

c-Met: A Promising Therapeutic Target in Bladder Cancer

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Abstract: Mesenchymal-epithelial transition factor (c-Met) belongs to the tyrosine kinase receptor family and is overexpressed in various human cancers. Its ligand is hepatocyte growth factor (HGF), and the HGF/c-Met signaling pathway is involved in a wide range of cellular processes, including cell proliferation, migration, and metastasis. Emerging studies have indicated that c-Met expression is strongly associated with bladder cancer (BCa) development and prognosis. Therefore, c-Met is a potential therapeutic target for BCa treatment. Recently, the aberrant expression of noncoding RNAs was found to play a significant role in tumour progression. There is a close connection between c-Met and noncoding RNA. Herein, we summarized the biological function and prognostic value of c-Met in BCa, as well as its potential role as a drug target. The relation of c-Met and ncRNA was also described in the paper.

Keywords: c-Met, HGF, noncoding RNA, bladder cancer, review

Introduction

Bladder cancer (BCa) is one of the most common malignant carcinomas. According to global cancer statistics, there were 54,939 newly diagnosed cases of BCa in 2018, ranking 12th among all kinds of cancers in both sexes and 6th among those in males.¹ BCa is usually classified into two categories: non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC).² At present, nearly 75% of cases are NMIBC, and 25% are MIBC or metastatic disease.³ The 5-year survival rate for MIBC patients with active treatment is approximately 60%, and the 5-year recurrence rate of NMIBC ranges from 50% to 70%.³ BCa is a complex disease and has been a global burden for a long time. However, current treatments for BCa show limited benefits. Many more studies are required to obtain a more advanced understanding of BCa development and more effective therapeutic strategies. The aberrant activities of mesenchymal-epithelial transition factor (c-Met) have been observed in multiple human malignancies including BCa. In the present study, we reviewed the biological function and prognostic value of c-Met, miRNAs in relation to c-Met, as well as the potential role of c-Met as a drug target in BCa.

The Structure and Biological Function of c-Met

c-Met was first identified in the mid-1980s.^{4,5} The c-Met-encoding gene is located at chromosome 7q21-31 and is a well-characterized oncogene.⁶ c-Met, also known as Met, HGFR, AUTS9, RCCP2 and DFNB97, belongs to the receptor tyrosine kinase family and is present predominantly in epithelial cells.⁷ The mature c-Met protein is a disulfide-linked heterodimer composed of a highly glycosylated 45-kDa extracellular α -subunit and a 145-kDa transmembrane β -subunit. The extracellular portions of c-Met consist of three domains, an N-terminal SEMA domain (sematophorin domain), a cysteine-rich portion (plexin sematophorin domain) and four IPT domains (immunoglobulin-like plexin transcription

factor), and the intracellular portions include a tyrosine kinase catalytic domain, a juxta-membrane domain and carboxy-terminal sequences.^{8,9}

Hepatocyte growth factor (HGF) is a high-affinity ligand for c-Met^{10,11} and was first discovered in 1984.¹² HGF is the representative of a family of plasminogen-related cytokines named scatter factors and is expressed in cells of mesenchymal origin.¹³ The encoding gene of HGF is located at chromosome 7q11.2–21, and the mature form of HGF contains an N-terminal domain, four Kringle domains, and a domain homologous to the chymotrypsin family of serine proteases.¹⁴ Universally, dimerization (the binding of two monomers) is the underlying regulatory mechanism for the activation of tyrosine kinase receptors,¹⁵ and HGF binds to c-Met via this mechanism. HGF contains two receptor-binding sites: a binding site with high affinity for the IPT3 and IPT4 domains of c-Met, and a binding site with low affinity site for the SEMA domain.⁸ Upon binding to biologically active HGF, c-Met undergoes dimerization, and Tyr residues Y¹²³⁴ and Y¹²³⁵ within the activation loop undergo trans-autophosphorylation.¹⁶ Then, Tyr residues Y¹³⁴⁹ and Y¹³⁵⁶ within the C-terminal segment undergo autophosphorylation, providing offers binding sites and facilitating the recruitment of downstream signaling effector molecules.

These binding proteins with enzymatic activity include phosphatidylinositol 3-kinase (PI3K), phospholipase C- γ 1 (PLC- γ 1), the nonreceptor tyrosine kinase Src and signal transducer and activator of transcription 3 (STAT3), as well as adaptor proteins without enzymatic activity, such as growth factor-bound protein 2 (GRB2) and GRB2-associated binding protein 1 (GAB1).^{5,8,16,17} These effectors therefore exert effects related to the scattering effect, proliferative action, morphogenetic action and many other biological functions.¹⁸ The downstream signaling pathways include the Ras pathway, PI3K pathway, Wnt/ β -catenin signaling pathway and other pathways. Under normal physiological conditions, HGF/c-Met functions in embryogenesis, tissue regeneration, wound healing, and the formation of nerves and muscles,¹⁹ the progression of which is partially mediated by the tumour suppressor p53.^{20,21} In addition, it is well established that c-Met may activate signals in an HGF-independent manner.^{22,23} Accumulating evidence has demonstrated that HGF/c-Met dysregulation and c-Met gene mutations, amplifications and overexpression contribute to a plethora of human diseases, especially cancers, such as lung cancer, liver cancer, and BCa.^{24–27}

The Role of c-Met in BCa

Early in the 1990s, studies revealed that the HGF/c-Met pathway was involved in BCa development in animal models and in vivo. The expression of HGF/c-Met was increased during the progression of rat bladder carcinogenesis induced with N-butyl-N-(4-hydroxybutyl)-nitrosamine.²⁸ HGF in rats promoted the proliferation and growth of nontumorigenic cell lines, whereas in tumorigenic cell lines, HGF stimulated cell invasion and migration by either a paracrine or an autocrine mechanism.²⁹ These results suggested that HGF acted as a mitogen in a nontumorigenic cell line but as an invasion and migration factor in tumorigenic cell lines.²⁹ Researchers compared the HGF content in urine between patients with BCa and healthy controls and found that HGF was significantly elevated in BCa patients. Therefore, there seems to be a positive association between BCa invasion and HGF content.^{30,31}

c-Met overexpression was observed in 25% (31 of 123) of locally advanced or metastatic bladder urothelial carcinomas, compared with 8.3% (2 of 24) of nonbladder urothelial carcinomas.³² An analysis of 97 high-grade BCa samples revealed that the frequency of c-Met gene alteration was 2%.³³ Lee et al reviewed several studies and reported genetic aberrations in HGF and c-Met genes at an average combined frequency of 10% in 528 BCa cases.³⁴ Furthermore, Lee et al measured the cellular c-Met protein content in 12 BCa-derived cell lines, and the values ranged from 12 to 133 ng/mg total protein. The same study demonstrated that autocrine HGF/c-Met signaling was rare in BCa, while paracrine signaling was likely dominant.³⁴ However, Parr and Jiang revealed a complete HGF autocrine regulatory cycle in BCa cells.³⁵ Although c-Met and HGF exhibited different expression patterns in different types of BCa cells, these studies revealed enhanced expression of c-Met. c-Met has also been observed to play a facilitating role in BCa cell proliferation, invasion, and migration but an inhibitory role in apoptosis.^{36,37}

