REVIEW ARTICLE

Clinical significance of circulating tumor cells in blood from patients with gastric cancer

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Abstract

Circulating tumor cells (CTC) have been focused on as a target for detecting occult tumors, predicting therapeutic responses and prognoses, and monitoring postoperative recurrence in the clinical management of patients with various malignancies, including gastric cancer. Recent advances in molecular diagnostic tools have contributed to high sensitivity and specificity for the detection of CTC. A conspicuous disparity exists in the incidence of CTC among studies. However, a close relationship has been reported between positivity for CTC and well-known prognostic clinicopathological factors including depth of tumor invasion, lymph node metastasis, stage, and lymphatic and venous invasion in patients with gastric cancer. According to most studies published on the clinical impact of CTC, the presence of CTC negatively affects the prognosis of patients with gastric cancer. Moreover, the study of CTC based on a meta-analysis demonstrated their importance as a poor prognostic indicator. In clinical management, pre- and post-therapeutic monitoring of CTC using liquid biopsy may be useful for early detection of subclinical patients or disease recurrence, prediction of tumor progression, and administrative control of adjuvant chemotherapy. Although their functional properties remain unclear, molecular profiling of CTC may contribute to the development of personalized treatment that effectively inhibits tumor progression in patients with advanced gastric cancer. We herein review the clinical significance of CTC as a promising blood marker and therapeutic target in patients with gastric cancer.

KEYWORDS
circulating tumor cell, gastric cancer, liquid biopsy, prognosis, tumor progression

1 | INTRODUCTION

Gastric cancer is the fourth most common malignancy in the world and the second leading cause of cancer death.[1] Advances in

diagnostic tools and techniques have resulted in a high incidence of patients with early gastric cancer. Endoscopic resection techniques such as endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) have been extensively carried out on selected

patients with early gastric tumors and free of lymph node metastasis. However, there are clinical limits for accurate tumor detection and diagnoses using preoperative examinations such as endoscopy, endoscopic ultrasonography (EUS), computed tomography (CT), and positron emission tomography-computed tomography.[2–4] Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are now commonly used as established serum markers in the clinical management of patients with gastric cancer. Nevertheless, the sensitivity and specificity of detecting patients with early gastric cancer are clinically insufficient and few candidate blood markers have clinical utility for overcoming these key problems.[5]

Five-year survival rates of patients with International Union Against Cancer stages IIIA, IIIB, and IV gastric cancers are 30.8–54.0%, 16.1–36.5%, and 9.2–23.9%, respectively.[6,7] Furthermore, advances in chemotherapy have contributed to improvements in the prognosis of patients with advanced gastric cancer.[8] However, difficulties are associated with predicting tumor responses to chemotherapy and disease recurrence after surgery in patients with advanced stage cancer. Although blood monitoring using serum CEA and CA19-9 has been conventionally introduced for the managements of patients with gastric cancer, serum levels of conventional blood markers do not necessarily coincide with tumor behavior.[9] Therefore, surrogate blood markers are needed clinically to monitor tumor aggressiveness in real time. Moreover, liquid biopsy using blood specimens has the clinical benefit of being a simple and repeatable sampling tool.

In 1869, the presence of circulating tumor cells (CTC) in peripheral blood was proposed by Ashworth.[10] CTC are generally isolated from primary tumors or metastatic sites and these cells flow in the bloodstream of patients with malignancies.[11] To date, CTC have been focused on as a target for detecting occult tumors, predicting therapeutic responses and prognoses, and monitoring postoperative recurrence in the clinical management of patients with various malignancies, including gastric cancer.[12,13] Non-invasive liquid biopsy has enabled CTC to be characterized and their numbers assessed. Therefore, the assessment of CTC using liquid biopsy may support new perspectives for the diagnosis and treatment of patients with gastric cancer.

The present review will focus on the clinical significance of CTC as an important therapeutic target in gastric cancer, including recent topics.

2 | DETECTION OF CTC

Many investigators have reported several approaches for the detection of CTC in patients with gastric cancer. Representative detection methods have been classified into two categories: polymerase chain reaction (PCR)-based methods and cytometric-based methods.

Reverse transcription-polymerase chain reaction (RT-PCR) is one of the PCR-based methods. A RT-PCR assay permits the molecular detection of CTC by assessing the mRNA expression of tumor-associated markers. Moreover, quantitative RT-PCR (qRT-PCR) is a promising tool for quantifying mRNA copy numbers. The greatest advantage of the RT-PCR assay is its high sensitivity for the molecular detection of CTC. We previously investigated its sensitivity using an *in vitro* model system with serially diluted gastric tumor cells mixed with peripheral blood cells from healthy donors.[14] The findings of this cell spiking study showed that the RT-PCR assay detected 10 tumor cells/10^7 donor-derived peripheral blood cells. Additionally, a recent RT-PCR system has the ability to assess multiple gene expressions for the detection of CTC in one run. However, several investigators identified some limitations in the clinical application of RT-PCR assays to the detection of CTC.[15,16] False-positive results associated with RT-PCR may be yielded as a result of the illegitimate expression of targeted genes by normal cells and epidermal contamination in blood collecting or processing.[15] Furthermore, false-negative results may be obtained as a result of the heterogeneous expression of the targeted markers.[16] Further studies are needed in order to resolve the problems associated with the detection of CTC using RT-PCR-based methods.

