Common molecular markers between circulating tumor cells and blood exosomes in colorectal cancer: a systematic and analytical review

Somayeh Vafaei1,2,3, Fahimeh Fattahi1,2, Marzieh Ebrahimi3, Leila Janani4, Ahmad Shariftabrizi5, Zahra Madjd1,2,6

1Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran; 2Student Research Committee, Iran University of Medical Sciences, Tehran, Iran; 3Department of Stem Cells and Developmental Biology, Cell Science Research Center, Roiyin Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran; 4Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran; 5Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA; 6Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran

Abstract: Nearly half of patients with colorectal cancer (CRC), the third leading cause of cancer deaths worldwide, are diagnosed in the late stages of the disease. Appropriate treatment is not applied in a timely manner and nearly 90% of the patients who experience metastasis ultimately die. Timely detection of CRC can increase the five-year survival rate of patients. Existing histopathological and molecular classifications are insufficient for prediction of metastasis, which limits approaches to treatment. Detection of reliable cancer-related biomarkers can improve early diagnosis, prognosis, and treatment response prediction and recurrence risk. Circulating tumor cells (CTCs) and exosomes in peripheral blood can be used in a liquid biopsy to assess the status of a tumor. Exosomes are abundant and available in all fluids of the body, have a high half-life and are released by most cells. Tumor-derived exosomes are released from primary tumors or CTCs with selective cargo that represents the overall tumor. The current systematic review highlights new trends and approaches in the detection of CRC biomarkers to determine tumor signatures using CTC and exosomes. When these are combined, they could be used to guide molecular pathology and can revolutionize detection tools. Relevant observational studies published until July 24, 2019 which evaluated the expression of tumor markers in CTCs and exosomes were searched in PubMed, Scopus, Embase, and ISI Web of Science databases. The extracted biomarkers were analyzed using String and EnrichR tools.

Keywords: colorectal cancer, circulating tumor cell, CTC, exosomes, diagnosis, prognosis, biomarker, systematic review

Introduction

Colorectal cancer (CRC) is the third highest cause of cancer deaths worldwide.1,2 The time of diagnosis directly influences the overall survival rate of patients. The five-year survival rates are estimated to decrease 12.5% after the occurrence of metastasis vs for localized cancer. Histological examination of tumor tissue is the gold standard for diagnosis, but is invasive, time-consuming, and nonrepeatable over time. There is a need for new methods that are simple, non-invasive, and inexpensive to provide clear clinical evidence and improve early detection or predict a response to treatment.3,4

Serum biomarkers such as carcinoembryonic antigens (CEAs) and carbohydrate antigen 19-9 (CA19-9) along with multi-target stool DNA tests represent the concrete implementation of non-invasive methods for CRC screening.5,6 There is urgent need for more reliable molecular markers that demonstrate the heterogeneity of cancer
cells during progression. The use of biological fluids as sources of nucleic acid-biomarkers for liquid biopsies in oncology has clinical promise.\textsuperscript{7,8} Molecular characterization of cancer signatures also can provide relevant information for personalized treatment of tumors.\textsuperscript{9,10} Circulating tumor cells (CTCs) and exosomes are shed from a tumor mass and enter the bloodstream. They can provide a metastatic niche for the invasion and migration of a tumor, so detection of their markers is critical.\textsuperscript{11} Ashworth et al, first identified CTCs as valuable indicators of cancer progression.\textsuperscript{12} CTCs detach from the primary tumor, intravasate into the bloodstream, evade immune detection, survive and extravasate into the microvessels of target tissue to establish a micro-metastatic niche.\textsuperscript{13} They have been identified in many cancers, including colon cancer. CTCs in the bloodstream may exist as single cells with a different EMT phenotypes or as clusters that bind to platelets or macrophages or are reactivated as stromal cells.\textsuperscript{14,15} The presence and number of CTCs before and during treatment are a strong independent predictor of shorter progression-free survival and overall survival of CRC patients.\textsuperscript{16} In spite of their advantages, researchers believe that the most challenging obstacles related to research on CTCs are their extremely low numbers, short lifetimes, fragility, and their heterogeneity and plasticity. The investigation of specific and reliable markers for their detection or isolation is an undeniable issue.\textsuperscript{17} Extracellular vesicles (EVs) generally include microvesicles (100–350 nm), apoptotic bodies (500–1000 nm), and exosomes (30–150 nm).\textsuperscript{18} Exosomes are nanovesicles with membrane-bound phospholipids which introduced and confirmed by Pan et al,\textsuperscript{19} and are actively secreted by mammalian cells into body fluids such as urine, plasma, and saliva. Exosomal cargo includes lipids, proteins, DNA, and RNA (mRNA, miRNA, long non-coding RNA) that are selected according to their roles. Exosomes involved in many biological processes, especially intercellular communication, establish a premetastatic niche by carrying oncogenic elements that suppress host immune responses.\textsuperscript{20} Exosomes are abundant, have high half-lives and are released by most cells. This is in contrast with CTCs, which are tumor specific, rare, fragile, have a short life and are difficult to isolate. It is possible to design a molecular marker common between the exosomes and CTCs for better understanding of the metastasis process. American Society of Clinical Oncology suggests circulating exosomes may provide an alternative platform for monitoring disease progression as opposed to CTCs.\textsuperscript{21} Several ongoing studies have aimed at quantifying a stress protein or other biomarkers in the blood and urine for monitoring and early diagnosis of malignant solid tumors (https://clinicaltrials.gov). The current analytical review is the first to explore similar molecular mechanisms and pathways between CTCs and Exosomes. In this systematic review, all molecular mechanisms that can potentially apply to the diagnosis and prognosis of CRC using CTCs and exosomes are discussed.

Materials and methods

Search strategy for literature mining

Observational studies evaluating the expression of circulating CRC cells and exosomes markers from 1980 to July 24, 2019 were electronically searched for in the PubMed, Scopus, Embase, and ISI Web of Science databases. The search syntax was modified for each database in accordance with their rules, the Mesh terms and keywords as listed in detail in Table 1.

The authors (S. Vafaei and F. Fattahi) searched and identified eligible studies and excluded all irrelevant articles after reviewing the publication titles and abstracts. Duplicate publications were excluded. Discrepancies were resolved between the two reviewers by consensus and by consulting the other authors. Next, the full text of the selected publications was retrieved and fully reviewed. This systematic review has been carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.\textsuperscript{22}

Publication inclusion criteria

The inclusion criteria for this systematic review followed the criteria of population, intervention, control, and outcomes. Observational studies (case-control) investigating CTC and exosomes mRNA and gene markers for the diagnosis and prognosis of CRC patient samples were included if they met the following criteria:

1. The article must be published in English and the full text must be available.
2. Studies included those on CRC patient blood samples and human blood for CTC, although tissue or cell lines for exosomes were done because exosomes research is rare and in its initial stages.
3. Expression of mRNA and gene markers in patient specimens or cell lines was detected by established molecular methods.
Table 1 Search strategy of CTC and exosome in colorectal cancer

