Treg function and suppressing the function of cytotoxic $CD8^+$ T cells [128–131]. Additionally, the expression of CXCL12

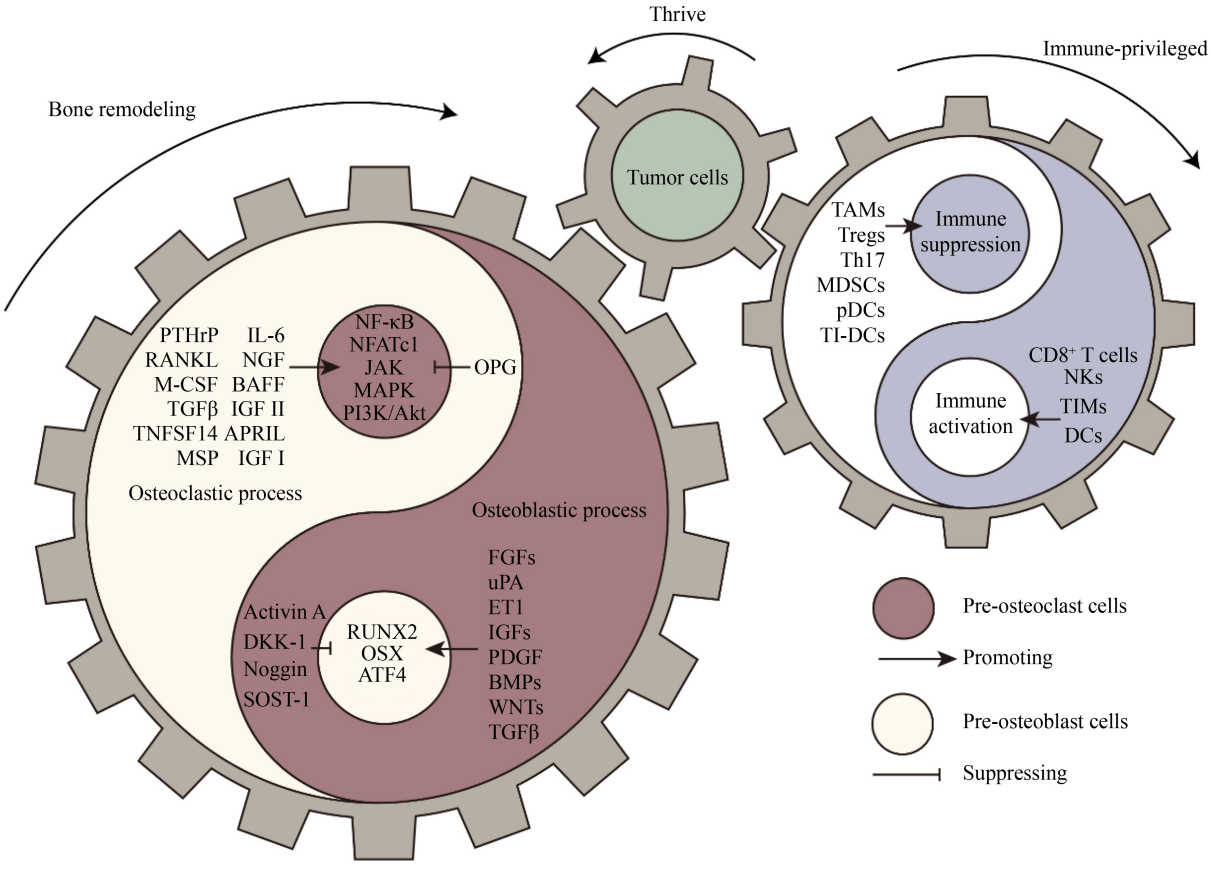

Fig. 4 Disturbed homeostasis of the bone microenvironment driven by tumor cells. Bone-metastasized tumor cells trigger the imbalances of immune-suppressive/immune-active and osteoclastic/osteoblastic activities to escape from immune elimination and initiate excessive bone reconstruction. Thriving tumor cells, the immune-privileged microenvironment, and uncoupled bone remodeling benefit each other through various bioactive molecules to perpetuate the destructive vicious cycle. Abbreviations: TAMs, tumor associated macrophages; Tregs, regulatory T cells; Th17, $CD4^+$ T helper cells 17; MDSCs, myeloid-derived suppressor cells; DCs, dendritic cells; pDCs, plasmacytoid DCs; TI-DCs, tumor-infiltrating DCs; NK cells, natural killer cells; TIMs, tumor inflammatory macrophages.