Table 1 summarizes studies reported since 2001 on CTC assessed using PCR-based methods in blood specimens from patients with gastric cancer.[16–34] In RT-PCR assays, cytokeratin (CK) and CEA are commonly selected as gene target markers for CTC. Both genes are epithelial-specific antigens that are expressed in the normal cells of gastrointestinal tissues or most tumor cells, including gastric cancer.[35,36] Recent studies reported the clinical utility of new molecular markers for RT-PCR assays to detect CTC in the peripheral blood of patients with gastric cancer.[37] Survivin has been attracting attention as a promising blood marker for CTC in gastric cancer.[25,26,32] Survivin is a member of the inhibitor of apoptosis gene family and plays an important role in tumor progression.[38] It has been shown to control tumor apoptosis, promote proliferation, and enhance angiogenesis by a vascular endothelial growth factor signaling pathway.[39,40] Furthermore, survivin is overexpressed in the tumor cells of various malignant neoplasms, including gastric cancer.[41] Liu *et al.*, in a meta-analysis of 1365 patients with gastric cancer from 16 eligible studies, demonstrated a close relationship between strong survivin expression in primary tumor sites and a poor prognosis.[42] Consequently, survivin has potential as an indicator for monitoring CTC in patients with gastric cancer. In contrast, we recently reported the clinical availability of B7-H3 and B7-H4 as blood biomarkers of CTC in patients with gastric cancer.[28,31] These molecules are members of the B7 family and regulate T-cell-mediated immune responses.[43,44] The signaling pathway between B7 family members and their CD28 receptors on activated T cells has a marked impact on the immune surveillance system.[43,44] Although B7-H3 is considered to have two opposing characteristics as a co-inhibitory or co-stimulatory mediator in T-cell-mediated immunity, B7-H4 is known to function as a negative modulator of immune responses.[43] Immunohistochemical studies showed that B7-H3 and B7-H4 were abundantly expressed in the primary tumor cells of patients with gastric cancer.[45,46] Accordingly, these immune checkpoint molecules have potential as CTC-targeted markers to predict tumor responses to chemotherapy and prognoses in the clinical

TABLE 1 PCR-based studies on circulating tumor cells in pre- and post-operative blood specimens obtained from patients with gastric cancer

Year	Study	No. patients	Median age, years (range)	UICC stage	Method	Marker	Blood volume for tests (mL)	mRNA levels as the cut-off value	No. of healthy donors	Specificity (%)	No. patients with CTC (%)	5-year survival (High/positive vs Low/negative)	P-value	Prognostic significance
2001	Miyazono et al.[17]	57	64.4	I–IV	RT-PCR	CEA	5	–	15	100.0	21 (36.8)	–	–	–
2003	Sumikura et al.[18]	106	63.3 (30–87)	–	RT-PCR	CEA	5	Undescribed	–	100.0	43 (41)	–	–	–
2005	Ikeguchi et al.[19]	59	66.3 (26–86)	I–IV	qRT-PCR	CEA	1.5	–	15	100.0	27 (45.8) †after surgery	–	0.744	No
2005	Illert et al.[20]	70	69 (41–87)	I–IV	RT-PCR	CK-20	9	–	–	–	28 (40)	42.8% vs 74.8%	0.0363	Yes
2005	Seo et al.[21]	46	58 (31–78)	I–III	RT-PCR	CEA	–	–	13	100.0	24 (52.2)	–	–	–
2006	Uen et al.[16]	52	60 (34–84)	I–IV	RT-PCR	c-Met	4	–	36	94.4	32 (61.5)	–	0.0178	–
2006	Uen et al.[16]	52	60 (34–84)	I–IV	RT-PCR	MUC-1	4	–	36	91.7	37 (71.2)	–	0.0352	–
2006	Wu et al.[22]	64	60.5 (36–84)	I–IV	High-throughput colorimetric membrane-array	hTERT	4	–	80	82.5	52 (81.3)	All 4 markers (+) vs all 4 markers (–)	0.0223	Yes
2006	Wu et al.[22]	64	60.5 (36–84)	I–IV	High-throughput colorimetric membrane-array	CK-19	4	–	80	85.0	50 (78.1)	–	–	–
2006	Wu et al.[22]	64	60.5 (36–84)	I–IV	High-throughput colorimetric membrane-array	CEA	4	–	80	76.3	53 (82.8)	–	–	–
2006	Wu et al.[22]	64	60.5 (36–84)	I–IV	High-throughput colorimetric membrane-array	MUC-1	4	–	80	83.8	54 (84.4)	–	–	–
2008	Koga et al.[23]	69	65.9	I–IV	qRT-PCR	CK-19	10	103	14	100.0	8 (11.6)	50.0% vs 79.0%	0.0347	–
2008	Koga et al.[23]	69	65.9	I–IV	qRT-PCR	CK-20	10	20	14	100.0	10 (15.5)	51.9% vs 78.9%	0.049	–
2008	Mimori et al.[24]	810	63.0	I–IV	qRT-PCR	MT1-MMP	1	–	29	–	185 (22.8)	–	–	–
2008	Yie et al.[25]	55	58 (26–77)	I–IV	qRT-PCR	Survivin	2	1.07	86	100.0	25 (45.4)	–	0.026	Yes