Since c-Met and HGF are recognized as characteristic molecules in BCa, more studies have focused on the role of HGF/c-Met in BCa and the underlying mechanisms. The significance of c-Met overexpression was compared with p53 nuclear accumulation (TP53) in primary BCa. The results showed that c-Met expression was strongly correlated with histologic grade, stage classification, tumour size, nodular tumour growth, and disease progression, but not TP53 status,

in BCa.³⁸ Additionally, c-Met overexpression, but not TP53 status, could indicate a poor long-term survival rate. These findings together demonstrated that c-Met plays a more important role than p53 in bladder carcinogenesis and progression.³⁸ Antibody arrays were also used to demonstrate that c-Met expression was associated with pathological stage, tumour grade, and the survival rate of BCa patients.³⁹ Another mechanism was introduced by Sim et al, in which the regulation of the TGF β signaling pathway plays an essential role in BCa. Specifically, HGF/c-Met activates the phosphorylation of SMURF2 induced by c-SRC, dissociating SMAD7 from SMURF2 and facilitating the interactions between C2 and HECT domains, which silences SMURF2. SMURF2 acts like a switch between MAPK and TGF β signaling pathways. The inhibition of SMURF2 enhances the membrane TGF β receptor, and the upregulation of the TGF β signaling pathway eventually causes EMT and enhances BCa invasiveness.⁴⁰

c-Met belongs to a family of receptor tyrosine kinases (RTKs) that share sequence and structural homology; other family members include RON, AXL and PDGFR.⁴¹ Coexpression of c-Met with RON, AXL or PDGFR is commonly found in urothelial cells.^{36,42–44} Cheng et al observed that the overexpression of c-Met and RON was positively associated with the histological grade, nonpapillary contour, tumour size and muscle invasion of BCa.⁴³ The coexpression of c-Met and RON is positively associated with the progression of transitional-cell carcinoma of the bladder and with poor overall survival in patients with superficial BCa.⁴³ According to reports, the enhanced expression of AXL and PDGFR is correlated with the aggressiveness and prognosis of several tumours.^{36,45,46} The expression of AXL and PDGFR is greatly altered in BCa and can distinguish between MIBC and NMIBC.³⁷ Further research showed that expression of c-Met network members (c-Met-AXL-PDGFR) was significantly associated with the disease progression of NMIBC and with the overall survival of MIBC and that c-Met activates AXL and PDGFR- α through a ras- and Src-independent pathway.³⁶ These findings suggest a new hypothesis that the c-Met-AXL-PDGFR network could be a candidate prognostic marker for predicting BCa development.³⁷

Kim et al demonstrated that the downregulation of c-Met could inhibit proliferation and induce cell apoptosis in BCa. Moreover, c-Met silencing suppressed cell motility by downregulating MMP2 and MMP9.³⁷ Miyata et al found that among different c-Met phosphorylation sites, pY¹³⁴⁹ was the only significant factor for high pT stage.⁴⁷ Their subsequent studies suggested that two c-Met tyrosine residues, pY1234/pY1235 and pY1349, were related to muscular infiltration and metastasis, while c-Met itself was only significantly associated with muscular infiltration.⁴⁸ In contrast, Iyer et al found a nonsignificant association between HGF/c-Met immunoreactivity and tumour stage, grade and overall patient survival.³⁴ In parallel, Yeh et al found that c-Met expression did not correlate with tumour grade or lymph node involvement.³⁶ The variability in c-Met expression in BCa could be due to differences in sample size, evaluation methods and tissue sample conditions.⁴⁹

There are various types of BCa, and studies have demonstrated that the HGF/c-Met signaling pathway may be involved in multiple types of BCa. Neuroendocrine BCa is a rare type of BCa. c-Met expression was observed in serum-free cultures, animal models and primary tissue sections; moreover, c-Met inhibitors could greatly decrease tumour growth.⁵⁰ HGF could enhance the proliferation, migration, and wound healing of human neurogenic bladder smooth muscle cells and urothelial cells and critically mediate the growth of neuroendocrine BCa spheroids in vitro.^{50,51} Another rare BCa, hepatoid adenocarcinoma of the bladder, also expresses HGF and c-Met.⁵² HGF expression was observed in papillary transitional cell carcinoma with notable immunoreactivity but weak immunoreactivity in nodular tumours, while c-Met immunostaining was consistently detected in both papillary and nodular tumours.⁵³

In addition to the strong association between cellular c-Met and BCa, urinary soluble c-Met (sMet) and subunits of c-Met might be potential biomarkers for BCa. There is a clear difference in urinary sMet levels between BCa patients and nontumour patients, and urinary sMet levels are able to distinguish MIBC from NMIBC with moderate sensitivity and specificity.⁵⁴ Subunits of c-Met are also involved in BCa. P145^{met}, the β -subunit of c-Met, in which tyrosine residues 1003, 1234 and 1235 are phosphorylated in the process of the serum-independent growth of human BCa “5637” cell line, is required for “5637” cell survival.⁵⁵ This progress is mediated by the transmembrane signaling cascade via EGFR ligand(s). As mentioned above, studies conducted by Miyata et al revealed correlations between c-Met tyrosine residues and BCa progression. Furthermore, they demonstrated possible links between pY1349 c-Met and cancer-related substances such as cyclooxygenase-2 (COX-2), haem oxygenase-1 (HO-1) and programmed death ligand 1 (PD-L1).⁴⁸ Intriguingly, the upstream mediator of pY1349 c-Met might be SRY-box 18 (SOX18), which was proven to promote

carcinogenesis and progression of BCa. In such a study, Huaqi et al observed an augmented level of phosphorylated pY1349 c-Met in SOX18-elevated BCa cells, and after applying cabozantinib, a c-Met suppressor, the migration capability of SOX18-elevated BCa cells was dramatically compromised.⁵⁶