| Search strategy                                                                 | No. of papers |
|---------------------------------------------------------------------------------|---------------|
| **SCOPUS**                                                                       |               |
| 1 (TITLE-ABS-KEY (cecum OR colon OR sigmoid OR rectum OR anal)) AND (TITLE-ABS-KEY ((neoplasm OR cancer OR tumor OR tumors OR carcinoma)) OR (TITLE-ABS-KEY ((colorectal AND neoplasms OR crc)))) | 258,569       |
| 2 (TITLE-ABS-KEY (circulating AND tumor AND cell)) OR (TITLE-ABS-KEY (circulating AND neoplastic AND cells)) OR (TITLE-ABS-KEY (neoplasm AND micro-metastasis)) OR (TITLE-ABS-KEY (ctc OR ctm OR dtc)) | 60,012        |
| 3 ((TITLE-ABS-KEY (gene AND expression AND profiling)) OR (TITLE-ABS-KEY (messenger AND rna)) OR (TITLE-ABS-KEY (ma OR transcriptome OR mrna))) AND ((TITLE-ABS-KEY (early AND diagnosis)) OR (TITLE-ABS-KEY (early AND detection)) OR (TITLE-ABS-KEY (prognosis OR diagnosis OR biomarkers OR screening OR diagnostic OR prognosis OR prognostic))) AND ((TITLE-ABS-KEY (extracellular AND vesicles)) OR (TITLE-ABS-KEY (cell-derived AND microparticles)) OR (TITLE-ABS-KEY (extracellular AND vesicles)) OR (TITLE-ABS-KEY (ev OR microvesicle OR exosomes))) | 209,207       |
| 4 (TITLE-ABS-KEY (extracellular AND vesicle)) OR (TITLE-ABS-KEY (cell-derived AND microparticles)) OR (TITLE-ABS-KEY (extracellular AND vesicles)) OR (TITLE-ABS-KEY (ev OR microvesicle OR exosomes)) | 274,615       |
| **PUBMED**                                                                       |               |
| 1 (((Colorectal Neoplasms[Title/Abstract] OR “Colorectal Neoplasms”[Mesh] OR CRC[Title/Abstract]) OR (“Cecum”[Mesh] OR “Colon”[Mesh] OR “Colon, Sigmoid”[Mesh] OR “Rectum”[Mesh] OR “Anal Canal”[Mesh]) AND (“Neoplasms”[Mesh] OR “Carcinoma”[Mesh])) OR ((cecum[Title/Abstract] OR colon[Title/Abstract] OR sigmoid[Title/Abstract] OR rectum[Title/Abstract] OR anus[Title/Abstract]) AND (neoplasm[Title/Abstract] OR cancer[Title/Abstract] OR tumor[Title/Abstract] OR tumors[Title/Abstract] OR carcinoma[Title/Abstract])) | 251,819       |
| 2 (“Neoplastic Cells, Circulating”[Mesh] OR Circulating Tumor Cell[Title/Abstract] OR “Neoplasm Micrometastasis”[Mesh] OR CTC[Title/Abstract] OR CTC[Title/Abstract] OR CTC[Title/Abstract]) | 20,001        |
| 3 (“Prognosis”[Mesh] OR “Diagnosis”[Mesh] OR “Early Diagnosis”[Mesh] OR “Early Detection of Cancer”[Mesh] OR “Biomarkers, Tumor”[Mesh] OR (“screening”[Title/Abstract] OR “early detection”[Title/Abstract] OR “Diagnosis”[Title/Abstract] OR “Diagnostic”[Title/Abstract] OR “Prognosis”[Title/Abstract] OR “Predictive”[Title/Abstract] OR “Gene, Messenger”[Mesh] OR “RNA”[Mesh] OR “Transcriptome”[Mesh] OR “Gene Expression Profiling”[Mesh] OR “mRNA” OR “RNA” OR “Transcriptome” OR “gene expression profiling”)) | 376,269       |
| 4 (“extracellular vesicles”[Mesh] OR “Cell-Derived Microparticles”[Mesh] OR “EV” OR “microvesicle” OR “extracellular vesicle” OR “Exosomes”[Mesh] OR Exosome) | 41,831        |
Table 1 (Continued).

| Search strategy                                                                 | No. of papers |
|---------------------------------------------------------------------------------|---------------|
| 1 & 2 & 3 Search ((((((Colorectal Neoplasms[Title/Abstract] OR “Colorectal Neoplasms”[Mesh] OR CRC[Title/Abstract]) OR ((“Cecum”[Mesh] OR “Colon”[Mesh] OR “Colon, Sigmoid”[Mesh] OR “Rectum”[Mesh] OR “Anal Canal”[Mesh]) AND (“Neoplasms”[Mesh] OR “Carcinoma”[Mesh])) OR ((“Cecum”[Title/Abstract] OR colon[Title/Abstract] OR sigmoid[Title/Abstract] OR rectum[Title/Abstract] OR anus[Title/Abstract]) AND (neoplasm[Title/Abstract] OR cancer[Title/Abstract] OR tumor[Title/Abstract] OR tumors[Title/Abstract] OR carcinoma[Title/Abstract]))) AND (((“Neoplastic Cells, Circulating”[Mesh] OR Circulating Tumor Cell[Title/Abstract] OR “Neoplasm Micrometastasis”[Mesh] OR CTC[Title/Abstract] OR CTM[Title/Abstract] OR DTC[Title/Abstract]) AND (((“Prognosis”[Mesh] OR “Diagnosis”[Mesh] OR “Early Diagnosis”[Mesh] OR “Early Detection of Cancer”[Mesh] OR “Biomarkers, Tumor”[Mesh] OR (“screening”[Title/Abstract] OR “early detection”[Title/Abstract] OR “Diagnostic”[Title/Abstract] OR “Diagnosis”[Title/Abstract] OR “Prognostic”[Title/Abstract] OR “Prognosis”[Title/Abstract] OR “Prognostic”[Title/Abstract]) AND (“RNA, Messenger”[Mesh] OR “RNA”[Mesh] OR “Transcriptome”[Mesh] OR “Gene Expression Profiling”[Mesh] OR “mRNA” OR “RNA” OR “Transcriptome” OR “gene expression profiling”))) Filters: Humans; English | 164            |
| 1 & 2 & 4 Search ((((((Colorectal Neoplasms[Title/Abstract] OR “Colorectal Neoplasms”[Mesh] OR CRC[Title/Abstract]) OR ((“Cecum”[Mesh] OR “Colon”[Mesh] OR “Colon, Sigmoid”[Mesh] OR “Rectum”[Mesh] OR “Anal Canal”[Mesh]) AND (“Neoplasms”[Mesh] OR “Carcinoma”[Mesh])) OR ((“Cecum”[Title/Abstract] OR colon[Title/Abstract] OR sigmoid[Title/Abstract] OR rectum[Title/Abstract] OR anus[Title/Abstract]) AND (neoplasm[Title/Abstract] OR cancer[Title/Abstract] OR tumor[Title/Abstract] OR tumors[Title/Abstract] OR carcinoma[Title/Abstract]))) AND (((“Neoplastic Cells, Circulating”[Mesh] OR Circulating Tumor Cell[Title/Abstract] OR “Neoplasm Micrometastasis”[Mesh] OR CTC[Title/Abstract] OR CTM[Title/Abstract] OR DTC[Title/Abstract]) AND (((“Prognosis”[Mesh] OR “Diagnosis”[Mesh] OR “Early Diagnosis”[Mesh] OR “Early Detection of Cancer”[Mesh] OR “Biomarkers, Tumor”[Mesh] OR (“screening”[Title/Abstract] OR “early detection”[Title/Abstract] OR “Diagnostic”[Title/Abstract] OR “Diagnosis”[Title/Abstract] OR “Prognostic”[Title/Abstract] OR “Prognosis”[Title/Abstract] OR “Prognostic”[Title/Abstract]) AND (“RNA, Messenger”[Mesh] OR “RNA”[Mesh] OR “Transcriptome”[Mesh] OR “Gene Expression Profiling”[Mesh] OR “mRNA” OR “RNA” OR “Transcriptome” OR “gene expression profiling”))) AND (((“extracellular vesicles”[Mesh] OR “Cell-Derived Microparticles”[Mesh] OR “EV” OR “microvesicle” OR “extracellular vesicle” OR “Exosomes”[Mesh] OR Exosomes)))) Filters: Humans; English | 66             |

**Embase**

| Search                                                                 | No. of papers |
|------------------------------------------------------------------------|---------------|
| 1 (cecum OR sigmoid OR rectum OR anal) AND (neoplasm OR cancer OR tumor OR tumors OR carcinoma) OR “colorectal cancer” OR crc | 323,384       |
| 2 ctc OR ctm OR dtc OR (circulating AND neoplastic AND cells) OR (circulating AND tumor AND cell) OR (neoplasm AND “micro-metastasis”)| 54,423        |
| 3 (early AND diagnosis) OR (early AND detection) OR biomarkers OR screening OR diagnostic OR prognosis OR prognostic AND (messenger AND ma) OR (gene AND expression AND profiling) OR mRNA OR transcriptome | 101,305       |
| 4 “membrane microparticle” OR “exosome”                                | 25,614        |

**Web of Science**

| Search                                                                 | No. of papers |
|------------------------------------------------------------------------|---------------|
| 1 T=(Cecum OR Colon OR Colon Sigmoid OR Rectum OR Anal) AND (neoplasm OR cancer OR tumor OR tumors OR carcinoma) OR T1=(Colorectal Neoplasms OR CRC) | 43,039        |
| 2 TS=(Circulating Neoplastic Cells OR Circulating Tumor Cell OR Neoplasm Micrometastasis OR CTC OR CTM OR DTC) | 44,339        |
| 3 TS=(Prognosis OR Diagnosis OR Early Diagnosis OR Early Detection OR Biomarkers OR screening OR Diagnostic OR Prognosis OR Prognostic) AND TS=(Messenger RNA OR RNA OR Transcriptome OR Gene Expression Profiling OR mRNA) | 138,133       |
| 4 TS=(extracellular vesicles OR Cell-Derived Microparticles OR EV OR microvesicle OR extracellular vesicle OR Exosomes) | 212,089       |
4. Studies demonstrated the correlation between mRNA profiling using isolation, detection, or validation methods, included sample type and size and other clinical parameters of diagnosis and prognosis, tumor stage and the frequency of estimated marker expression.