in osteoclasts recruits HSCs, Tregs, and metastatic seeds to the endosteum [116,120].

Molecular mechanisms of HCC BM

In addition to the common changes that contribute to BM, i.e., EMT, angiogenesis, and the vicious cycle inside the bone marrow [132], some intrinsic genetic alterations and molecular expression patterns that contribute to HCC BM (Fig. 5) have been discovered (Table 3) [133–138].

Next-generation sequencing revealed mutations in two pairs of primary HCC and corresponding bone lesions. The deletions of ABL1, KIT, and EGFR or the missense mutations of CTNNB1, ERBB1, and KRAS were found in primary HCC and in paired BM, whereas the mutations of ALK, APC, and FGFR1 were observed only in primary HCC with mutations of DEAR, AKT1, and CDH1 being unique to HCC bone lesions [138]. A total of 22 genes in tumors and 45 genes in peritumor tissues were found to be differentially expressed between patients with HCC with and without BM. Among these genes, the high expressions of intratumoral connective tissue growth factor (CTGF), intratumoral interleukin-11 (IL-11), and peritumoral matrix metallopeptidase-1 (MMP-1) were independent prognostic factors for the progression to BM in patients with HCC [137]. CXCR4 was reported to be more highly expressed in patients with HCC and BM than in those without BM, and the high scores of CXCR4 in primary HCC predicted an increased risk for developing BM with decreased OS [141]. The same group that reported the above results further developed a clinicopathological prediction model consisting of vascular invasion, tumor–node–metastasis staging, CXCR4, CTGF, and IL-11, to predict the BM risk of patients with HCC [139]. Frizzled-related protein (FRZB) was found to be highly expressed in HCC specimens with BM and upregulated in bone metastatic lesions relative to in paired primary HCC tumor tissues, indicating that FRZB might play a key role in the BM of HCC [136]. Interestingly, they also revealed that perineural density was higher in HCC BM than in the corresponding primary liver tumor by using the same patient samples [142]. Patients with HCC and the high

expression of RNF219 had shorter BM-free survival time than other patients. Mechanistically, the excessive activation of the RNF219/α-catenin/LGALS3 axis in HCC cells enhanced pro-osteolytic interactions with preosteoclasts, promoting osteoclastogenesis and BM formation, which in turn aggravated SREs. The administration of verteporfin, a small-molecule antagonist of the YAP–TEA interaction, decreased the RNF219-induced upregulation of LGALS3