(Continues)

TABLE 1 (Continued)

Year	Study	No. patients	Median age, years (range)	UICC stage	Method	Marker	Blood volume for tests (mL)	mRNA levels as the cut-off value	Patients vs Healthy donors		No. patients with CTC (%)	5-year survival (High/positive vs Low/negative)	P-value	Prognostic significance
									No. of healthy donors	Specificity (%)				
2009	Bertazza et al.[26]	70	68 (28–90)	I–IV	qRT-PCR	Survivin	6	–	–	–	69 (98.6)	14 mo vs 41 mo (median OS)	0.036	Yes
2009	Kita et al.[27]	846	61.5 (27–87)	I–IV	qRT-PCR	uPAR	1	–	25	100.0	404 (47.8)	–	–	–
2010	Arigami et al.[28]	94	68 (35–87)	I–IV	qRT-PCR	B7-H4	5	0	22	100.0	71 (75.5)	60.4% vs 87.2%	0.04	–
2010	Qiu et al.[29]	123	59 (28–84)	I–IV	qRT-PCR	CEA	5	100	30	100.0	45 (36.6)	43.9% vs 74.1% (3-year DFS)	0.001	Yes
2010	Saad et al.[30]	30	55 (31–72)	I–IV	qRT-PCR	CK-18	2	–	–	–	15 (50.0)	15.2 mo vs 35.9 mo (mean DFS)	<0.001	Yes
2011	Arigami et al.[31]	95	68 (35–87)	I–IV	qRT-PCR	B7-H3	5	–	21	90.0	77 (81)	57.1% vs 76.4%	0.02	Yes
2011	Cao et al.[32]	98	–	I–IV	qRT-PCR	Survivin	6	1.25	30	100.0	45 (45.9)	84.3% vs 53.1% (3-year DFS)	<0.001	Yes
2013	Arigami et al.[33]	93	68 (35–87)	I–IV	qRT-PCR	STC-2	5	–	22	–	43 (46.2)	58.4% vs 80.9%	0.014	–
2013	Kang et al.[34]	118	–	I–IV	qRT-PCR	hTERT	6	0.18	58	87.0	66.0	33.4% vs 54.2%	<0.001	Yes

CEA, carcinoembryonic antigen; CK, cytokeratin; c-Met, hepatocyte growth factor receptor; CTC, circulating tumor cells; DFS, disease-free survival; hTERT, human telomerase reverse transcriptase; mo, months; MT1-MMP, membrane-type-1 matrix metalloproteinase; MUC, mucin; OS, overall survival; qRT-PCR, quantitative reverse-transcription–polymerase chain reaction; RT-PCR, reverse transcription–polymerase chain reaction; STC, stanniocalcin; UICC, International Union Against Cancer; uPAR, urokinase-type plasminogen activator receptor; –, undescribed.

management of patients with gastric cancer. In the near future, the advent of new blood markers is anticipated for the development of an RT-PCR-based approach to monitor CTC using liquid biopsy.

Table 2 summarizes studies reported since 2007 on CTC assessed by cytometric-based methods using blood specimens from patients with gastric cancer.[47–55] The CellSearch system (Janssen Diagnostics, Raritan, NJ, USA) is one of the representative CTC detection assays using a cytometric-based method. This system has been approved by the American Food and Drug Administration (FDA) as a diagnostic tool for detecting CTC in patients with metastatic breast, colorectal, and prostate cancer.[56] In the CellSearch system, CTC are captured based on enrichment using antibody-coated magnetic beads with epithelial-cell adhesion molecules and discrimination using fluorescently labeled antibodies against CK and CD45. We investigated the presence or absence of CTC in peripheral blood cells from patients with gastric cancer using the CellSearch system.[51] The findings obtained showed that CTC were morphologically detected using the CellSearch system, particularly in patients with unresectable advanced or recurrent gastric cancers. Recently, a new size-based separation system has been developed for enrichment and cultivation of CTC.[55] The greatest appeal of this system is that it can easily separate viable CTC from peripheral blood. Moreover, we can assess functional properties by culture of enriched viable CTC. Accordingly, the size-based filtration system may be focused as a novel tool for isolating viable CTC.

3 | INCIDENCE OF CTC

The incidence of CTC ranges between 11.6% and 98.6% in studies based on PCR-based methods (Table 1).[16-34] The gap observed in the incidence of CTC among each study may be as a result of differences in the clinicopathological backgrounds of enrolled patients, target markers, blood volumes assessed by PCR, and the cut-off values for mRNA levels. However, the incidence of CTC ranged between 36.6% and 52.2% in five RT-PCR studies targeting CEA, which is one of the conventional PCR markers for the detection of CTC.[17–19,21,29] According to studies assessed in this review article, positive rates of serum CEA ranged between 24.3% and 26.3%.[21,29,31] These results suggest that sensitivity of CEA mRNA levels is higher than those of serum CEA levels. Furthermore, in a large-scale study on 846 patients with stages I–IV gastric cancer, Kita *et al.*[27] reported that positivity for CTC using a qRT-PCR assay with the urokinase-type plasminogen activator receptor was 47.8% (404/846). In contrast, specificity ranged between 76.3% and 100% in these studies.[16–19,21–23,25,27–29,31,32,34] These findings indicate that PCR-based methods have clinical availability for discriminating between healthy donors and patients with gastric cancer.