5. Study characteristics (first author surname, publication year, and study design) were included.

**Publication exclusion criteria**

Exclusion criteria included:

1. Evidence and article on CTC and exosomes covering review articles, seminars, letters, expert opinions, book chapters, meeting records, commentaries, and clinical guidelines.
2. In-vitro or in-vivo experimental studies.
3. Articles that were not published in English.
4. Full text of the article not available.

Exclusion criteria for CTC articles were:

1. Studies performed only on cell lines or tissue samples.
2. Studied housekeeping genes, such as glyceraldehyde-3-phosphate dehydrogenase, actin beta, β2-microglobulin, as they are not specific markers for CTC detection and expressed in all cells.
3. Bioinformatics analysis or data mining without experimental confirmation of the introduced biomarkers.
4. Therapy gaudiness based on the CTC results (per-operative and postoperative) in predicting the clinical outcome, not counting for drug effect on the expression of CTC genes.
5. The study only tested the spiked cell lines in human blood donors and not the actual patients.

In exosome studies, because of the limited data, we reviewed all articles on all markers that were introduced using the cell lines, tissue, or blood, even those only introduced through bioinformatics means without experimental confirmation.

**Risk of bias (quality) assessment**

The quality of each study was assessed using the Newcastle–Ottawa Scale (NOS), a well-known scale for assessing the quality and risk of bias in observational studies. NOS gives a score between 0 (minimum) and 9 (maximum). Studies with a NOS score >6 were considered to be of high quality, making them possible for use as potential moderators in meta-regression analysis.

**Statistical analysis**

Because the studies included were not sufficiently similar in terms of study design, experimental techniques, and heterogeneity of genetic variants, a meta-analysis was not performed.

**Bioinformatics approach to systematic search**

Molecular pathology is a valuable tool in the development of a cancer signature. The initially extracted markers in this article were subjected to STRING (https://string-db.org/) for better understanding of the significantly related pathway and secondary data were enriched using the EnrichR (amp.pharm.mssm.edu/Enrichr/) web tool. The GO project provided ontologies to describe the attributes of the gene products in the non-overlapping domains of molecular biology. Molecular function describes activities (such as catalytic or binding activities) at the molecular level. Biological processes describe biological goals accomplished by one or more ordered assemblies of molecular functions. Cellular component describes the locations of subcellular structures and macromolecular complexes.

**Results**

**Literature**

The initial search retrieved a total of 607 studies using the search strategy. After primary selection, 497 papers were
excluded because they were duplicates, had irrelevant titles or were paper abstracts. Eventually, 110 studies were selected for further evaluation. The schematic of the design and the reasons for exclusions are summarized in Figures 1 and 2 for CTC and exosomes, respectively.

Clinical applications of CTCs and exosomes in CRC as diagnostic markers

CTCs

Antigen expression of circulating cells and their specific phenotypes affects the progression of cancer and patient survival; thus, the focus was on CTC molecular markers that could lead to the detection of CTC rather than isolation in blood samples. CTC detection methods included real-time polymerase chain reaction (RT-PCR), flow cytometry, fluorescence in situ hybridization, and immunocytochemistry. Isolation methods included Cellsearch, OncoQuick, Filration, magnetic-activated cell sorting, fluorescence-activated cell sorting, Adnatest Colon Cancer Select and Detect, CELLlection electrophoresis assay, and microfluidic devices.

When attempting to find more reliable markers for CTCs in CRC cases, 6 out of 39 articles described only CK20 mRNA as the target gene, which is not transcribed in normal hematopoietic cells. It has previously been reported through immunohistochemistry by Moll et al,25–30 and has been seen in control blood samples through sensitivity assay and sampling,29 in addition to CK20, CA19-9, and CEA, which is used in clinics routinely for CRC detection, also has been introduced as a marker of CTC in CRC. Six of 39 studied examined CEA alone31–36 or in association with markers such as CK19,37,38 anti-epithelial cell adhesion molecule (EPCAM)39–43 and transmission electron microscopy (TEM)-8.44

Wong et al, used a sensitivity assay for the detection of CTCs and nodal metastases using CD44 splice variants as a tumor marker.45 It has been proven that RT-PCR in combination with positive isolation of epithelial tumor cells (addition of Ber-EP4
immunomagnetic) and negative isolation of non-epithelial cells (CD45 immunomagnetic beads used to deplete leukocytes from MNC) could improve detection.\textsuperscript{30,36} Guanylyl-cyclase C (GCC) is another marker introduced to detect rare epithelial circulating metastatic cancer cells.\textsuperscript{46-48}

After 2004, researchers focused on multi-marker panels in literature or data mining as listed in Table 2.\textsuperscript{49-56} Besides these, novel markers such as serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), SERPINB5,\textsuperscript{57} epidermal growth factor receptor (EGFR),\textsuperscript{58-60} epithelial cell transforming sequence 2 oncogene (ECT2),\textsuperscript{61} FAM172A,\textsuperscript{62} A3 receptor\textsuperscript{63} have been examined as well as other markers, especially through bioinformatics analysis.\textsuperscript{64}

### Exosomes

Exosome isolation methods consisted of ultracentrifugation, commercial kits, and a combination of several methods based on their physical, chemical, immunological, and molecular markers. Characterization of exosomes was also achieved based on morphology, such as with scanning electron microscopy and TEM, based on size, such as with dynamic light-scattering and nanoparticle tracking assay or based on molecular profiling through conventional enzyme-linked immunosorbent assay, polymerase chain reaction, and Western blotting.

Exosomes carry molecular markers such as DNA, RNA, and proteins. Many reports indicate that exosomes contain miRNAs;\textsuperscript{65-68} moreover, blood EVs contain a substantial fraction of intact mRNAs\textsuperscript{69-72} and a large number of assembling spliced junctions-circRNAs\textsuperscript{73} and long non-coding RNAs.\textsuperscript{72,74,75} Exosomal proteins belong to the following functional groups: tetraspanins, including CD63 antigen (CD63), CD9 antigen (CD9), CD81 antigen (CD81), heat shock proteins (HSC70 and HSC90), and endosomal sorting complexes required for transport proteins such as Alix and TSG101, found in a wide range of exosomes.\textsuperscript{76} The size of the extra vesicles varied and could influence gene expression. Larger vesicles (<100 nm) exhibited the greatest amount of EPCAM in extracted exosomes of HCT116 (CRC cell line) cells.\textsuperscript{77} The level of glypican-1 was evaluated in exosomes of patients before and after surgical treatment.\textsuperscript{78}

KRTAP5-4 and MAGEA3 mRNA in the serum of patients could be used as diagnostic biomarkers to detect CRC.\textsuperscript{79} Ct-OATP1B3 mRNA was present in EVs derived from HCT116, HT-29, and SW480 cells that were declared to be serum-based CRC biomarkers.\textsuperscript{80} Huang et al, introduced UBC, H3F3A, HIST2H2AA3, AKT3, and HSPA1B as hub genes in bioinformatic analysis to serve as diagnostic markers and therapeutic targets of CRC in the future.\textsuperscript{81} Table 3 shows all of these results.

### Clinical applications of CTCs and exosomes in CRC as prognostic markers

CTCs

Many researchers had discovered prognostic markers related to CRC as a beneficial tool for the detection of CTC. Five papers reported only CK20-positive as a prognostic marker. It caused significantly shorter survival in patients than the CK 20-negative marker.\textsuperscript{82-86} However, some studies emphasized only on CEA as a marker (five articles)\textsuperscript{87-91} and several studies also introduced both CK20 and CEA as prognostic markers. In most articles, CK20 and/or CEA were accompanied by markers such as CK19,\textsuperscript{97-102} GCC,\textsuperscript{96,103,104} Prominin 1 (CD133),\textsuperscript{95,100,105,106} EPCAM,\textsuperscript{107-109} survivin,\textsuperscript{110,111} ProtM,\textsuperscript{112} mucin 1 (MUC 1),\textsuperscript{105} and mucin 2 (MUC 2),\textsuperscript{99} and telomerase reverse transcriptase (hTERT).\textsuperscript{101,113,114}

Douard et al, showed that the expression of carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5; formerly CEA)\textsuperscript{102,115} and CEACAM7 (formerly CGM2)\textsuperscript{115,116} was more sensitive than use of a single marker in detecting CTCs, in contrast to the other studies, Bessa et al, showed that assessment of CTCs using RT-PCR CEA before surgery does not have prognostic value for CRC patients.\textsuperscript{117}

Some articles examined markers that had been investigated previously, such as EGFR,\textsuperscript{107,118-121} Plastin3,\textsuperscript{122,123} anterior gradient-2,\textsuperscript{102,124,125} leucine-rich repeat-containing-G-protein-coupled receptor 5,\textsuperscript{102,109,126-128} double cordin-like kinase 1,\textsuperscript{109,127} twist family bHLH transcription factor 1,\textsuperscript{110,129} and aldehyde dehydrogenase 1\textsuperscript{105,129} as prognostic markers in CRC through CTC.