Shintani et al took an in-depth look at the relationship between cabozantinib and c-Met in BCa, and their findings suggested that cabozantinib significantly downregulated the expression of matrix metalloproteinase 1 (MMP1), which was induced by HGF/c-Met.⁵⁷

The prognostic role of c-Met in BCa has been analysed and confirmed by several studies. Our previous meta-analysis included 8 studies with a total of 1336 cases and demonstrated that c-Met upregulation was associated with shorter overall survival (OS) in BCa patients. Additionally, an immunohistochemical analysis of 26 BCa specimens indicated that the phosphorylation of c-Met mediated by matriptase was strongly correlated with an unfavourable outcome.⁵⁸

Noncoding RNA/c-Met Regulatory Axis in BCa

Dysregulated noncoding RNAs lead to a variety of pathological events and diseases, including BCa. Evidence has shown that miR-409-3p is downregulated in BCa. Enforced expression of miR-409-3p inhibited BCa cell migration and invasion but had no effect on cell proliferation. Mechanistically, miR-409-3p targets the 3'-untranslated region (UTR) of c-Met and reduces the expression of c-Met, which inhibits the downstream proteins MMP2 and MMP9, leading to reduced degradation of the basement membrane and extracellular matrix. Naturally, the overexpression of c-Met could partially reverse miR-409-3p-mediated cancer cell migration and invasion.⁵⁹ In addition, the same research group identified that the expression level of miR-101 was negatively correlated with BCa development by targeting c-Met.⁶⁰ The migration and invasion of BCa cells are significantly inhibited by decreases in c-Met expression in a miR-101 overexpression-induced manner.

In 2016, Xu et al found that miR-433 was frequently downregulated in BCa tissues.⁶¹ Enhanced expression of miR-433 inhibited cell proliferation, motility and epithelial-mesenchymal transition (EMT). c-Met and CREB1 are direct target genes of miR-433. Silencing c-Met could repress cell motility and EMT by regulating the AKT/GSK-3 β /SNAIL signaling pathway, which phenocopied the effect of miR-433 overexpression. Similarly, CREB1 could also exert such a function. CREB1 may regulate c-Met by mediating MITF. Moreover, miR-409-3p was partially involved in the miR-433-induced inhibition of cell migration and invasion.⁶¹ This result implies the possibility of reciprocal regulation among c-Met, miR-433 and miR-409-3p.

There is another c-Met gene network mediated by miRNAs involved in BCa development. Similar to miR-433 and miR-409-3p, miR-323a-3p is downregulated in BCa, and enhanced expression of miR-323a-3p clearly reduced cell motility by regulating EMT progression.⁶² Both c-Met and SMAD3 are targeted by miR-323a-3p, which ultimately exerts effects on SNAIL. miR-323a-3p suppression partially rescued the knockdown of c-Met-induced and SMAD3-induced inhibition of EMT progression. Thus, the miR-323a-3p/c-Met/SMAD3/SNAIL circuit is involved in EMT progression in BCa. Moreover, this study revealed the mutual regulation between miR-323a-3p, miR-433, miR-409 and c-Met.⁶² In addition, the correlations between miR-323a and c-Met were confirmed by other scientists in different BCa cell lines.⁶³

The expression of miR-23b and miR-27b was significantly reduced in BCa tissues.⁶⁴ Correspondingly, the overexpression of miR-23b and miR-27b exerted inhibitory effects on cell proliferation, migration and invasion. Mechanistically, miR-23b and miR-27b directly repress c-Met and EGFR by binding to the 3'-UTR of the target mRNAs, therefore affecting the downstream signaling pathways.

As mentioned above, miR-433, miR-409-3p and miR-323a-3p all mediate the progression of BCa by targeting c-Met. These miRNAs are all transcribed from the miRNA cluster in the DLK-DIO3-imprinted domain.⁶⁵ Therefore, it is reasonable to propose that there are other candidate miRNAs transcribed from the DLK-DIO3-imprinted domain that play important roles in BCa. Li et al demonstrated that miR-381-3p, which is transcribed from the DLK-DIO3-imprinted domain, has great regulatory effects on BCa development.⁶⁵ Accordingly, miR-381-3p is downregulated in BCa, while the overexpression of miR-381-3p is able to inhibit BCa cell proliferation and migration. c-Met, together with CDK6 and CCNA2, is directly targeted by miR-381-3p, which explains why miR-381-3p plays an inhibitory role in BCa cell proliferation and migration.

Numerous studies have identified a number of miRNAs involved in BCa progression and metastasis, including miR-34a,⁶⁶ miR-206,⁶⁷ miR-200^{68,69} and many others. The target genes of these miRNAs include CDK4 and CDK6. In addition, certain interactions between c-Met and these miRNAs have been identified.^{70–74} c-Met is involved in these miRNA-related circuits and may be a target of these miRNAs. Currently, whether c-Met works in conjunction with miR-34a, miR-206, the miR-200 family, etc. in BCa remains elusive and therefore provides us with a promising research topic. The miRNA/c-Met axis in BCa is shown in Figure 1.