In a study on 57 patients with stages I–IV gastric cancer, Miyazono *et al.* reported that the positive rates for CEA mRNA expression before and after gastrectomy were 8.8% (5/57) and 33.3% (19/57), respectively.[17] Moreover, they demonstrated a close relationship between the presence or absence of CEA mRNA

expression and disease recurrence, such as liver metastases.[17] The findings of this study suggest that surgical maneuvers enhance the metastatic process from the detachment of primary tumor cells into the systemic circulation in patients with gastric cancer. Therefore, sequential evaluations based on pre- and postoperative PCR-based assays are anticipated to monitor disease recurrence in patients with gastric cancer.

According to studies based on cytometric-based methods, the incidence of CTC ranges between 10.8% and 79.5% (Table 2).[47–55] The CellSearch system was previously used to detect CTC in four (44.4%) out of nine studies using cytometric-based assays. The findings of these CellSearch studies demonstrated that the incidence of CTC ranged between 10.8% and 18.4% and between 32.7% and 60.2% in patients with stages I–IV and stage IV, respectively.[48,49,51,53] These findings indicate that incidence of CTC is higher in patients with than in those without distant metastasis.

The incidences of CTC as determined by PCR assay and cytometric-based methods in patients with stage I were 12.5–58.3% and 27.5–69.2%, respectively.[16,17,20,22,24,25,29,47,50,51,55] These results suggest that patients with early tumors tend to display a low incidence of CTC compared with those with advanced tumors. Consequently, the clinical significance of CTC in early gastric cancer remains controversial at present. However, according to a systematic review, positive rates of serum CEA and CA 19-9 in patients with stage I were 13.7% and 9.0%, respectively.[5] These findings indicate that PCR-based or cytometric-based tools for CTC have a high sensitivity for detecting early tumors in comparison with conventional serum CEA or CA 19-9 markers. These abilities will assist clinical management in patients with early gastric cancer.

4 | RELATIONSHIP BETWEEN CLINICOPATHOLOGICAL FACTORS AND CTC

To date, many investigators have reported a close relationship between positivity for CTC and well-known prognostic factors, such as tumor size, depth of tumor invasion, lymph node metastasis, stage, lymphatic and venous invasion.[16–34,47–55] In a study on 94 patients with gastric cancer, we reported that the presence or absence of CTC evaluated by a qRT-PCR assay for the expression of B7-H4 correlated with the depth of tumor invasion, lymph node metastasis, stage, lymphatic invasion, and venous invasion ($P = 0.006$, $P = 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.01$, respectively).[28] In a study on 148 gastric cancer patients receiving surgical treatment, Uenosono *et al.*[51] reported that CTC assessed by the CellSearch system correlated with the depth of tumor invasion, lymph node metastasis, distant metastasis, stage, lymphatic invasion, and venous invasion ($P = 0.009$, $P < 0.0001$, $P = 0.012$, $P = 0.0002$, $P = 0.0003$, and $P = 0.006$, respectively). These findings suggest that blood assessments for the detection of CTC have the clinical power to predict tumor progression and malignant aggressiveness in patients with gastric cancer.

TABLE 2 Cytometric-based studies on circulating tumor cells in blood specimens obtained from patients with gastric cancer

Year	Study	No. patients	Mean age, years (range)	UICC stage	Enrichment	Detection	Marker	Blood volume for tests (mL)	No. CTC as the cut-off value	No. healthy donors	Specificity (%)	No. patients with CTC (%)	5-year survival (High/positive vs Low/negative)	P-value	Prognostic significance
2007	Pituch-Noworolska et al.[47]	57	55.0	I–IV	FACS	ICC	CK-8, CK-18, CK-19	Undescribed	≥3	–	–	31 (54.4%)	–	≥0.05	No
2008	Hiraiwa et al.[48]	27	–	IV	CellSearch	ICC	EpCAM, CK-8, CK-18, CK-19	7.5	≥2	41	100	15 (55.6%)	–	0.039	Yes
2010	Matsusaka et al.[49]	52	62 (24–78)	IV	CellSearch	ICC	EpCAM, CK-8, CK-18, CK-19	7.5	≥4	–	–	17 (32.7%)	3.5 mo vs 11.7 mo (median OS)	<0.001	Yes
2012	Ito et al.[50]	65	58.8 (33–76)	I–IV	TelomeScan (GFP)	ICC	EpCAM	7.5	≥5	–	–	–	–	0.0021	–
2013	Uenosono et al.[51]	148	–	I–IV	CellSearch	ICC	EpCAM, CK-8, CK-18, CK-19	7.5	≥1	15	100	16 (10.8%)	–	<0.0001	Yes
2013	Uenosono et al.[51]	103	–	IV	CellSearch	ICC	EpCAM, CK-8, CK-18, CK-19	7.5	≥1	15	100	62 (60.2%)	248 days vs 582 days (median OS)	0.0044	–
2015	Li et al.[52]	44	56 (25–87)	I–IV	CanPatrol	RNA-ISH	KRT-8, KRT-18, KRT-19, EpCAM	5	≥1	10	100	35 (79.5%)	–	–	–
2015	Okabe et al.[53]	136	66.0	I–IV	CellSearch	ICC	EpCAM, CK-8, CK-18, CK-19	7.5	≥1	–	–	25 (18.4%)	–	0.016	Yes
2015	Yuan et al.[54]	31	62 (35–78)	II–IV	FACS	ICC	CD44	2	–	–	–	14 (45.2%)	–	–	–
2016	Kolostova et al.[55]	22	–	I–IV	MetaCell	ICC	CK-18, CK-19, CK-20, CK-7, EpCAM, MUC-1, HER-2, EGFR	8	–	–	–	13 (59%)	–	–	–