Table 3 Biomarkers for the prediction of diagnosis and prognosis of HCC BM

Gene	Subject	Expression pattern	Biological function/molecular mechanism	Potential application
CTGF [137,139]	Primary tumor	Highly expressed in patients with HCC and BM	–	Risk factor of BM
IL-11 [137,139]	Primary tumor	Highly expressed in patients with HCC and BM	–	Risk factor of BM
MMP-1 [137,140]	Peritumor/HCC cell lines	Highly expressed in patients with HCC and BM/in osteotropic cells lines	–	Risk factor of BM
CXCR4 [139,141]	Primary tumor	Highly expressed in patients with HCC and BM	–	Risk factor of BM
FRZB [136]	BM	Highly expressed in bone metastatic lesions	–	–
PNI [142]	BM	High density in bone metastatic lesions	–	–
RNF219 [134]	Primary tumor/BM/HCC cell lines	Highly expressed in patients with HCC and BM/in bone metastatic lesions/in osteotropic cell lines	Promotes osteoclastogenesis by upregulating LGALS3 in a YAP1/β-catenin complex-dependent manner	Therapeutic target
LGALS3 [134]	Primary tumor/BM/HCC cell lines	Highly expressed in patients with HCC and BM/in bone metastatic lesions/in osteotropic cell lines	Promotes osteoclastogenesis and aggravate SREs	Therapeutic target
miR-34a [143]	Serum/primary tumor	Low expression in the serum and in the primary tumor of patients with HCC and BM	Promotes the migration and invasion of HCC cells by upregulating SMAD4 to further activate TGFβ signaling and upregulate its downstream effectors	Risk factor of BM and therapeutic target
lnc34a [144]	Serum/primary tumor	Highly expressed in the serum and primary tumor of patients and HCC and BM	Suppresses miR-34a expression epigenetically through DNMT3A/PHB2 and HDAC1 and sponging miR-34a	Risk factor of BM
lncZEB1-AS1 [145]	Primary tumor	Highly expressed in patients with HCC and BM	Promotes the migration and invasion of HCC cells by sponging miR-302b to activate PI3K/AKT signaling and increase EGFR expression	Therapeutic target
H19 [133]	Primary tumor/BM/HCC cell lines	Highly expressed in patients with HCC and BM/in bone metastatic lesions/in osteotropic cell lines	Promotes the migration and invasion of HCC cells by sponging miR-200b-3p and inducing osteoclastogenesis through the PPP1CA/p38MAPK axis	Therapeutic target
CCL2 [146]	CAFs	Highly expressed in CAFs in primary site	Promotes the migration of HCC cells by activating hedgehog signaling	Therapeutic target
CCL5 [146]	CAFs	Highly expressed in CAFs in primary site	Promotes the migration of HCC cells by activating hedgehog signaling	Therapeutic target
CCL7 [146]	CAFs	Highly expressed in CAFs in primary site	Promotes the invasion of HCC cells by activating TGFβ signaling	Therapeutic target
CXCL16 [146]	CAFs	Highly expressed in CAFs in primary site	Promotes the invasion of HCC cells by activating TGFβ signaling	Therapeutic target
MAPK14 [147]	BMECs	Excessive activation in BMECs	Upregulates ADAM17 expression	Therapeutic target
ADAM17 [147]	BMECs	Highly expressed in BMECs	Promotes the secretion of CX3CL1	Therapeutic target
CX3CL1 [147]	HCC/BMECs	Highly expressed in bone metastatic lesions/in BMECs	Promotes the migration and invasion of HCC cells by activating PIK3CA/AKT1 and RHOA/ROCK2 signaling	Therapeutic target

(*Continued*)

Gene	Subject	Expression pattern	Biological function/molecular mechanism	Potential application
CX3CL1R [147]	HCC	Highly expressed in bone metastatic lesions	Promotes the migration and invasion of HCC cells by activating PIK3CA/AKT1 and RHOA/ROCK2 signaling	Therapeutic target

Abbreviations: HCC, hepatocellular carcinoma; BM, bone metastases; CTGF, connective tissue growth factor; IL-11, interleukin-11; MMP-1, matrix metallopeptidase-1; CXCR4, C-X-C motif chemokine receptor 4; FRZB, frizzled-related protein; PNI, perineural density; RNF219, ring finger protein 219; LGALS3, galectin 3; YAP1, Yes1 associated transcriptional regulator; SREs, skeleton-related events; SMAD4, SMAD family member 4; TGFβ, transforming growth factor β; DNMT3A, DNA methyltransferase 3 α; PHB2, prohibitin 2; HDAC1, histone deacetylase 1; lnc, long non-coding RNA; PI3K, phosphatidylinositol 3-kinase; AKT, AKT serine/threonine kinase; EGFR, epidermal growth factor receptor; PPP1CA, protein phosphatase 1 catalytic subunit α; MAPK, mitogen activated kinase-like protein; CCL, chemokine (C-C motif) ligand; CAFs, cancer-associated fibroblasts; CXCL, C-X-C motif chemokine ligand; BMECs, bone marrow endothelial cells; ADAM17, ADAM metallopeptidase domain 17; CX3CL1, C-X3-C motif chemokine ligand 1; CX3CL1R, CX3CL1 receptor; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; RHOA, ras homolog family member A; ROCK2, Rho associated coiled-coil containing protein kinase 2.