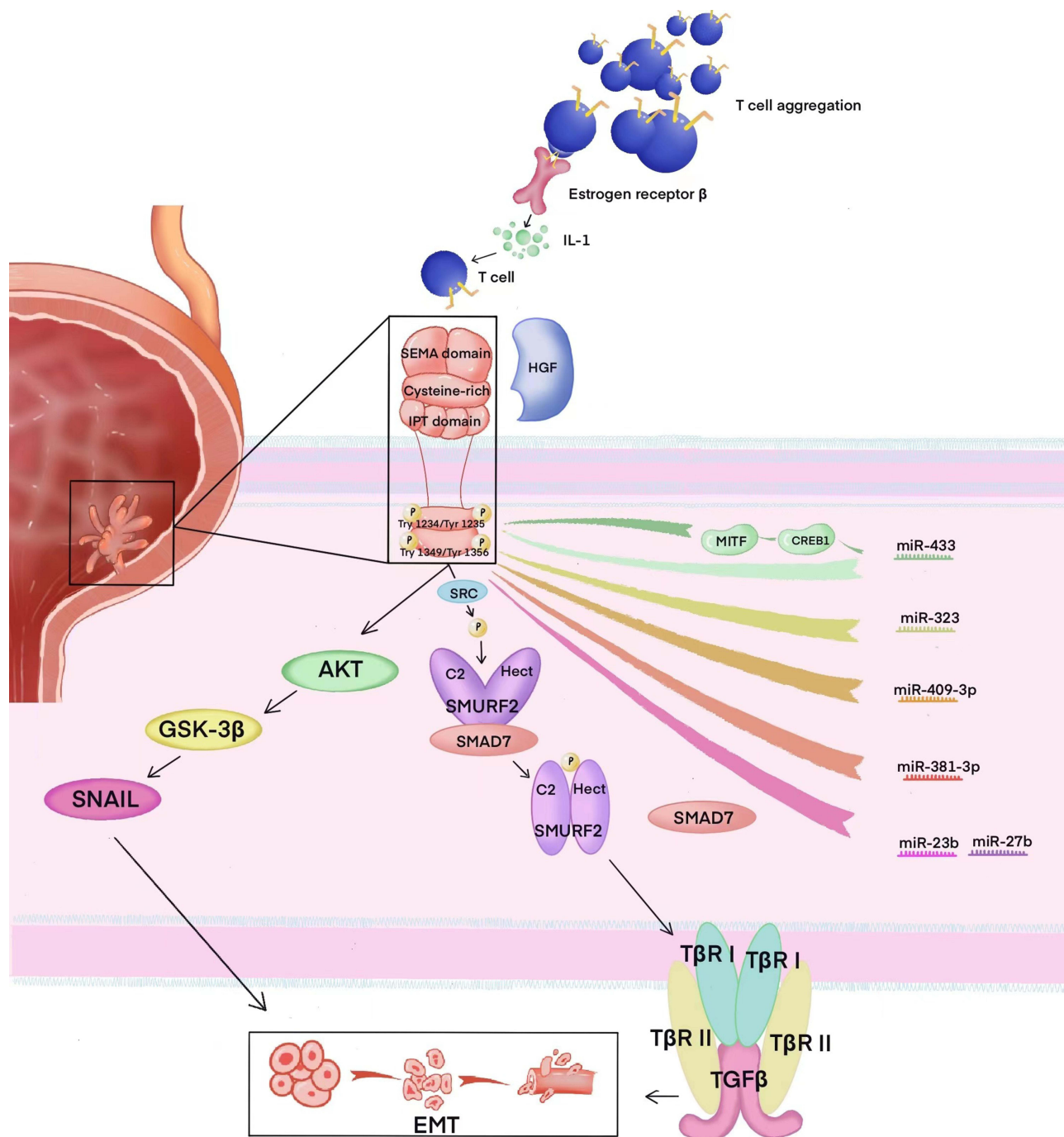


Figure 1 C-Met structure, relevant miRNAs and important signaling pathways involved in HGF/c-Met regulation in BCa. HGF/c-Met-TGFβ signaling: HGF/c-Met activates the phosphorylation of SMURF2 induced by c-SRC, dissociating SMAD7 from SMURF2 and facilitating the interactions between C2 and HECT domains, which in turn puts SMURF2 in a closed inactive status. The silence of SMURF2 stabilizes TβR and upregulates the TGFβ signaling pathway and eventually causes EMT.

c-Met and Tumor Microenvironment in BCa

Recent years, numerous studies have demonstrated that tumor microenvironment plays a crucial role in the carcinogenesis and progression in multiple neoplasms including BCa.⁷⁵ Tao et al discovered an elevated infiltration of CD4+T cells surrounding BCa cells, which promoted the progression of BCa. The underlying mechanisms were revealed that CD4+T cells triggered estrogen receptor beta (ER β) pathway and ER β enhanced the expression of c-Met in an IL-1 dependent or independent manner.⁷⁶ This is the first literature that disclosed the interaction between c-Met and BCa microenvironment, providing us a novel insight into the immunotherapy of BCa.

c-Met Inhibitors in BCa

Generally, c-Met inhibitors could be categorized into three classes: c-Met tyrosine kinase inhibitors (TKIs), HGF antagonists targeting c-Met and HGF-neutralizing antibodies.⁷⁷ Small molecule TKIs block intracellular signaling pathways in tumor cells, most of which are competitors for ATP binding. Type I inhibitors bind to proteins through a U-shaped structure while type II inhibitors are multi-target agents and function in a more extended spatial conformation. Type III inhibitors are non-ATP competitive agents.⁷⁸ Table 1 summarized the representative c-Met inhibitors with clinical significance.

Table 1 Representative c-Met Inhibitors with Clinical Significance

Name	Type	Mechanism	Clinical Trials of c-Met Cancer Types	Other Targets
Cabozantinib	Nonselective TKI	ATP competitive	RCC (Phase 2), HCC (Phase 4), Cervical Cancer (Phase 2), CRC (Phase 1), NSCLC (Phase 2), PSCC (Phase 2), Neuroendocrine Tumors (Phase 1), UC (Phase 2), Prostate Cancer (Phase 2), Breast Cancer (Phase 2), Esophageal Cancer (Phase 2), Melanoma (Phase 2), Thyroid Cancer (Phase 3), AML (Phase 1), HNSCC (Phase 2), Pancreatic Cancer (Phase 2), BCa (Phase 2)	VEGFR2, FLT3, KIT, AXL, RET
Crizotinib	Nonselective TKI	ATP competitive	Gastric Cancer (Phase 2), NSCLC (Phase 4), Prostate Cancer (Phase 1), Lymphoma (Phase 2)	ALK, ROS1
Foretinib	Nonselective TKI	ATP competitive	HCC (Phase 1), Breast Cancer (Phase 2), NHSCC (Phase 2), RCC (Phase 2), NSCLC (Phase 1)	KDR
Golvatinib	Nonselective TKI	ATP competitive	Gastric Cancer (Phase 1), Solid Tumors (Phase 1)	VEGFR2
Tivantinib	Nonselective TKI	Non-ATP competitive	HCC (Phase 3), NSCLC (Phase 2), CRC (Phase 2), Solid Tumors (Phase 1), Pancreatic Neoplasms (Phase 2)	-
Savolitinib	Selective TKI	ATP competitive	NSCLC (Phase 3), Prostate Cancer (Phase 2), Solid Tumors (Phase 1), RCC (Phase 3), CRC (Phase 2), Gastric Adenocarcinoma (Phase 2)	-
Capmatinib	Selective TKI	ATP competitive	HCC (Phase 2), Solid Tumors (Phase 2), Oesophageal Adenocarcinoma (Phase 2), NSCLC (Phase 3),	-
Tepotinib	Selective TKI	ATP competitive	NSCLC (Phase 2), Solid Tumors (Phase 2), Brain Tumor (Phase 1), HCC (Phase 1)	-
Rilotumumab	Monoclonal antibody	HGF	Gastric Cancer (Phase 3), Solid Tumors (Phase 1), NSCLC (Phase 2), Malignant Glioma (Phase 2), Prostate Cancer (Phase 1), RCC (Phase 2)	-
Ficlatuzumab	Monoclonal antibody	HGF	NHSCC (Phase 1), Pancreatic cancer (Phase 1), AML (Phase 1), NSCLC (Phase 2)	-
Onartuzumab	Monoclonal antibody	c-Met	Solid Tumors (Phase 3), HCC (Phase 1), Glioblastoma (Phase 2), Solid Tumors (Phase 1), NSCLC (Phase 3), Gastric Cancer (Phase 3), Breast Cancer (Phase 2), CRC (Phase 2)	-