CanPatrol, SurExam, Guangzhou, China; CellSearch, Janssen Diagnostics, Raritan, NJ, USA; MetaCell, MetaCell s.r.o., Ostrava, Czech Republic; TelomeScan, Oncolys BioPharma Inc., Tokyo, Japan. CK, cytokeratin; CTC, circulating tumor cells; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; FACS, fluorescence-activated cell sorting; GFP, green fluorescent protein; HER, human epidermal growth factor receptor; ICC, immunocytochemistry; ISH, in situ hybridization; KRT, keratin; mo, months; MUC, mucin; OS, overall survival; UICC, International Union Against Cancer; –, undescribed.

5 | PROGNOSTIC IMPACT OF CTC

A large number of studies have investigated the clinical significance of CTC in patients with various malignancies, such as esophageal cancer, colorectal cancer, and pancreatic cancer.[57–59] Similarly, many investigators have assessed the prognostic impact of CTC in patients with gastric cancer, and most studies have suggested a close relationship between the presence of CTC and a poor prognosis.[16–34,47–55]

In a qRT-PCR study of 123 gastric cancer patients with stages I–IV, Qiu et al.[29] reported that 3-year disease-free survival (DFS) rates in patients who were positive or negative for CEA mRNA were 43.9% and 74.1%, respectively ($P = 0.001$). A multivariate analysis identified CEA mRNA positivity as an independent prognostic factor ($P = 0.02$).[29] Moreover, the sensitivity and specificity of CEA mRNA expression for predicting disease recurrence were 56.8% and 74.7%, respectively.[29] However, the sensitivity and specificity of the serum CEA status were 31.8% and 79.7%, respectively.[29] They concluded that the presence or absence of CTC by qRT-PCR detection for CEA mRNA was a promising predictor for disease recurrence in patients with gastric cancer.[29] In contrast, in a qRT-PCR study on 59 gastric cancer patients with stages I–IV, Ikeguchi and Kaibara reported that there were no significant differences in overall survival (OS) rates among patients with or without CEA mRNA expression ($P = 0.744$).[19] In that study, CTC were assessed using a qRT-PCR assay on blood specimens after gastrectomy.[19] The findings obtained indicated that CTC were destroyed shortly after gastrectomy.[19] They hypothesized host-related immunological defense mechanisms as one of the reasons for these findings.[19]

Cao et al.[32] focused on survivin as a novel blood marker of CTC in a qRT-PCR study on 98 gastric cancer patients with stages I–IV.[32] They reported that 3-year DFS rates in patients who were positive or negative for survivin mRNA were 53.1% and 84.3%, respectively ($P < 0.001$). Furthermore, a multivariate analysis identified the status of survivin mRNA as an independent prognostic factor ($P < 0.001$).[32] In a study on 55 gastric cancer patients with stages I–IV, Yie et al. showed that the specificity, sensitivity, and accuracy of survivin-expressing CTC for predicting disease recurrence were 100%, 100%, and 84.6%, respectively.[25] Bertazza et al.[26] compared survivin with other blood markers, such as CEA, CK-19, and vascular endothelial growth factor (VEGF), in order to select the most suitable mRNA marker for predicting clinical outcomes in a qRT-PCR study on 70 gastric cancer patients with stages I–IV. Univariate and multivariate analyses identified only the status of survivin mRNA expression as an independent prognostic factor.[26] These studies suggest that qRT-PCR assays for survivin expression support the planning of strategic treatment, particularly in patients with advanced gastric cancer who occasionally develop disease recurrence.

In recent years, immunotherapy has begun to attract attention as a drug treatment for patients with several malignant neoplasms.[60] According to the findings of a phase 1b trial on immunotherapy for patients with advanced gastric cancer, the anti-programmed cell death protein 1 (PD-1) antibody pembrolizumab was found to be safe and exerted antitumor effects.[61] Although PD-1 is one of the representative molecules for immune checkpoints, we focused on other immune checkpoint molecules, such as B7-H3 and B7-H4.[28,31] We investigated the prognostic impact of B7-H3 and B7-H4 in the peripheral blood of patients with stages I–IV gastric cancer.[28,31] In a qRT-PCR-based study on 95 patients with gastric cancer, 5-year OS rates in patients who strongly or weakly expressed B7-H3 were 57.1% and 76.4%, respectively ($P = 0.02$).[31] Additionally, multivariate analyses selected the status of B7-H3 expression as an independent prognostic factor ($P = 0.046$).[31] In a B7-H4 study on 94 patients with gastric cancer, 5-year OS rates in patients who were positive or negative for mRNA expression were 60.4% and 87.2%, respectively ($P = 0.04$).[28] Our findings propose that the evaluation of B7-H3 and B7-H4 mRNA expression in blood specimens is useful as a CTC-associated tool for predicting the prognosis of patients with gastric cancer.