Gradilone et al, assessed CK19 (75%), CK20 (8%), and EGFR (25%) expression in CTCs of some malignant tumors, including CRC samples, by RT-PCR followed by southern blot hybridization. They reported no correlation between prognostic values of CTCs and clinical manifestations of CRC.\textsuperscript{130}

Histone-like protein (HLM),\textsuperscript{120} tenasin C,\textsuperscript{121} aquaporin (AQ5),\textsuperscript{131} plakophilin 3, tyrosinase, prostate-specific antigen,\textsuperscript{132} universal MAGE-A,\textsuperscript{133} disheveled segment polarity protein 1 (DVL1),\textsuperscript{134} CD47,\textsuperscript{135} and
### Table 2 The biomarkers which worked for diagnostic of CRC in circulating tumor cells

| Biomarker | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker | Cutoff | Patients (number/type) | Patient stage | Author/year | CTCs positive rate | PMID       |
|-----------|----------------------------------------|-------------------------------------|----------------|--------|------------------------|---------------|-------------|---------------------|------------|
| CK20      | Nested RT-PCR¹                        | SWI 116, HT29 cell spiking          | –              | 5 mL   | 57 patients, 2 controls/Blood | I–IV³         | Soeth, 1996.28   | 35%               | 8,797,868 |
|           | Nested RT-PCR                         | A818-4 cell spiking                 | –              | 5 mL   | 39 patients, 12 controls/Blood | I–IV          | Soeth, 1997.27   | 24%               | 9,242,433 |
| RT-PCR    | HT29 cell spiking, Immunohistochemistry| PBGD                                | 5–10 m        | 30 patients, 16 controls/Blood | I–IV          | Vlems, 2002.29  | 30%               | 12,032,226 |
| CD45 Immune magnetic beads/or Ber-EP4 immuno magnetic beads | LS174T cell spiking                | 5 mL                                | 40 patients, 10 controls/Blood | A–D Dukes³   | Guo, 2005.30    | 80.0%, 82.5%, 72.5% | 16,048,578 |
| RT-PCR    | –                                      | –                                   | 5–10 mL       | 58 patients, 12 controls (abnormal)/ Blood | A–C Dukes | Zhang, 2005.25 | 44.8% to 69.0% | 15,637,763 |
| RT-PCR    | CEA, CKI 9                             |                                    | 15 mL         | 57/Blood | A–D Dukes | Katsumata, 2006.26 | 42.1% | 17,058,136 |
| CEA       | CD45 Immune magnetic beads and/or Ber-EP4 immuno magnetic beads | LS174T cell spiking                | 5 mL           | 25 patients, 10 controls/Blood | A–D Dukes | Guo, 2004.36   | 25.0%, 83.3%, 88.9% | 15,490,093 |
| RT-PCR    | Southern blotting, Colo201, HT1 16, HT29, and HT115 cell spiking | –                                   | 14 mL         | 31 patients, 22 controls/Blood | Liver metastasis | Jonas, 1996.31 | 58% | 9,014,772 |
| RT-PCR    | Cell spiking                           | –                                   | 10 mL         | 95 patients, 11 controls/Blood | I–IV        | Castells, 1998.32 | 41% | 9,823,981 |
| RT-PCR    | Colo201 cell spiking                   | –                                   | 14 mL         | 24 patients, 9 controls/Blood | B, C, D Dukes | Noh, 1999.33   | 41.1% | 10,642,939 |

(Continued)
Table 2 (Continued).

| Biomarker, Technique of isolation/detection of CTC | Technique of validation/related one | Related marker | Cutoff | Patients (number/type) | Patient stage | Author/year | CTCs positive rate | PMID               |
|--------------------------------------------------|------------------------------------|----------------|--------|------------------------|--------------|-------------|--------------------|--------------------|
| Nested RT-PCR                                     | In-vivo assay                       | CA19.9, CA72-4 | 7 mL   | 51 patients, 40 controls, 18 patients with benign colorectal disease/Blood | A–D Dukes | Guadagni, 2001 | 67%               | 11,289,125         |
| RT-PCR                                           | HT29 and LS147T cell spiking, Sequence analysis | CK20           | 20 mL  | 32 patients, 17 controls/Blood | –             | Hampton, 2002 | 36%               | 12,420,218         |
| CEA, CK19                                         | Semi-quantitative RT-PCR            | –              | 20 mL  | 33 patients, 26 controls/Blood | B–D Dukes | Wong, 2001 | 64%, 88%          | 11,121,864         |
| RT-PCR                                           | –                                  | –              | 3 mL   | 53 patients, 25 controls/Blood | I–III       | Silva, 2002 | 73.6%, 32%        | 11,889,075         |
| CEA, EPCAM. Adnatest ColonCancerSelect & Detect.  | Multiplex RT-PCR                   | –              | –      | 50 patients, 40 controls/Blood | I–III       | Mourtzikou, 2012 | 66%, 6%           | 10.6051/jissn.2224–3992.2012.01.070 |
| EPCAM                                            | Multigene qRT-PCR, flow cytometry  | CK19, CK20, CEA, EGFR | 7.5 mL | 49 patients/Blood | I–IV       | Cohen, 2006 | 80%               | 16,945,168         |
| EPCAM                                            | Microfluidic device, FISH, Cellsearch | Pan CK, EPCAM | 2 mL   | 5 patients, 200 controls/blood With metastasis | – | Gogoi, 2016 | 100%              | 26,808,060         |
| EPCAM                                            | CTC-chip                           | NCI-H1650 cell spiking | –      | 2.7 mL | 10 patients/Blood | Advanced | Nagrath, 2007 | 67%               | 18,097,410         |
| CEA, TEM-B                                       | RT-PCR                             | MAD-MB231 and HT29 cell spiking | 5 mL  | 40 patients, 40 controls/Blood | I–III | Raeesossadati, 2011 | 55%, 22.5%        | 21,573,768         |
| CD44                                             | RT-PCR                             | Southern blotting, HCT116 cell spiking, Restriction enzyme analysis | –      | 15 mL | 24 patients, 8 controls/Blood | B, C Dukes | Wong, 1997 | 16%               | 10.1046/j.1365–2168.1997.02685 |

(Continued)
Table 2 (Continued).

| Biomarker | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker | Cutoff | Patients (number/type) | Patient stage | Author/year | CTCs positive rate | PMID       |
|-----------|----------------------------------------|------------------------------------|----------------|--------|------------------------|---------------|-------------|-------------------|------------|
| GCC       | Nested RT-PCR                          |                                    | PSA, PSMA, CEA, CK-19, CK-20, mucin 1, GA733.2 | -      | 24 patients, 20 controls/Blood | D Dukes       | Fava, 2001.46 | 100%              | 11,579,116 |
|           | Nested Duplex RT-PCR                   | Immuno histochemistry               | CD31           | 10 mL  | 58 patients, 41 controls/Blood | B-D Dukes     | Tien, 2001.47 | 52%               | 11,410,499 |
|           | Nested Duplex RT-PCR                   | CCL-220 cell spiking, Immunohistochemistry, Western blotting | –              | 10 mL  | 68 patients, 41 controls/Blood | A-D Dukes     | Tien, 2004.48 | 58.8%             | 15,192,312 |
| BMP4, CycD, FAM3D, GPA33, ZPX2, LGALS4, TACSTD1, hTERT, TFF3, TM4SF3, UGT1A9, VIL1, FLJ20127. | RT-PCR                               | –                                  | B2M            | 10–15 mL | 16 pooled patients, 16 controls/Blood | I-IV         | Solmi, 2004.49 | ~100%, 100%, 100%, 100%, 100%, 100%, 100%, 100%, 100%, 100%, 100%, 87.5%, 83%, 36.3% | 15,375,555 |
| CK-20, CEA, CK-19, REG4, uPA, TIAM1 | RT-PCR                               | –                                  | –              | –      | 80 patients, 98 controls/Blood | I-II         | Yeh, 2006.50   | 82.5%, 78.8%, 82.5%, 80.0%, 78.8%, 80.0% | 16,391,796 |
| TMEM69, RANBP3, PRSS22 | Microarray screening, QRT-PCR       | –                                  | –              | 10–15 mL | 2 patients, 4 controls/ Blood | TNM stage     | Solmi, 2006.51 | ~3-fold          | 17,054,783 |