Abbreviations: TKI, tyrosine kinase inhibitor; ATP, adenosine-triphosphate; RCC, renal cell carcinoma; HCC, hepatocellular carcinoma; UC, urothelial carcinoma; PSCC, penile squamous cell carcinoma; NSCLC, non-small cell lung cancer; AML, acute myeloid leukemia; HNSCC, Head and neck squamous cell carcinomas; BCa, bladder cancer; HGF, Hepatocyte growth factor; CRC, colorectal cancer; VEGFR2, vascular endothelial growth factor receptor-2; FLT3, FMS-like tyrosine kinase 3; KIT, tyrosine protein kinase kit; AXL, AXL receptor tyrosine kinase; RET, rearranged during transfection; ALK, anaplastic lymphoma kinase; ROS1, proto-oncogene 1, receptor tyrosine kinase; c-Met, mesenchymal-epithelial transition factor.

Table 2 Clinical Trials of c-Met Inhibitors in Patients with BCa

Drug	NCT Number	Status	Brief Description	Phase	Number Enrolled
Cabozantinib	NCT04289779	Recruiting	Cabozantinib plus atezolizumab as neoadjuvant treatment for MIBC	2	42
	NCT03824691	Recruiting	Cabozantinib Plus Durvalumab for advanced and chemotherapy-treated BCa: ARCADIA Study	2	122
	NCT03534804	Recruiting	Cabozantinib plus pembrolizumab cisplatin-ineligible advanced BCa	2	39
	NCT01688999	Ongoing	Cabozantinib monotherapy for advanced urothelial cancer	2	69
	NCT03866382	Recruiting	Cabozantinib plus nivolumab and ipilimumab for rare genitourinary tumors	2	224
	NCT05092958	Recruiting	Cabozantinib plus avelumab for metastatic urothelial cancer: MAIN-CAV Study	3	654
Crizotinib	NCT02612194	Terminated	Crizotinib monotherapy for c-Met or RON-Positive metastatic urothelial cancer	2	8

Abbreviations: MIBC, muscle-invasive bladder cancer; BCa, bladder cancer; c-Met, mesenchymal-epithelial transition factor; RON, receptor originated from Nantes.

In BCa, cabozantinib, a nonselective TKI against c-Met and others such as VEGFR2, FLT3, KIT, AXL, and RET is undergoing in-depth clinical research currently. As mentioned above, scientists revealed that cabozantinib could block MMP1 expression mediated by HGF/c-Met.⁵⁷ A Phase II clinical trial of cabozantinib and durvalumab combination therapy in patients with metastatic disease after platinum-based chemotherapy initially showed that the combination therapy was safe and achieved an objective response rate of 43.8%.⁷⁹ Another TKI with multiple targets including c-Met named crizotinib might be promising in clinical application. A preclinical xenograft study suggested that both crizotinib and cabozantinib significantly suppressed HGF/c-Met induced tumor cell proliferation and invasion in multiple BCa cell lines.³⁴ Several clinical trials are underway on cabozantinib and crizotinib in BCa treatment, relevant details are listed in Table 2.

Conclusion

In summary, we evaluated the present knowledge regarding c-Met and its biological and potential therapeutic role in BCa. Currently, c-Met is regarded as a proto-oncogene and facilitates a wide range of biological functions, including cell proliferation, growth, migration, invasion, and angiogenesis, through interaction with its ligand HGF. Enhanced expression of c-Met is positively related to the development of various types of BCa and a poor prognosis. Although there is no final conclusion, it has been observed in many studies that c-Met overexpression is closely related to the histological grade, nonpapillary contour, tumour size and muscle invasion of BCa. Emerging evidence supports that c-Met could be a novel and important biomarker for BCa. As epigenetic regulators, miRNAs perform biological functions by inhibiting gene expression. The aberrant expression of miRNAs has been identified in the development of human diseases, including BCa. Studies have demonstrated that c-Met is the target of various miRNAs. By downregulating the expression of c-Met, aberrantly expressed miRNAs support the survival and metastasis of BCa cells and thus participate in BCa development. Currently, c-Met inhibitors represented by cabozantinib have been widely used in anti-cancer treatment and demonstrates broad prospects for clinical application in BCa.