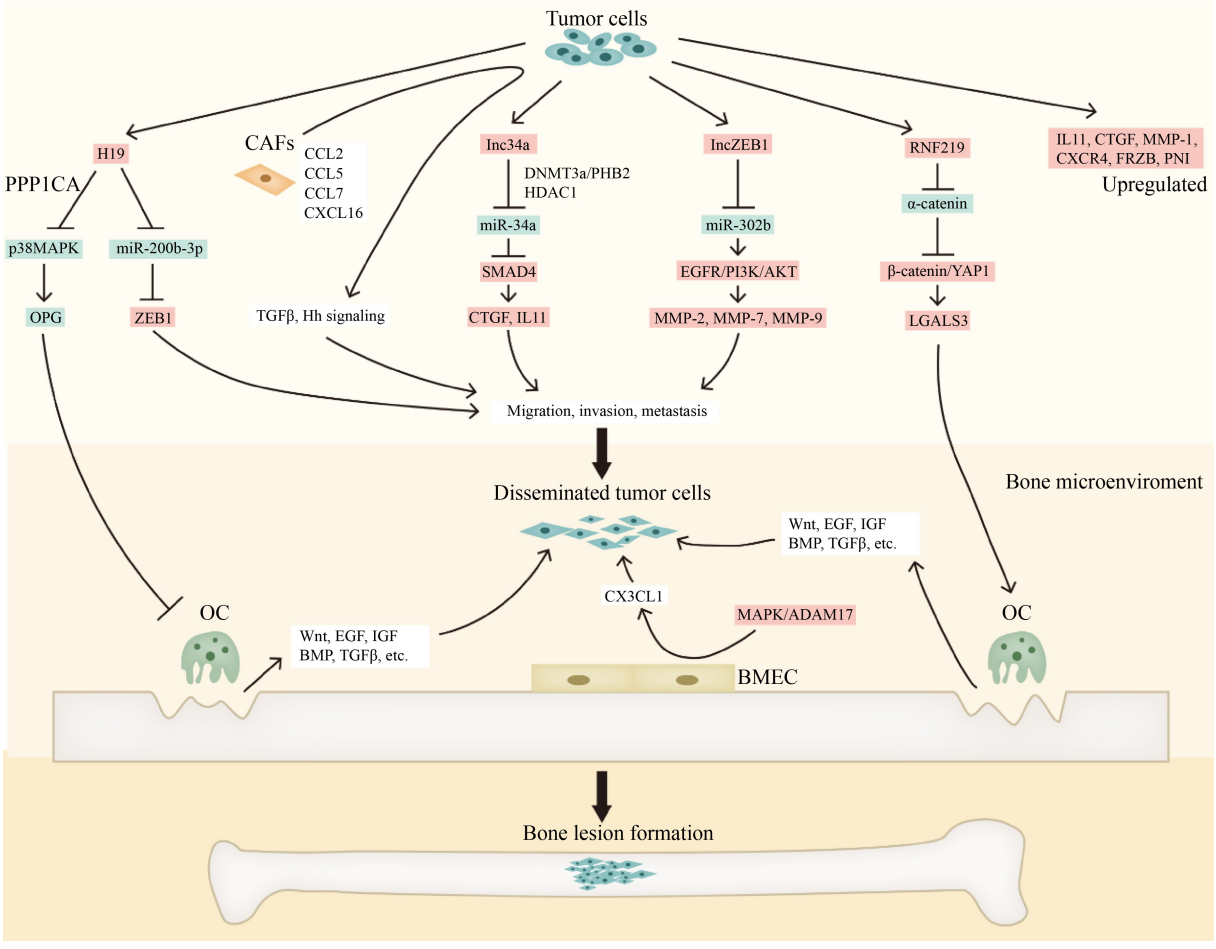

Fig. 5 Molecular mechanism of HCC BM. Elevated expression levels of H19, lnc34a, lncZEB1, and RNF219 in HCC cells contribute to strengthened bone metastatic ability by enhancing the migration, invasion, and metastasis ability of HCC cells and inducing osteolytic activities in bone. CCL2, CCL5, CCL7, and CXCL16 secreted by CAFs in primary sites activate Hh and TGFβ signaling in HCC cells to facilitate their metastatic capacities. High levels of IL11, CTGF, and MMP-1 have been found in primary HCCs with bone lesions and predict bone metastatic events. Various cytokines released from bone remodeling and bone stromal cells, such as BMECs, support the outgrowth of disseminated HCC cells in bone. Abbreviations: CAFs, cancer-associated fibroblasts; BMEC, bone marrow endothelial cells; OC, osteoclasts.

and retarded RNF219/LGALS3-induced osteoclastogenesis, thus blocking the initiation and progression of HCC BM [134].

Noncoding RNAs also play a role in the HCC BM process. Higher serum levels of long noncoding RNA 34a (lnc34a), together with reduced serum and tumor levels of miR-34a, have been reported as independent risk factors for the development of BM in patients with BM [143,144]. lnc34a epigenetically decreases miR-34a expression by recruiting DNMT3A/PHB2 and HDAC1, which together

sponge miR-34a [135]. The downregulation of miR-34a enhances the migration and invasion of cancer cells and activates the TGFβ-induced osteotropic transcription of the downstream genes by upregulating the expression of SMAD4 [135]. lncRNA zinc finger E-box binding homeobox 1 antisense 1 (lncZEB1-AS1) sponges miR-302b to activate PI3K/AKT signaling and increase EGFR expression, thereby promoting HCC BM. Univariate and multivariate analyses demonstrated that