(Continued)
Table 2 (Continued).

| Biomarker | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker | Cutoff | Patients (number/type) | Patient stage | Author/year | CTCs positive rate | PMID |
|-----------|----------------------------------------|------------------------------------|----------------|--------|------------------------|--------------|-------------|-------------------|------|
| LOC644844, FABPI, CEACAM5, MUC13, GUCA2A, ABP1, SLC26A3 | Digital Gene Expression Display (DGED), RT-PCR | – | – | 5 mL | 8 patients, 9 controls/Blood | – | Lauriola, 2010 | – | 20,596,680 |
| SERPINBS | qRT-PCR | SW480 and T84 cell spiking | VSN11, DPEP1, STC1 | 5 mL | 818 patients, 4 IBD, 8 controls, 36 control without malignant disease/Blood | TNM stage | Findeisen, 2008 | 36% | 18,949,363 |
| CK20, CK19, EGFR | Multiplex-PCR | – | – | 6 mL | 81 patients, 38 controls/Blood | 0–IV | Vaiopoulos, 2014 | – | 24,922,677 |
| CK20, CEA, EGFR | Nested RT-PCR | – | – | – | 36 patients, 18 controls/Blood | I–IV | Teama, 2010 | 41.7, 61.1%, 66.7% | 10.1016/j.ejmhg.2009.10.001 |
| EGFR | AdnaTest Colon Cancer Select, AdnaTest Colon Cancer detect | COLO 205, HCC-2998, HCT-116, LoVo, WiDr, CACO-2, HT-29, SW-480, T84, DLD-1, SW-948, SW-1116 cell spiking, IHC, Multiplex RT-PCR | EPCAM, CEA | 15 mL | 20 patients, 22 controls/Blood | TNM stage | Lankiewicz, 2008 | 18% | 18,936,523 |
| ECT2 | Nested qPCR | – | CEA | 4 mL | 90 patients, 151 controls/blood | I–IV | Chen, 2017 | – | 28,362,321 |
| FAM172A | Filtration | In situ hybridization | EpCAM, CK8, CK18, CK19, Vimentin, Twist, CD45 | 5 mL | 45/Blood | I–IV | Cui, 2017 | 75.6% | 28,618,931 |
| A3 adenosine receptors | Real-time RT-PCR | Immunocytochemistry | – | 40 mL | 30/Blood | I–IV | Gessi, 2003 | – | 15,355,922 |

(Continued)
| Biomarker | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker | Cutoff | Patients (number/type) | Patient stage | Author/year | CTCs positive rate | PMID |
|-----------|----------------------------------------|------------------------------------|----------------|--------|------------------------|--------------|-------------|-------------------|------|
| TGFβ1, APP, CD9, CLU, ITGB5, LIMS1, RSU1, TIMP1, TLN1, VCL, BMP6. | CELLection™, Agilent expression arrays | Real-time RT-PCR | EPCAM | 7.5 mL | 28 patients, 10 controls/Blood | Primary and metastasis | Barbazan, 2012 | 22,811,761 |
| VIL1, TBX20, GPA33, FAM132A | CELLection™ | Real-time RT-PCR, HT29 and HCT116 cell spiking | CD45<sup>−</sup>, EPCAM | 7.5 mL | 44 patients, 22 controls/Blood | IV | Barbazan, 2012 | 77.2% | 22,304,365 |
| TSPAN8, LGALS5. | qRT-PCR | TRAM based data set meta-analysis | EPCAM, SPINK1, COL3A1, CEACAM5, COL1A2, CDH1, CKT18, SLC26A3, REG1A, FNI, LUM, CEACAM6, CK20 | 5 mL | 67 patients, 67 controls/Blood | I–III | Rodia, 2016 | – | 26,993,598 |
| LOXL3, ZEB2, VIL1, TIMP1, CLU, TLN1 | AdnaTest colon cancer | – | CD45<sup>−</sup>, EPCAM, CK 8, 18, and/or 19 | 7.5 mL | 50 patients/Blood | Advanced | Alonso-Alconada, 2017 | – | 29,058,262 |
| VIL1, CLU, TIMP1, LOXL3 and ZEB2 | CELLection™ | qRT-PCR | EPCAM | 7.5 mL | 50 patients/Blood | Barbazan, 2012 | – | 24,752,533 |

Abbreviations: RT-PCR, real-time polymerase chain reaction; Controls, healthy volunteer/donors; I–IV, TNM classification of malignant tumors (TNM); A–D Dukes. Dukes staging system is a classification system for colorectal cancer.
| Biomarker                     | Technique of exosome isolation                  | Technique of exosome validation | Technique of markers detection or validation | Related marker | Patients (number/type)                                             | Patient stage | Author/year: PMID | PMID  |
|------------------------------|-------------------------------------------------|--------------------------------|---------------------------------------------|----------------|-------------------------------------------------------------------|---------------|-------------------|-------|
| KRTAP5-4, MAGEA3             | Centrifugation syringe filter                    | TEM, NTA, light microscope   | Bioinformatic Analysis, RT-PCR               | IncRNA         | 30 patients, 30 control/Blood                                      | I-IV          | Dong, 2016.79     | 27,197,301 |
| GPC1                         | ExoCapTM                                        | TEM, Flow cytometry, Western blot analysis | miR-96-5p, miR-149, miR-182-5p               | 102 patients, 89 control/tissue and Blood, Cell line (HT-29 & HCT-116), Mouse | I-II          | Li, 2017.78       | 28,233,416 |
| EPCAM                        | PEG                                             | ELISA, SEM                    | qRT-PCR, SEM, DLS, ELISA                    |                | HCT-116 Cell line                                                |               | Manri, 2016.77   | 27,917,441 |
| UBC, H3F3A, HIST2H2AA3, AKT3, HSPA1B | Exosome Isolation kit (Thermo Fisher Scientific), PVDF filter and Differential centrifugation | TEM, Western blotting         | qRT-PCR, Western blotting                   |                | 29 patients, 49 control/tissue and Blood                         |               | Huang, 2018.81   | doi:10.21037/tcr.2018.05.32 |
| OATP1B3                      | Exosome Isolation kit (Thermo Fisher Scientific), PVDF filter and Differential centrifugation | TEM, Western blotting         | qRT-PCR, Western blotting                   |                | HCT116, HT-29, and SW480 cell line, Blood of Mouse               |               | Morio, 2018.86   | 29,491,222 |

**Abbreviations:** TEM, transmission electron microscopy; NTA, nanoparticle tracking analyzer; PEG, polyethylene glycol polymer; ELISA, enzyme-linked immunosorbent assay; SEM, scanning electron microscope; DLS, dynamic light-scattering.
CD44 variant exon 9 (CD44v9) were proposed as markers in a smaller number of articles. The heterogeneity of CTC markers led some researchers to focus on multi-marker panels in data mining as listed in Table 4.101,105,109,110,114,125,129,137–139

Exosomes
Some prognosis markers have nearly the same functional patterns as molecular markers related to CRC. Studies have reported on colorectal exosome prognostic markers such as ALIX (ALG 2-interacting protein X), Hsp60, Hsp70, CEA, ATP-binding cassette transporter G1 (ABCG1), copine III (CPNE3), and ΔNp73 in cancer patients.

Tauro et al, used multiple isolation methods to detect known exosome markers such as ALIX, TSG101, HSP70, and other specific and novel markers listed in Table 5.141 Chen et al, applied bioinformatic analysis for introduction of two panels and validated them.146 Chiba et al, reported that exosomes derived from CRC cell lines contain mRNA, microRNA, and natural antisense RNA as listed in Table 5.71

Risk of bias (quality) assessment
All articles related to CTC (39 diagnosis-related and 57 prognosis-related) were assessed by NOS case-control guidelines as reported in Table S1. Of the diagnosis-related articles (40% of the total), 43%, 43%, and 14% scored 7, 6, and 5, respectively. Of the diagnosis-related articles (60% of the total), 49%, 31.5%, 14%, and 1.5% scored 7, 6, 5, and 4, respectively; and 4% could not be scored.

All articles related to exosomes (Five diagnosis-related and nine prognosis-related) were assessed by the NOS case-control guidelines in Table S2. Of the diagnosis-related articles (36% of total), 20%, 40%, and 40% scored 7, 6, and 5, respectively. Of the prognosis-related articles (64% of total), 67%, 22%, and 11% scored 7, 6, and 5, respectively. The 0–3 and 8–9 scores were not given out in these studies, so the NOS number varied from 4 to 7. About 99.3% of systematically imported articles scored over 5, 20% of the articles scored 5, and 79.7% scored 6 or 7.