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Disclosure

The authors declare that they have no conflicts of interest.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424. doi:10.3322/caac.21492

2. Luke C, Tracey E, Stapleton A, Roder D. Exploring contrary trends in bladder cancer incidence, mortality and survival: implications for research and cancer control. *Intern Med J*. 2010;40(5):357–362. doi:10.1111/j.1445-5994.2009.01980.x
3. Kamat AM, Hahn NM, Efstathiou JA, et al. Bladder cancer. *Lancet*. 2016;388(10061):2796–2810. doi:10.1016/S0140-6736(16)30512-8
4. Cooper CS, Park M, Blair DG, et al. Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature*. 1984;311(5981):29–33. doi:10.1038/311029a0
5. Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G. Targeting MET in cancer: rationale and progress. *Nat Rev Cancer*. 2012;12(2):89–103. doi:10.1038/nrc3205
6. Giordano S, Ponzetto C, Di Renzo MF, Cooper CS, Comoglio PM. Tyrosine kinase receptor indistinguishable from the c-Met protein. *Nature*. 1989;339(6220):155–156. doi:10.1038/339155a0
7. Ilangumaran S, Villalobos-Hernandez A, Bobbala D, Ramanathan S. The hepatocyte growth factor (HGF)-MET receptor tyrosine kinase signaling pathway: diverse roles in modulating immune cell functions. *Cytokine*. 2016;82:125–139. doi:10.1016/j.cyt.2015.12.013
8. Stamos J, Lazarus RA, Yao X, Kirchhofer D, Wiesmann C. Crystal structure of the HGF beta-chain in complex with the Sema domain of the Met receptor. *EMBO J*. 2004;23(12):2325–2335. doi:10.1038/sj.emboj.7600243
9. Organ SL, Tsao MS. An overview of the c-Met signaling pathway. *Ther Adv Med Oncol*. 2011;3(1 Suppl):S7–S19. doi:10.1177/1758834011422556
10. Naldini L, Vigna E, Narsimhan RP, et al. Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-Met. *Oncogene*. 1991;6(4):501–504.
11. Bottaro DP, Rubin JS, Faletto DL, et al. Identification of the hepatocyte growth factor receptor as the c-Met proto-oncogene product. *Science*. 1991;251(4995):802–804. doi:10.1126/science.1846706
12. Nakamura T, Nawa K, Ichihara A. Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. *Biochem Biophys Res Commun*. 1984;122(3):1450–1459. doi:10.1016/0006-291X(84)91253-1
13. Tamagnone L, Comoglio PM. Control of invasive growth by hepatocyte growth factor (HGF) and related scatter factors. *Cytokine Growth Factor Rev*. 1997;8(2):129–142. doi:10.1016/S1359-6101(97)00007-5
14. Weidner KM, Hartmann G, Naldini L, et al. Molecular characteristics of HGF-SF and its role in cell motility and invasion. *Exs*. 1993;65:311–328.
15. Heldin CH. Dimerization of cell surface receptors in signal transduction. *Cell*. 1995;80(2):213–223. doi:10.1016/0092-8674(95)90404-2
16. Hass R, Jennek S, Yang Y, Friedrich K. c-Met expression and activity in urogenital cancers - novel aspects of signal transduction and medical implications. *CCS*. 2017;15(1):10. doi:10.1186/s12964-017-0165-2
17. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol*. 2003;4(12):915–925. doi:10.1038/nrm1261
18. Trovato M, Campenni A, Giovinazzo S, Siracusa M, Ruggeri RM. Hepatocyte growth factor/c-Met axis in thyroid cancer: from diagnostic biomarker to therapeutic target. *Biomark Insights*. 2017;12:1177271917701126. doi:10.1177/1177271917701126
19. Cui JJ. Targeting receptor tyrosine kinase MET in cancer: small molecule inhibitors and clinical progress. *J Med Chem*. 2014;57(11):4427–4453. doi:10.1021/jm401427c
20. Grugan KD, Vega ME, Wong GS, et al. A common p53 mutation (R175H) activates c-Met receptor tyrosine kinase to enhance tumor cell invasion. *Cancer Biol Ther*. 2013;14(9):853–859. doi:10.4161/cbt.25406
21. Muller PA, Trinidad AG, Timpson P, et al. Mutant p53 enhances MET trafficking and signaling to drive cell scattering and invasion. *Oncogene*. 2013;32(10):1252–1265. doi:10.1038/onc.2012.148
22. Liu X, Newton RC, Scherle PA. Development of c-Met pathway inhibitors. *Expert Opin Investig Drugs*. 2011;20(9):1225–1241. doi:10.1517/13543784.2011.600687
23. Tulasne D, Foveau B. The shadow of death on the MET tyrosine kinase receptor. *Cell Death Differ*. 2008;15(3):427–434. doi:10.1038/sj.cdd.4402229
24. Christensen JG, Burrows J, Salgia R. c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. *Cancer Lett*. 2005;225(1):1–26. doi:10.1016/j.canlet.2004.09.044
25. Gumustekin M, Kargi A, Bulut G, et al. HGF/c-Met overexpressions, but not met mutation, correlates with progression of non-small cell lung cancer. *Pathol Oncol Res*. 2012;18(2):209–218. doi:10.1007/s12253-011-9430-7
26. Goyal L, Muzumdar MD, Zhu AX. Targeting the HGF/c-Met pathway in hepatocellular carcinoma. *Clin Cancer Res*. 2013;19(9):2310–2318. doi:10.1158/1078-0432.CCR-12-2791
27. Matsumoto R, Tsuda M, Wang L, et al. Adaptor protein CRK induces epithelial-mesenchymal transition and metastasis of bladder cancer cells through HGF/c-Met feedback loop. *Cancer Sci*. 2015;106(6):709–717. doi:10.1111/cas.12662
28. Inui M, Nishi N, Yasumoto A, et al. Enhanced gene expression of transforming growth factor- α and c-Met in rat urinary bladder cancer. *Urol Res*. 1996;24(1):55–60. doi:10.1007/BF00296735
29. Tamatani T, Hattori K, Iyer A, Tamatani K, Oyasu R. Hepatocyte growth factor is an invasion/migration factor of rat urothelial carcinoma cells in vitro. *Carcinogenesis*. 1999;20(6):957–962. doi:10.1093/carcin/20.6.957
30. Joseph A, Weiss GH, Jin L, et al. Expression of scatter factor in human bladder carcinoma. *J Natl Cancer Inst*. 1995;87(5):372–377. doi:10.1093/jnci/87.5.372
31. Rosen EM, Joseph A, Jin L, et al. Urinary and tissue levels of scatter factor in transitional cell carcinoma of bladder. *J Urol*. 1997;157(1):72–78. doi:10.1016/S0022-5347(01)65286-8
32. Millis SZ, Bryant D, Basu G, et al. Molecular profiling of infiltrating urothelial carcinoma of bladder and nonbladder origin. *Clin Genitourin Cancer*. 2015;13(1):e37–e49. doi:10.1016/j.clgc.2014.07.010
33. Iyer G, Al-Ahmadie H, Schultz N, et al. Prevalence and co-occurrence of actionable genomic alterations in high-grade bladder cancer. *J Clin Oncol*. 2013;31(25):3133–3140. doi:10.1200/JCO.2012.46.5740
34. Lee YH, Apolo AB, Agarwal PK, Bottaro DP. Characterization of HGF/Met signaling in cell lines derived from urothelial carcinoma of the bladder. *Cancers*. 2014;6(4):2313–2329. doi:10.3390/cancers6042313
35. Parr C, Jiang WG. Expression of hepatocyte growth factor/scatter factor, its activator, inhibitors and the c-Met receptor in human cancer cells. *Int J Oncol*. 2001;19(4):857–863.
36. Yeh CY, Shin SM, Yeh HH, et al. Transcriptional activation of the Axl and PDGFR- α by c-Met through a ras- and Src-independent mechanism in human bladder cancer. *BMC Cancer*. 2011;11(1):139. doi:10.1186/1471-2407-11-139