lncZEB1-AS1 is an independent risk factor for BM in patients with HCC [145]. In our own previous studies, we successfully established an animal model of HCC BM and isolated osteotropic HCC cell subpopulations by *in vivo* selection [133,140]. The expression levels of MMP-1, PTHrP, and CTGF were significantly elevated in osteotropic HCC cells relative to those in their ancestor cells. We further demonstrated that H19, a well-recognized lncRNA, promoted HCC BM by enhancing the mobility of HCC cells and modulating their interactions with osteoclasts. The high expression of H19 sponges miR-200b-3p to induce EMT, which confers HCC cells with increased mobility to spread from primary sites and reduces OPG expression by inactivating the p38MAPK signaling pathway, further enhancing the HCC-induced osteolytic process [133].

Beyond changes in liver cancer cells, alterations in the microenvironment at the primary site and bone marrow contribute to HCC progression and BM. CAFs in the liver promote HCC BM by secreting CCL2, CCL5, CCL7, and CXCL16 to activate hedgehog and TGFβ signaling [146]. The excessive activation of MAPK14 in bone marrow endothelial cells induces ADAM17-regulated CX3CL1 expression, which promotes the spinal metastasis of HCC cells with high CX3CLR expression by activating the PIK3CA/AKT1 and RHOA/ROCK2 signaling pathways [147].

Challenges and perspectives

Regardless of the increasing endeavors and impressive achievements in studies on HCC BM, the underlying pathological and molecular mechanisms, not to mention curative therapies, of this disease are far from clear. Here, we wish to emphasize the following four challenges facing the further investigation of HCC BM: ideal animal models, bone stromal cell involvement, novel drug targets, and effective drug delivery systems.