Bioinformatics approach to systematic results
This systematic search identified 66 CTC gene markers for the diagnosis of CRC, 65 CTC gene markers for prognosis with repetition, 10 exosome gene markers for diagnosis of CRC, and 35 exosome gene markers for prognosis as shown in Tables 2–5.

Protein–protein interaction network via STRING analysis
In the gene network, biochemical functions and identified pathways were obtained from gene expression data, and the results are shown in Figures 3 and 4 and supplementary Table S3 (online resources). Surprisingly, the cellular components of exosomes and CTC highlight extracellular space, region and exosome, plasma membrane, and cell junction. Their molecular function highlights cell adhesion molecule binding and protein binding. Biological processes included regulation of cellular component movement, assembly, localization, organization, and response to external stimuli.

Gene ontology
The results of EnrichR web tools in supplementary Table S4 (online resources) can be used to accurately understand the molecular pathways. The common pathways in biomarkers such as proteoglycans in cancer, focal adhesion pathways in cancer, integrin, Rap1, MAPK signaling pathways, angiogenesis, p53 pathways, and viral processes were similar and related to cancer.

Discussion
CRC is a common malignancy that often has a poor prognosis.147 The tumor microenvironment contributes to its progression and cross-talk between cancer cells and exosomes play a critical role in this dynamic network.149 Their identification and characterization are important steps to improve understanding of cellular and molecular cancer metastasis. Tracking of tumor-associated molecular markers in the blood can be used to assess the presence of residual disease, recurrence, and resistance.150 This systematic review highlights new trends and approaches in CRC biomarker discovery using CTC and exosomes.

Evidence related to diagnosis of CRC by means of CTC markers was addressed in 38 articles (Table 2) and 54 articles discussed prognosis of CRC using CTC markers (Table 4). Only 14 articles examined exosomes, five about diagnosis and nine about prognosis (Tables 3 and 5). Our results show that the most common markers introduced in CTCs were CEA (35 of 94 studies) and CK20 (33 of 94 studies), especially using quantitative real-time polymerase chain reaction. Most markers investigated for
Table 4 The biomarkers which worked for prognostic of CRC in circulating tumor cells

| Biomarker | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker | Cutoff (mL) | Patients (number/type) | Patient stage | Author/year | CTCs positive rate | PMID |
|-----------|----------------------------------------|------------------------------------|----------------|------------|------------------------|---------------|-------------|-------------------|------|
| CK20      | RT-PCR                                 | Colo205 cell spiking               | -              | 2          | 8 patients, 3 controls/Blood | III–IV        | Funaki, 1997.84 | 36%               | 9,048,967 |
|           | RT-PCR                                 | HT29 cell spiking                  | -              | 10 cells/2 mL | 26 patients, 12 controls/Blood | B, C Dukes stage | Wyld, 1998.82 | 48%               | 9,645,353 |
|           | RT-PCR                                 |                                    | -              | 10          | 108 patients, 38 controls/Blood | I–IV          | Hinz, 2012.83 | 25%               | 22,395,998 |
|           | qRT-PCR                                |                                    | -              | 5           | 95 patients, 23 controls/Blood | I–IV          | Samija, 2013.85 | -                 | 23,558,939 |
|           | RT-PCR                                 |                                    | -              | 5           | 95 patients, 23 controls/Blood | I–IV          | Kust, 2016.86 | -                 | 27,144,776 |
| CEA       | RT-PCR                                 | Southern blot hybridization        | -              | 7           | 69 patients, 16 controls/Blood | I–IV          | Piva, 2000.87 | 34%               | 11,096,345 |
|           | qRT-PCR                                | COLM-2 cell spiking                | -              | 5–7 mL      | 99 patients, 20 controls/Blood | I–III         | Ito, 2002.88 | 44.4%             | 12,065,095 |
|           | RT-PCR                                 |                                    | -              | 5           | 108 patients, 76 controls/Blood | III–IV        | Kanellos, 2006.89 | 11.1%            | 16,788,936 |
|           | Membrane arrays                        | RT-PCR                             | -              | 4           | 141 patients/Blood          | II–III        | Lu, 2011.90 | 33.3%             | 21,343,933 |
|           | CellSearch (EPCAM)                     | CellTracks® Analyzer II            | CD45<sup>+</sup> | 7.5 mL      | 20 patients/Blood           | I–III         | Thorsteinsson, 2011.91 | 5%              | 21,378,346 |

(Continued)
| Biomarker | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker | Cutoff | Patients (number/type) | Patient stage | Author/year | CTCs positive rate | PMID |
|-----------|----------------------------------------|-----------------------------------|----------------|--------|------------------------|--------------|-------------|-------------------|------|
| CK20, CEA. | RT-PCR | Colo320 cell spiking | – | 10 mL | 52 patients, 10 controls/Blood | I–IV | Yamaguchi, 2000.92 | 38.4%, 36.5% | 10,862,196 |
| RT-PCR | HT29 or HT115 cell spiking | – | 14 mL | 33 patients, 70 controls/Blood | I–IV | Mathur, 2001.93 | 85% | 11,417,979 |
| RT-PCR | LS 180 and C205; ATCC CL-187 and CCL-222 cell spiking | – | 12 mL | 39 patients, 13 controls (abnormal)/Blood | I–III | Guller, 2002.94 | 28% | 12,454,515 |
| qRT-PCR | – | – | – | 167 patients, 25 controls/Blood | I–IV | Inuma, 2006.95 | 22% | 16,391,782 |
| qRT-PCR | HT29 cell spiking | CA19-9 | 10 mL | 46 patients, 23 controls/Blood | I–IV | Liu, 2012.96 | 65.21%, 36.95% | 22,414,974 |
| CK20, CK19 | RT-PCR | Cell Spiking | K-ras, p53 | 20 mL | 35 patients, 23 controls/Blood | I–IV | Nakamori, 1997.98 | 26% | 9,378,009 |
| CK20, CEA, CK19. | Nested RT-PCR | – | – | – | 62 patients, 12 controls/Blood | I–IV | Huang, 2003.97 | 35.5%, 48.4%, 51.6% | 12,684,893 |
| CK20, GCC | RT-PCR | – | CEA, CA199 | 5 mL | 100 patients, 5 controls/Blood | I–III | Liu, 2017.103 | – | 28,418,917 |
| qRT-PCR | – | CEA | 5 mL | 69 patients, 23 controls/Blood | I–III | Liu, 2013.104 | 23,150,200 |
| CK, CEA, CD133. | qRT-PCR | – | CK19, CK20 | 10 mL | 735 patients/Blood | B–C Dukes | Inuma, 2011106 | 24.52% | 21,422,427 |

(Continued)
| Biomarker       | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker                          | Cutoff | Patients (number/type)                  | Patient stage | Author/year | CTCs positive rate | PMID         |
|-----------------|--------------------------------------|-----------------------------------|----------------------------------------|--------|----------------------------------------|---------------|-------------|-------------------|--------------|
| CK20, CEA, CK19, CD133 | qRT-PCR                             |                                   |                                        | –      | 197 patients, 20 controls (benign diseases)/Blood | B–C Dukes     | Shimada, 2012.100 | 63%               | 22,267,181   |
| CEA, EPCAM.     | CellSearch, TRC method               | DLD1 cell spiking                 |                                        | 7.5 mL | 67 patients/Blood                       | Metastatic    | Sato, 2012.108  | 9.0 ±23.4%, 64.3% | 21,732,137   |
| CK20, CEA, Survivin. | CD45 immuno magnetic beads + Ber-EP4 immuno magnetic beads | Lovo cell spiking, Real-time RT-PCR |                                        | 10 mL  | 156 patients, 40 benign patients, 40 healthy/Blood | A–D Dukes     | Shen, 2008.111  | 47.4%, 39.1%, 57.7% | 18,845,519   |
| CK20,CEA, ProxM. | Real-time RT-PCR                     |                                   |                                        | 10 mL  | 129 patients, 47 controls/Blood          | 0–IV          | Schuster, 2004.112 | 88%, 86%; 17% | 14,639,606    |
| CK19, CK20, MUC1, MUC2. | Immunobead RT-PCR                   |                                   |                                        | 20 mL  | 94 patients, 20 controls/Blood           | A–D Dukes     | Hardingham, 2000.99 | 20%               | 10,719,724    |
| CK-19, CK-20, CEA, hTERT. | Membrane arrays                      | RT-PCR                            |                                        | 4 mL   | 72 patients, 30 controls/Blood           | I–IV          | Wang, 2006.113   | 66.7%, 52.8%, 72.2%, 69.4% | 16,736,329   |
| CGM2 (CEACAM7)  | RT-PCR                              |                                    |                                        | 20 mL  | 78 patients, 115 controls/Blood          | A–D Dukes     | Douard, 2001.116  | 59%               | 11,331,451    |