In a CellSearch study on 136 gastric cancer patients with stages I–IV, Okabe et al.[53] reported that progression-free survival was significantly shorter in patients with than in those without CTC ($P = 0.016$). All other studies based on the CellSearch system demonstrated that CTC had an influence on prognosis.[48,49,51] These findings suggest that the presence or absence of CTC has an effect on the prognosis of patients with gastric cancer, even in cytometric-based methods.

In a meta-analysis on 19 studies regarding CTC from patients with gastric cancer, Wang et al. reported that positivity for CTC correlated with poor OS (HR: 2.42, 95% CI: 1.94–3.02, $P < 0.001$).[62] Although further clinical studies including a molecular analysis of CTC are needed to reach definitive conclusions on this matter, it is highly likely that CTC negatively affect the prognosis of patients with gastric cancer.

6 | FUTURE PERSPECTIVES FOR CTC AS A PROMISING BLOOD MARKER AND THERAPEUTIC TARGET

The advent of new blood markers for the detection of CTC is anticipated in the clinical management of patients with gastric cancer. Potential markers for CTC may be clinically identified in the near future and, thus, it may become possible to discriminate subclinical patients with early tumors and accurately predict tumor progression and prognosis in patients with gastric cancer. Additionally, the assessment of CTC in blood may improve the selection of patients for neoadjuvant systemic chemotherapy in pre-therapeutic management of gastric cancer. Moreover, the postoperative monitoring of CTC by liquid biopsy may be useful for the early detection of disease recurrence and the administrative control of adjuvant chemotherapy. As technologies have been developed for the isolation and enrichment of tumor cells, we will be able to easily isolate viable CTC from blood specimens using promising markers.

The functional properties of CTC currently remain unclear. The main reason for this is that ex vivo cultures of CTC represent a challenging approach in clinical experiments. However, recent advances

in basic research have contributed to elucidating CTC-associated biological behaviors in patients with several malignancies. Yamamoto et al.[63] developed a novel method for the *ex vivo* culturing of CTC using a fibroblast feeder layer and magnetic coculture protocol. They cultured CTC isolated from the blood specimens of metastatic mouse models and obtain three CTC-derived cell lines.[63] They demonstrated that the malignant behavior of CTC-derived cell lines were more aggressive than that of the original cells.[63] Furthermore, Alix-Panabières et al.[64] reviewed functional studies on CTC using *in vitro* cultures and *in vivo* xenograft models. They concluded that CTC-derived cell lines and xenograft models are promising tools for examining the molecular properties of CTC and identifying new therapeutic targets.[64]