The limited access to clinical HCC BM specimens and ethical concerns emphasize the importance of an ideal and reproducible animal model for investigations on HCC BM. Given the extremely low incidence of spontaneous BM from orthotopic HCC, tumor cells are frequently directly injected into the bone cavity or into blood routes directed to bones. Specifically, the intraosseous access [148], left ventricle [140,148], vena cava [149], tail vein [150] and iliac artery [151,152] are commonly used for the introduction of cancer cells into the bone marrow. However, none of the models established through the above approaches can mimic all of the natural steps of HCC BM. Effective models should be able to reproduce the anatomical, genetic, and phenotypic changes during cancer cell diastasis from the primary site; survival in blood circulation; and settling and thriving in the bone marrow. To our knowledge, cancer cells in metastatic lesions acquire alterations that increase their tendency to metastasize to specific sites, and this preference can be strengthened by serial *in vivo* selection [153]. Thus, the preselection of HCC cell subpopulations with high propensity for BM may contribute to BM from orthotopic organs [154]. Specimens or organoids from patients with HCC and BM maintain the distinctive genomic alterations and functional heterogeneity of the original tissues that are prone to metastasize to the bone, and the xenografts of patient-derived specimens or organoids may contribute to spontaneous HCC BM in animal models [155,156].

The usage of immunodeficient mice for inoculating human cancer cells completely excludes immune cells, and the bone microenvironment in humans differs from that in mice. An animal model named NOD/SCID-hu, which is established by implanting human fetal bones or adult human ribs into nonobese diabetic/severe combined immunodeficient mice, realizes tissue- and species-specific metastasis [157]. In this animal model, the injected circulating prostate tumor cells form visible tumors only in human bone implants rather than in the mouse skeleton [158]. Humanized mice are also a promising tool for immune system investigation and for eliminating the discrepancy between humans and mice [159,160].

The bone marrow is a hemostatic organ with highly heterogeneous cells. Thus, beyond the interaction between HCC cells and osteoclasts or osteoblasts that have been intensively studied [133,134], other bone stromal cells deserve further attention. Studies have demonstrated that BMSCs can modulate HCC progression by transferring microRNAs through exosomes [105,161]. The involvement of the perineural system is implicated by the presence of more peripheral nerves in HCC bone lesions than in the corresponding primary liver tumor [142]. Studies on the BM of other tumor cells, bone marrow endothelial cells, hematopoietic cells, and adipose stem cells indicate that myeloid and immune cells may participate in HCC BM [94]. Additionally, combined single-cell and spatial transcriptomics have revealed the molecular, cellular, and spatial organization of the bone marrow niche; such a revelation opens a new avenue for the investigation of the communication between cancer cells and bone stromal cells [162]. Additional profound studies aimed at bone

stromal cells are needed to obtain a further comprehensive understanding of HCC BM.

Another challenge is the efficient delivery of the therapeutic doses of drugs to tumor sites inside the bone marrow given the relatively low vascularization of bone tissue and the presence of physical barriers [151,163]. The administration of chemotherapeutic agents at high doses or frequency often leads to severe side effects and exerts dose-limiting toxicity on healthy tissues [164]. The affinity of BPs for the bone hydroxyapatite matrix has been utilized for the selective delivery of small-molecule drugs, cancer-targeting drugs and nanoparticles to the bone marrow [151,165]. Enclosing the BP-modified HER2 monoclonal antibody Tras or gold nanorods inside mesoporous silica nanoparticles results in the high efficiency of delivery to the bone marrow [151,166]. EVs have been established to mediate the communication among cells and have been revealed by various studies to be superior to conventional synthetic carriers in drug delivery [167]. Tumor-derived exosomes prepare pre-metastatic niches and determine organotropic metastasis via integrins [168]. Accordingly, exosomes or EVs with adequate organotropism are promising carriers for the delivery of bone- and tumor-targeted drugs to the bone marrow.

Acknowledgements

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Compliance with ethics guidelines

Zhao Huang, Jingyuan Wen, Yufei Wang, Shenqi Han, Zhen Li, Xuemei Hu, Dongling Zhu, Zhenxiong Wang, Junnan Liang, Huifang Liang, Xiao-ping Chen, and Bixiang Zhang declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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