(Continued)
Table 4 (Continued).

| Biomarker | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker | Cutoff | Patients (number/type) | Patient stage | Author/year | CTCs positive rate | PMID |
|-----------|----------------------------------------|----------------------------------|----------------|--------|------------------------|--------------|-------------|-------------------|------|
| CEACAMS, CEACAM7 | Immuno bead multiplex RT-PCR | – | HBB | 20 mL | 84 patients, 41 controls, 32 non CRC patients/Blood | I–IV | Douard, 2005. | 55%, 45% | 15,843,204 |
| EPCAM, EGFR. | Immuno magnetic selection (IMS), multiplex RT-PCR | T84, HT29, SW948 and SW1116 cell spiking | CEA | 5 mL | 76 patients, 106 controls/Blood | I–IV | Zieglschmid, 2007. | 88%, 12% | 17,649,779 |
| EGFR | RT-PCR | Immunohistochemistry (IHC) | CEA (45%), CK-19 (27%) | 5 mL | 38 patients, 38 controls/Blood | B, C, Dukes | De luca, 2000. | (73%) | 10,778,975 |
| EGFR | RT-PCR | – | – | 3 mL | 16 patients, 23 controls/Blood | Advanced-stage | Clarke, 2003. | 12.5% | 12,527,944 |
| EGFR, HLM | RT-PCR | Northern blotting, HT11C cell spiking | – | 3 mL | 1 patients, 9 controls/Blood | Metastatic | Fournier, 1999. | 100% | 10,446,991 |
| EGFR, Tenascin C. | – | – | – | 5 mL | 41 patients, 40 controls/Blood | I–IV | Gazzaniga, 2005. | 49% | 16,211,285 |
| PLS3 | RT-PCR | Fluorescent immunocytochemistry | CEA | – | 711 patients, 25 controls/Blood | Dukes A, B, C, and D | Yokobori, 2013. | 25% | 23,378,342 |
| PLS3, AQP5 | RT-PCR | Fluorescent immunocytochemistry | CD45 (–) | 10 mL | 177 patients, 25 controls/Blood | Dukes A, B, C, and D | Sugimachi, 2014. | - | 24,217,791 |
| PKP3, AGR2. | Bioinformatic analysis and RT-PCR | Gp5d, LoVo, DLD1, LS513, HT29, OJC4, OJC5, OJC6 cell spiking | S100A16, S100A6, LGALS4, CLDN3. | 10 mL | 21 patients and controls/Blood | III–IV | Valladares-Ayerbes, 2008. | 40%, 81.8% | 18,801,625 |

(Continued)
| Biomarker       | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker                                           | Cutoff  | Patients (number/type) | Patient stage | Author/year | CTGs positive rate | PMID       |
|-----------------|----------------------------------------|------------------------------------|----------------------------------------------------------|---------|------------------------|---------------|-------------|-------------------|------------|
| AGR2, LGR5      | qRT-PCR                                | –                                  | EpCAM, CK8, CK18, CK19, Twist1, Vimentin, AKT2, SNAIL, CD45 (−) | 10 mL   | 54 patients, 19 controls/Blood | I–IV          | Valladares-Ayerbes, 2012 | 84.9%, 90.5% | 22,605,983 |
| DCLK1, LGR5     | qRT-PCR                                | –                                  | EpCAM, CK8, CK18, CK19, Twist1, Vimentin, AKT2, SNAIL, CD45 (−) | 10 mL   | 58 patients, 58 controls/Blood | I–IV          | Mirzaei, 2015 | 63.7%            | 25,631,749 |
| LGR5            | mRNA ISH                               | –                                  | EpCAM, CK8, CK18, CK19, Twist1, Vimentin, AKT2, SNAIL, CD45 (−) | 5 mL    | 66 patients/Blood        | I–IV          | Wang, 2018 | 86.4%            | 29,949,050 |
| CK20, Tyrosinase, PSA | RT-PCR, Nucleic acid sequence-based amplification (NASBA assay) | HT-29 cell spiking, In vitro cell assay | EpCAM, CK8, CK18, CK19, Twist1, Vimentin, AKT2, SNAIL, CD45 (−) | 2 mL    | 12 patients, 8 controls/Blood | –              | Burchill, 2002 | –                | 11,857,020 |
| MAGE-A          | Electrochemiluminescence (ECL), RT-PCR | Sequencing analysis               | uMAGE-A, M-A1, M-A3, M-A12                              | 10 mL   | 12 patients, 20 controls/Blood | I–IV          | Miyashiro, 2001 | 29%              | 11,238,304 |
| DVL1            | Microarray and enzymatic chip array (WEnCA) | IHC                              | EpCAM, CK8, CK18, CK19, Twist1, Vimentin, AKT2, SNAIL, CD45 (−) | 4 mL    | 214 patients/Blood       | I–III         | Huang, 2013 | 55%              | 24,129,181 |
| Biomarker          | Technique of isolation/detection of CTC | Technique of validation.related one | Related marker                                                                 | Cutoff (umber/type) | Patient stage | Author/year   | CTCs positive rate | PMID          |
|--------------------|----------------------------------------|------------------------------------|--------------------------------------------------------------------------------|---------------------|---------------|---------------|-------------------|--------------|
| CD47               | Cellsearch                             | –                                  | EPCAM, CD45 (−)                                                              | 20–30 mL           | I–IV          | Steinert, 2014 | 14%               | 24,599,131    |
| CD44v9             | OncoQuick                              | qRT-PCR                            | −                                                                              | 20 mL              | I–IV          | Katoh, 2015   | 40%               | 25,550,556    |
| CK19, AGR2, CK8, CK9 | CellSearchn                           | −                                  | TSPAN8, LAD1, CK20, IGFBP5, GPX2, FABP1, S100A1, 6 CK8, PRSS8, CDX1, CEACA,M5, AKR1C3, RARRES2, REGIA, IGFBP4, CD44, TRIM2, CXCL1, SATB2, NQO1, CK19, MAPT, IGFBP3, COL4A1, FCGBP, SLC6A8, CDH5, CDH17, EGFR, S100P, HOXB9, CDH1, MACROD1, | 30 mL              | Metastatic colorectal cancer | Mostert, 2015 | 66%               | 25,655,581    |
| CK20, CEA, AGR2, MGB2, DLL4, EphA2, Her3, PDGFRα | qRT-PCR                              | −                                  | −                                                                              | 7.5 mL             | III–IV        | Bao, 2013     | 59%               | 23,990,866    |
| CK-20, CEA, CK-19, hTERT, TM4SF3, CK19 | Membrane arrays                       | RT-PCR                             | −                                                                              | 4 mL               | I–IV          | Wang, 2007    | 50%               | 17,406,027    |
|                    | RT-PCR                                |                                    | CEA, CK20, TACSTD1,                                                         | 10 mL              | I–IV          | Xi, 2007      | 96.4%              | 17,525,108    |

(Continued)
| Biomarker | Technique of isolation/detection of CTC | Technique of validation/related one | Related marker | Cutoff | Patients (number/type) | Patient stage | Author/year | CTCs positive rate | PMID  |
|-----------|----------------------------------------|------------------------------------|----------------|--------|-----------------------|---------------|-------------|-------------------|-------|
| DCLK1, LGR5, EpCAM, CK8, CK9, CK19, Vimentin, Twist | qRT-PCR, IHC | – | – | 10 mL | 78 patients and controls/Blood | I–IV | Mirzaei, 2016 | 26,383,518 |
| CanPatrol CTC enrichment | (ISH) assay | – | – | 5 mL | 38 patients, 27 controls/Blood | I–IV | Wu, 2015 | 67% | 25,909,322 |
| PSG2, ELAVL4, TK1, UBE2C, PDE6D, PSAT1, CHRNB1, BM11, CAP2, MMP13, OLM4, PTTG1, MYC, MET, MUC1, HMGB1, hTERT, BIRC5 | Enzyme immunoassay test kit | – | CEA | 3 mL | 298 patients/Blood | I–III | Chang, 2016 | - | 27,701,415 |
| PI3Kα, Akt-2, Twist1, ALDH1 | antiCD45 specific antibodies (Dynabeads, Invitrogen) | qRT-PCR and multiplex-PCR | – | 8 mL | 78 patients, 20 controls/Blood | I–IV | Ning, 2018 | 55% | 27,503,579 |
| CK19, MUC1, CD44, CD133, ALDH1 | CD45 Human MicroBeads (Miltenyi Biotec), enrichment of cytokeratin (Miltenyi Biotec) | Flowcytometry, CellSearch, qRT-PCR, Cytomorphology, PC3, MDA-MB-231 and SKBR3 cell spiking | – | 7.5 mL | 63 patients, 40 controls/Blood | I–III | Bahnassy, 2019 | (55.6%), (46.0%), (44.4%), (41.3%), (41.3%) | 30,578,762 |
| CEACAM5, CK19, AGR2, LGR5 | Inertial microfluidics combined with droplet digital PCR | qRT-PCR, HT-29 and LoVo cell spiking | – | 9 mL | Patients and controls/Blood | Advanced | Methai, 2019 | - | 31,304,099 |
Table 5 The biomarkers which worked for prognostic of CRC in exosome