Several researchers have proposed a close relation between CTC and cancer stem cell-like properties or epithelial mesenchymal transition in various malignancies, including gastric cancer.[54,56] Further understanding of their relationship might allow the progress of a new CTC-targeted therapy that controls hematogenous metastasis in patients with gastric cancer. Accordingly, the molecular profiling of CTC by liquid biopsy will contribute to the development of personalized treatment that effectively inhibits tumor progression in patients with advanced gastric cancer.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
2. Ahn HS, Lee HJ, Yoo MW, et al. Diagnostic accuracy of T and N stages with endoscopy, stomach protocol CT, and endoscopic ultrasonography in early gastric cancer. J Surg Oncol. 2009;99:20–7.
3. Choi J, Kim SG, Im JP, Kim JS, Jung HC, Song IS. Is endoscopic ultrasonography indispensable in patients with early gastric cancer prior to endoscopic resection? Surg Endosc. 2010;24:3177–85.
4. Tsujimoto H, Sugasawa H, Ono S, Ichikura T, Yamamoto J, Hase K. Has the accuracy of preoperative diagnosis improved in cases of early-stage gastric cancer? World J Surg. 2010;34:1840–6.
5. Shimada H, Noie T, Ohashi M, Oba K, Takahashi Y. Clinical significance of serum tumor markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association. Gastric Cancer. 2014;17:26–33.
6. Park JM, Kim YH. Current approaches to gastric cancer in Korea. Gastrointest Cancer Res. 2008;2:137–44.
7. Yamashita K, Sakuramoto S, Kikuchi S, Katada N, Kobayashi N, Watanabe M. Validation of staging systems for gastric cancer. Gastric Cancer. 2008;11:111–8.
8. Harada K, Mizrak Kaya D, Shimodaira Y, Ajani JA. Global chemotherapy development for gastric cancer. Gastric Cancer. 2017;20:92–101.
9. Webb A, Scott-Mackie P, Cunningham D, et al. The prognostic value of serum and immunohistochemical tumour markers in advanced gastric cancer. Eur J Cancer. 1996;32:63–8.
10. Ashworth TR. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. Aust Med J. 1869;14:146–9.
11. Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res. 2004; 10:6897–904.
12. Takeuchi H, Kitagawa Y. Circulating tumor cells in gastrointestinal cancer. J Hepatobiliary Pancreat Sci. 2010;17:577–82.
13. Sun YF, Yang XR, Zhou J, Qiu SJ, Fan J, Xu Y. Circulating tumor cells: advances in detection methods, biological issues, and clinical relevance. J Cancer Res Clin Oncol. 2011;137:1151–73.
14. Arigami T, Natsugoe S, Uenosono Y, et al. Lymphatic invasion using D2-40 monoclonal antibody and its relationship to lymph node micrometastasis in pN0 gastric cancer. Br J Cancer. 2005;93:688–93.
15. Lambrechts AC, Bosma AJ, Klaver SG, et al. Comparison of immunocytochemistry, reverse transcriptase polymerase chain reaction, and nucleic acid sequence-based amplification for the detection of circulating breast cancer cells. Breast Cancer Res Treat. 1999;56:219–31.
16. Uen YH, Lin SR, Wu CH, et al. Clinical significance of MUC1 and c-Met RT-PCR detection of circulating tumor cells in patients with gastric carcinoma. Clin Chim Acta. 2006;367:55–61.
17. Miyazono F, Natsugoe S, Takao S, et al. Surgical maneuvers enhance molecular detection of circulating tumor cells during gastric cancer surgery. Ann Surg. 2001;233:189–94.
18. Sumikura S, Ishigami S, Natsugoe S, et al. Disseminated cancer cells in the blood and expression of sialylated antigen in gastric cancer. Cancer Lett. 2003;200:77–83.
19. Ikeguchi M, Kaibara N. Detection of circulating cancer cells after a gastrectomy for gastric cancer. Surg Today. 2005;35:436–41.
20. Illert B, Fein M, Otto C, et al. Disseminated tumor cells in the blood of patients with gastric cancer are an independent predictive marker of poor prognosis. Scand J Gastroenterol. 2005;40:843–9.
21. Seo JH, Choi CW, Kim BS, et al. Follow-up study of peripheral blood carcinoembryonic antigen mRNA using reverse transcription-polymerase chain reaction as an early marker of clinical recurrence in patients with curatively resected gastric cancer. Am J Clin Oncol. 2005;28:24–9.
22. Wu CH, Lin SR, Yu FJ, et al. Development of a high-throughput membrane-array method for molecular diagnosis of circulating tumor cells in patients with gastric cancers. Int J Cancer. 2006; 119:373–9.
23. Koga T, Tokunaga E, Sumiyoshi Y, et al. Detection of circulating gastric cancer cells in peripheral blood using real time quantitative RT-PCR. Hepatogastroenterology. 2008;55:1131–5.
24. Mimori K, Fukagawa T, Kosaka Y, et al. A large-scale study of MT1-MMP as a marker for isolated tumor cells in peripheral blood and bone marrow in gastric cancer cases. Ann Surg Oncol. 2008; 15:2934–42.
25. Yie SM, Lou B, Ye SR, et al. Detection of survivin-expressing circulating cancer cells (CCCs) in peripheral blood of patients with gastric and colorectal cancer reveals high risks of relapse. Ann Surg Oncol. 2008;15:3073–82.
26. Bertazza L, Mocellin S, Marchet A, et al. Survivin gene levels in the peripheral blood of patients with gastric cancer independently predict survival. J Transl Med. 2009;7:111.
27. Kita Y, Fukagawa T, Mimori K, et al. Expression of uPAR mRNA in peripheral blood is a favourite marker for metastasis in gastric cancer cases. Br J Cancer. 2009;100:153–9.
28. Arigami T, Uenosono Y, Hirata M, et al. Expression of B7-H4 in blood of patients with gastric cancer predicts tumor progression and prognosis. J Surg Oncol. 2010;102:748–52.
29. Qiu MZ, Li ZH, Zhou ZW, et al. Detection of carcinoembryonic antigen messenger RNA in blood using quantitative real-time reverse transcriptase-polymerase chain reaction to predict recurrence of gastric adenocarcinoma. J Transl Med. 2010;8:107.

30. Saad AA, Awed NM, Abd Elkerim NN, et al. Prognostic significance of E-cadherin expression and peripheral blood micrometastasis in gastric carcinoma patients. Ann Surg Oncol. 2010;17:3059–67.

31. Arigami T, Uenosono Y, Hirata M, Yanagita S, Ishigami S, Natsugoe S. B7-H3 expression in gastric cancer: a novel molecular blood marker for detecting circulating tumor cells. Cancer Sci. 2011;102:1019–24.

32. Cao W, Yang W, Li H, et al. Using detection of survivin-expressing circulating tumor cells in peripheral blood to predict tumor recurrence following curative resection of gastric cancer. J Surg Oncol. 2011;103:110–5.

33. Arigami T, Uenosono Y, Ishigami S, et al. Clinical significance of stanniocalcin 2 expression as a predictor of tumor progression in gastric cancer. Oncol Rep. 2013;30:2838–44.

34. Kang Y, Zhang J, Sun P, Shang J. Circulating cell-free human telomerase reverse transcriptase mRNA in plasma and its potential diagnostic and prognostic value for gastric cancer. Int J Clin Oncol. 2013;18:478–86.

35. Gerhard M, Juhl H, Kalthoff H, Schreiber HW, Wagener C, Neumaier M. Specific detection of carcinoembryonic antigen-expressing tumor cells in bone marrow aspirates by polymerase chain reaction. J Clin Oncol. 1994;12:725–9.