37. Kim YW, Yun SJ, Jeong P, et al. The c-Met network as novel prognostic marker for predicting bladder cancer patients with an increased risk of developing aggressive disease. *PLoS One*. 2015;10(7):e0134552. doi:10.1371/journal.pone.0134552
38. Cheng HL, Trink B, Tzai TS, et al. Overexpression of c-met as a prognostic indicator for transitional cell carcinoma of the urinary bladder: a comparison with p53 nuclear accumulation. *J Clin Oncol*. 2002;20(6):1544–1550. doi:10.1200/JCO.2002.20.6.1544
39. Sanchez-Carbajo M, Socci ND, Lozano JJ, Haab BB, Cordon-Cardo C. Profiling bladder cancer using targeted antibody arrays. *Am J Pathol*. 2006;168(1):93–103. doi:10.2353/ajpath.2006.050601
40. Sim WJ, Iyengar PV, Lama D, et al. c-Met activation leads to the establishment of a TGFbeta-receptor regulatory network in bladder cancer progression. *Nat Commun*. 2019;10(1):4349. doi:10.1038/s41467-019-12241-2
41. Huff JL, Jelinek MA, Borgman CA, Lansing TJ, Parsons JT. The protooncogene c-sea encodes a transmembrane protein-tyrosine kinase related to the Met/hepatocyte growth factor/scatter factor receptor. *Proc Natl Acad Sci U S A*. 1993;90(13):6140–6144. doi:10.1073/pnas.90.13.6140
42. Lu TJ, Lu TL, Su IJ, Lai MD. Tyrosine kinase expression profile in bladder cancer. *Anticancer Res*. 1997;17(4a):2635–2637.
43. Cheng HL, Liu HS, Lin YJ, et al. Co-expression of RON and MET is a prognostic indicator for patients with transitional-cell carcinoma of the bladder. *Br J Cancer*. 2005;92(10):1906–1914. doi:10.1038/sj.bjc.6602593
44. Black PC, Brown GA, Inamoto T, et al. Sensitivity to epidermal growth factor receptor inhibitor requires E-cadherin expression in urothelial carcinoma cells. *Clin Cancer Res*. 2008;14(5):1478–1486. doi:10.1158/1078-0432.CCR-07-1593
45. Shieh YS, Lai CY, Kao YR, et al. Expression of axl in lung adenocarcinoma and correlation with tumor progression. *Neoplasia*. 2005;7(12):1058–1064. doi:10.1593/neo.05640
46. Choi YJ, Kim JH, Rho JK, et al. AXL and MET receptor tyrosine kinases are essential for lung cancer metastasis. *Oncol Rep*. 2017;37(4):2201–2208. doi:10.3892/or.2017.5482
47. Miyata Y, Sagara Y, Kanda S, Hayashi T, Kanetake H. Phosphorylated hepatocyte growth factor receptor/c-Met is associated with tumor growth and prognosis in patients with bladder cancer: correlation with matrix metalloproteinase-2 and -7 and E-cadherin. *Hum Pathol*. 2009;40(4):496–504. doi:10.1016/j.humpath.2008.09.011
48. Mukae Y, Miyata Y, Nakamura Y, et al. Pathological roles of c-Met in bladder cancer: association with cyclooxygenase-2, heme oxygenase-1, vascular endothelial growth factor-A and programmed death ligand 1. *Oncol Lett*. 2020;20(1):135–144. doi:10.3892/ol.2020.11540
49. Miyata Y, Asai A, Mitsunari K, et al. Met in urological cancers. *Cancers*. 2014;6(4):2387–2403. doi:10.3390/cancers6042387
50. Hofner T, Macher-Goeppinger S, Klein C, et al. Development and characteristics of preclinical experimental models for the research of rare neuroendocrine bladder cancer. *J Urol*. 2013;190(6):2263–2270. doi:10.1016/j.juro.2013.06.053
51. Yang B, Zhou L, Peng B, Sun Z, Dai Y, Zheng J. In vitro comparative evaluation of recombinant growth factors for tissue engineering of bladder in patients with neurogenic bladder. *J Surg Res*. 2014;186(1):63–72. doi:10.1016/j.jss.2013.07.044
52. Burgues O, Ferrer J, Navarro S, Ramos D, Botella E, Llobart-Bosch A. Hepatoid adenocarcinoma of the urinary bladder. *Virchows Archiv*. 1999;435(1):71–75. doi:10.1007/s004280050398
53. Li B, Kanamaru H, Noriki S, Fukuda M, Okada K. Differential expression of hepatocyte growth factor in papillary and nodular tumors of the bladder. *Int J Urol*. 1998;5(5):436–440. doi:10.1111/j.1442-2042.1998.tb00383.x
54. McNeil BK, Sorbellini M, Grubb RL 3rd, et al. Preliminary evaluation of urinary soluble Met as a biomarker for urothelial carcinoma of the bladder. *J Transl Med*. 2014;12:199. doi:10.1186/1479-5876-12-199
55. Yamamoto N, Mammadova G, Song RX, Fukami Y, Sato K. Tyrosine phosphorylation of p145met mediated by EGFR and Src is required for serum-independent survival of human bladder carcinoma cells. *J Cell Sci*. 2006;119(Pt 22):4623–4633. doi:10.1242/jcs.03236
56. Huaqi Y, Caipeng Q, Qiang W, Yiqing D, Tao X. The role of SOX18 in bladder cancer and its underlying mechanism in mediating cellular functions. *Life Sci*. 2019;232:116614. doi:10.1016/j.lfs.2019.116614
57. Shintani T, Kusuhara Y, Daizumoto K, et al. The involvement of hepatocyte growth factor-MET-matrix metalloproteinase 1 signaling in bladder cancer invasiveness and proliferation. Effect of the MET inhibitor, cabozantinib (XL184), on bladder cancer cells. *Urology*. 2017;101:169e7–170e13. doi:10.1016/j.urology.2016.12.006
58. Yamasaki K, Mukai S, Nagai T, et al. Matriptase-induced phosphorylation of MET is significantly associated with poor prognosis in invasive bladder cancer; an immunohistochemical analysis. *Int J Mol Sci*. 2018;19(12):12. doi:10.3390/ijms19123708
59. Xu X, Chen H, Lin Y, et al. MicroRNA-409-3p inhibits migration and invasion of bladder cancer cells via targeting c-Met. *Mol Cells*. 2013;36(1):62–68. doi:10.1007/s10059-013-0044-7
60. Hu Z, Lin Y, Chen H, et al. MicroRNA-101 suppresses motility of bladder cancer cells by targeting c-Met. *Biochem Biophys Res Commun*. 2013;435(1):82–87. doi:10.1016/j.bbrc.2013.04.042
61. Xu X, Zhu Y, Liang Z, et al. c-Met and CREB1 are involved in miR-433-mediated inhibition of the epithelial-mesenchymal transition in bladder cancer by regulating Akt/GSK-3beta/Snail signaling. *Cell Death Dis*. 2016;7:e2088. doi:10.1038/cddis.2015.274
62. Li J, Xu X, Meng S, et al. MET/SMAD3/SNAIL circuit mediated by miR-323a-3p is involved in regulating epithelial-mesenchymal transition progression in bladder cancer. *Cell Death Dis*. 2017;8(8):e3010. doi:10.1038/cddis.2017.331
63. Qiu J, Zeng FR, Fang Y, Li J, Xiao SY. Increased miR-323a induces bladder cancer cell apoptosis by suppressing c-Met. *Kaohsiung J Med Sci*. 2019;35(9):542–549. doi:10.1002/kjm2.12091
64. Chiyomaru T, Seki N, Inoguchi S, et al. Dual regulation of receptor tyrosine kinase genes EGFR and c-Met by the tumor-suppressive microRNA-23b/27b cluster in bladder cancer. *Int J Oncol*. 2015;46(2):487–496. doi:10.3892/ijo.2014.2752
65. Li J, Ying Y, Xie H, et al. Dual regulatory role of CCNA2 in modulating CDK6 and MET-mediated cell-cycle pathway and EMT progression is blocked by miR-381-3p in bladder cancer. *FASEB J*. 2018;33(1):1374–1388. doi:10.1096/fj.201800667R
66. Yu G, Yao W, Xiao W, Li H, Xu H, Lang B. MicroRNA-34a functions as an anti-metastatic microRNA and suppresses angiogenesis in bladder cancer by directly targeting CD44. *CR*. 2014;33:779. doi:10.1186/s13046-014-0115-4
67. Huang B, Zhai W, Hu G, et al. MicroRNA-206 acts as a tumor suppressor in bladder cancer via targeting YRDC. *Am J Transl Res*. 2016;8(11):4705–4715.
68. Wiklund ED, Bramsen JB, Hulf T, et al. Coordinated epigenetic repression of the miR-200 family and miR-205 in invasive bladder cancer. *Int J Cancer*. 2011;128(6):1327–1334. doi:10.1002/ijc.25461
69. Liu L, Qiu M, Tan G, et al. miR-200c inhibits invasion, migration and proliferation of bladder cancer cells through down-regulation of BMI-1 and E2F3. *J Transl Med*. 2014;12:305. doi:10.1186/s12967-014-0305-z