36. Soeth E, Vogel I, Röder C, et al. Comparative analysis of bone marrow and venous blood isolates from gastrointestinal cancer patients for the detection of disseminated tumor cells using reverse transcription PCR. Cancer Res. 1997;57:3106–10.

37. Zhang ZY, Ge HY. Micrometastasis in gastric cancer. Cancer Lett. 2013;336:34–45.

38. Srinivasula SM, Ashwell JD. IAPs: what's in a name? Mol Cell. 2008;30:123–35.

39. Tamm I, Wang Y, Sausville E, et al. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res. 1998;58:5315–20.

40. Tran J, Rak J, Sheehan C, et al. Marked induction of the IAP family antiapoptotic proteins survivin and XIAP by VEGF in vascular endothelial cells. Biochem Biophys Res Commun. 1999;264:781–8.

41. Andersen MH, Svane IM, Becker JC, Straten PT. The universal character of the tumor-associated antigen survivin. Clin Cancer Res. 2007;13:5991–4.

42. Liu JL, Gao W, Kang QM, Zhang XJ, Yang SG. Prognostic value of survivin in patients with gastric cancer: a systematic review with meta-analysis. PLoS ONE. 2013;8:e71930.

43. Zang X, Allison JP. The B7 family and cancer therapy: costimulation and coinhibition. Clin Cancer Res. 2007;13:5271–9.

44. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nat Rev Immunol. 2008;8:467–77.

45. Arigami T, Uenosono Y, Ishigami S, Hagihara T, Haraguchi N, Natsugoe S. Clinical significance of the B7-H4 coregulatory molecule as a novel prognostic marker in gastric cancer. World J Surg. 2011;35:2051–7.

46. Dai W, Shen G, Qiu J, Zhao X, Gao Q. Aberrant expression of B7-H3 in gastric adenocarcinoma promotes cancer cell metastasis. Oncol Rep. 2014;32:2086–92.

47. Pituch-Noworolska A, Kolodziejczyk P, Kulig J, et al. Circulating tumour cells and survival of patients with gastric cancer. Anticancer Res. 2007;27:635–40.

48. Hiraiwa K, Takeuchi H, Hasegawa H, et al. Clinical significance of circulating tumor cells in blood from patients with gastrointestinal cancers. Ann Surg Oncol. 2008;15:3092–100.

49. Matsusaka S, Chìn K, Ogura M, et al. Circulating tumor cells as a surrogate marker for determining response to chemotherapy in patients with advanced gastric cancer. Cancer Sci. 2010;101:1067–71.

50. Ito H, Inoue H, Sando N, et al. Prognostic impact of detecting viable circulating tumour cells in gastric cancer patients using a telomerase-specific viral agent: a prospective study. BMC Cancer. 2012;12:346.

51. Uenosono Y, Arigami T, Kozono T, et al. Clinical significance of circulating tumor cells in peripheral blood from patients with gastric cancer. Cancer. 2013;119:3984–91.

52. Li TT, Liu H, Li FP, et al. Evaluation of epithelial-mesenchymal transitioned circulating tumor cells in patients with resectable gastric cancer: relevance to therapy response. World J Gastroenterol. 2015;21:13259–67.

53. Okabe H, Tsunoda S, Hosogi H, et al. Circulating tumor cells as an independent predictor of survival in advanced gastric cancer. Ann Surg Oncol. 2015;22:3954–61.

54. Yuan D, Chen L, Li M, et al. Isolation and characterization of circulating tumor cells from human gastric cancer patients. J Cancer Res Clin Oncol. 2015;141:647–60.

55. Kolostova K, Matkowski R, Gürlich R, et al. Detection and cultivation of circulating tumor cells in gastric cancer. Cytotechnology. 2016;68:1095–102.

56. Andree KC, van Dalum G, Terstappen LW. Challenges in circulating tumor cell detection by the Cell Search system. Mol Oncol. 2016;10:395–407.

57. Iinuma H, Watanabe T, Mimori K, et al. Clinical significance of circulating tumor cells, including cancer stem-like cells, in peripheral blood for recurrence and prognosis in patients with Dukes' stage B and C colorectal cancer. J Clin Oncol. 2011;29:1547–55.

58. Reeh M, Effenberger KE, Koenig AM, et al. Circulating tumor cells as a biomarker for preoperative prognostic staging in patients with esophageal cancer. Ann Surg. 2015;261:1124–30.

59. Poruk KE, Valero V 3rd, Saunders T, et al. Circulating tumor cell phenotype predicts recurrence and survival in pancreatic adenocarcinoma. Ann Surg. 2016;264:1073–81.

60. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348:69–74.

61. Muro K, Chung HC, Shankaran V, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. Lancet Oncol. 2016;17:717–26.

62. Wang S, Zheng G, Cheng B, et al. Circulating tumor cells (CTCs) detected by RT-PCR and its prognostic role in gastric cancer: a meta-analysis of published literature. PLoS ONE. 2014;9:e99259.

63. Yamamoto S, Shimizu K, Fei J, et al. Ex vivo culture of circulating tumor cells using magnetic force-based coculture on a fibroblast feeder layer. Biotechnol J. 2016;11:1433–42.

64. Alix-Panabières C, Bartkowiak K, Pantel K. Functional studies on circulating and disseminated tumor cells in carcinoma patients. Mol Oncol. 2016;10:443–9.