70. Zhen Q, Liu J, Gao L, et al. MicroRNA-200a targets EGFR and c-Met to inhibit migration, invasion, and gefitinib resistance in non-small cell lung cancer. *Cytogenet Genome Res.* **2015**;146(1):1–8. doi:10.1159/000434741
71. Hong JH, Roh KS, Suh SS, et al. The expression of microRNA-34a is inversely correlated with c-MET and CDK6 and has a prognostic significance in lung adenocarcinoma patients. *Tumour Biol.* **2015**;36(12):9327–9337. doi:10.1007/s13277-015-3428-9
72. Dang Y, Luo D, Rong M, Chen G. Underexpression of miR-34a in hepatocellular carcinoma and its contribution towards enhancement of proliferating inhibitory effects of agents targeting c-MET. *PLoS One.* **2013**;8(4):e61054. doi:10.1371/journal.pone.0061054
73. Zheng Z, Yan D, Chen X, et al. MicroRNA-206: effective inhibition of gastric cancer progression through the c-Met pathway. *PLoS One.* **2015**;10(7):e0128751. doi:10.1371/journal.pone.0128751
74. Sun C, Liu Z, Li S, et al. Down-regulation of c-Met and Bcl2 by microRNA-206, activates apoptosis, and inhibits tumor cell proliferation, migration and colony formation. *Oncotarget.* **2015**;6(28):25533–25574. doi:10.18632/oncotarget.4575
75. Nie Z, Chen M, Wen X, et al. Endoplasmic reticulum stress and tumor microenvironment in bladder cancer: the missing link. *Front Cell Dev Biol.* **2021**;9:683940. doi:10.3389/fcell.2021.683940
76. Tao L, Qiu J, Slavin S, et al. Recruited T cells promote the bladder cancer metastasis via up-regulation of the estrogen receptor beta/IL-1/c-MET signals. *Cancer Lett.* **2018**;430:215–223. doi:10.1016/j.canlet.2018.03.045
77. Bouattour M, Raymond E, Qin S, et al. Recent developments of c-Met as a therapeutic target in hepatocellular carcinoma. *Hepatology.* **2018**;67(3):1132–1149. doi:10.1002/hep.29496
78. Pasquini G, Giaccone G. C-MET inhibitors for advanced non-small cell lung cancer. *Expert Opin Investig Drugs.* **2018**;27(4):363–375. doi:10.1080/13543784.2018.1462336
79. Marandino L, Raggi D, Calareso G, et al. Cabozantinib plus durvalumab in patients with advanced urothelial carcinoma after platinum chemotherapy: safety and preliminary activity of the open-label, single-arm, phase 2 ARCADIA trial. *Clin Genitourin Cancer.* **2021**;19(5):457–465. doi:10.1016/j.clgc.2021.04.001

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