| Biomarker | Technique of exosome isolation | Technique of exosome validation | Technique of markers detection or validation | Related marker | Patients (number/type) | Patient stage | Author/year: | PMID  |
|-----------|-------------------------------|---------------------------------|---------------------------------------------|----------------|----------------------|---------------|-------------|-------|
| Alix      | –                             | –                               | GSE3764, GSE10714, GSE4183, GSE18105, GSE4107, GSE9348, GSE8671, IHC | PGK1, PKM, ANXA5, ENO1, HSP90AB1, MSN | 72 patients, 27 controls, and 98 sample (literature bioinformatic) | I–IV         | Valcz G, 2016.140 | 27,150,162 |
| △Np73    | UC-Exo centrifugation 120,000 and PVDF filter | Acetylcholinesterase activity, flow cytometry quantification, transmission electron microscopy, Western blot analysis | qRT-PCR, Cell culture and transfection | CEA | 69 patients and control tissues, HCT 116 cell lines. | I–IV         | Soldevilla, 2013.70 | 24,067,531 |
| Hsp60    | UC-Exo                        | TEM AChEase: acetylcholinesterase assay, Western blot | IHC, ELISA, immunogold electron microscopy | Hsc70, Alix, CD57, CD68 | 57 patients and control tissues, 2 blood sample | I–III        | Campanella, 2015.143 | 26,060,090 |
| RPL13A, HMBS, TBP | UC-Exo                    | BCA, Western blotting | qRT-PCR | miR-21, miR-34, miR-143, miR-192, miR-215, miR-22 | 91 patients, 12 controls/tissue and blood | I–IV         | Chiba, 2012.71 | 22,895,844 |
| TSAP6, CEA | UC-Exo                         | Flow Cytometry, Western blotting | qRT-PCR, IHC, levels of circulating exosomes in plasma | – | – | I–IV | Silva, 2012.142 | 22,420,032 |
| Alix, TSG101, HSP70, CD9, CD81, ESCRT-III, VPS32/C/CHMP4C, VAMP2, ENB1, ENB2, EPHA2-8, EPHB1–4, CTNNB1, TNK, CRK, GRB2 | UC-Exo, DG-Exo: OptiPrep™ density gradient exosome, IAC-Exo: EpCAM immunosafinity capture | Western blotting, EM: Electron microscopy | GeLC–MS/MS (protein profiling) | LIM1863 cell line | – | Tauro, 2012.141 | 22,285,593 |

(Continued)
exosomes, in addition to CD9, CD81, ALIX, and TSG101, were including EPCAM and HSP, especially using ultracentrifugation. Comparison of 131 CTC markers and 45 exosomes markers showed only three common markers (CEA, CD9, and EPCAM) on the gene list as diagnostic and prognostic biomarkers.

A half-century-old investigation of CEA in CRC was the first step in the identification of a much larger family of 12 CEACAMs. Gene encoding CEA is a member of the immunoglobulin supergene family that plays a role in cell adhesion and tumor progression, even in protecting the colon from microbial infection. CEA is involved in the metastatic cascade process through positive regulation of cell migration and invasion; thus, the monitoring of CEA as a cost-effective and frequent indicator of recurrence of CRC has been investigated for years.

Integrin on tumor exosomes may play an important role in modulating organ-specific metastasis in cancer progression. CD9 is a member of the tetraspanin superfamily commonly detected in all types of exosomes involved in pathophysiologic processes such as cellular adhesion, growth, motility, cell–cell fusion, signal transduction, and tumor metastasis.

EPCAM is a membranous glycoprotein that is a CSC marker in tumor cells in the basolateral surface of most normal epithelial tissue and its role is to connect cells by means of calcium. The expression of this marker increases in benign and malignant tumors that arise from epithelial tissue. The first step in metastasis is the separation of cancerous cells from primary tumors. CEA, CD9, and EPCAM are closely correlated with tumor progression as a poor prognostic factor and is required for the survival of CTCs in some cancers.

Taken together, it appears that the signature of the CTC and exosome biomarkers are similar and follow common pathways; thus, exosomes can be applied as alternative tools for guiding better molecular pathology in the fight against cancer.

Precision medicine is changing clinical practice by tailoring treatment based on an individual’s genetic makeup. Recent studies have shown that CTC and circulating tumor DNA provide complementary information and the use of both approaches to study tumor metastasis is warranted. CTC and exosomes can pave a path as diagnostic and prognostic procedures using the heterogeneity of tumor sites as they are released into the blood from live origins and can be

| Biomarker | Technique of exosome isolation | Related marker | Technique of markers detection or validation | Patient stage | Author/Year | PMID |
|-----------|-------------------------------|----------------|---------------------------------------------|---------------|-------------|------|
| BCL7C, EEF1G, RAB13, RSP3, TPT1, SCAB1, SCD | UC-Exo, RIPA, RIPA, RIPA | - | A3-Exos and EPCAM-Exos (Dynabeads™), TEM, Western blotting | Chemo 2016 | Chen, 2016 | 27,917,920 |
| CPNE3 | UC-Exo | - | TEM, NTA, Western blotting | LIM1863 cell line | Chen, 2016 | 30,078,189 |
| ABCG1 | Polymer-based precipitation method | - | TEM, Zetasizer Nano ZSP, Western blotting | 92 patients, 32 controls/Blood | Sun, 2019 | 30,344,132 |

Abbreviation: UC-Exo, ultracentrifugation exosome.
analyzed at the DNA, RNA, and protein levels. It is undeniable that more investigation is needed to compare them, especially for cancer patients.

Various CTC isolating techniques each have its own advantages and disadvantages as to their CTC capture capacity and subgrouping of CTCs based on various markers. Similar problems also exist for exosomes, with a lack of a proven rapid and high-yield approach for extracting exosomes for downstream analysis. Microfluidic devices and bioinformatics analysis might play an important role in solving the current shortcomings of the liquid biopsy concept. Microfluidics, by using inertial focusing/hydrodynamics (laminar flow in microchannels) and applying spiral, acoustic, electrophoretic, and electromagnetic features passively separate CTCs and exosomes from the other background calls. Immobilizing specific antibodies either on micro-posts or in a herringbone design against their marker might be useful; it is easy to explore and yields quantitative readouts with high sensitivity, low cost, and minimal sample handling. Finally, although the potential clinical utility of these techniques is clear, more effort is needed to use the full potential of liquid biopsy in clinical settings.

Figure 3 Network and enrichment analysis visualization. Combined screenshots from the STRING website, showing results obtained upon entering a set of 131 proteins suspected to be involved in circulating tumor cell markers. According on kmeans clustering has been selected, the corresponding protein nodes in three categories automatically highlighted in colors.
Future perspectives
Currently, isolation and purification of tumor-derived exosome in a worm bag of EVs is technically cumbersome and also isolation of CTCs has its own limitations. Therefore, combined use of these two biomarkers together as a liquid biopsy requires large-scale clinical trials. Microfluidic devices and bioinformatics analysis might play an important role in solving the current shortcomings of the liquid biopsy. Additionally, cross talking of CTCs and tumor-derived exosomes in a tumor microenvironment should become a heated question in exploring the premetastatic niche. As such, more research is needed on CTCs and exosome’s overlapping molecular pathways to determine more effective biomarker signatures of CRC, especially in the metastatic form.

Acknowledgments
Systematic Review Network, Vice-Chancellor for Research and Technology, Iran University of Medical Sciences (Grant # 97-4-37-13921), funded this research. We would also like to thank the Royan Stem Cell Technology Company and our colleagues from both centers who provided insight and expertise that greatly assisted the research.

Disclosure
The authors report no conflicts of interest in this work.

References
1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108. doi:10.3322/caac.21262
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):5–29. doi:10.3322/caac.21254
3. Punt CJ, Koopman M, Vermeulen L. From tumour heterogeneity to advances in precision treatment of colorectal cancer. Nat Rev Clin Oncol. 2017;14(4):235. doi:10.1038/nrclinonc.2016.171
4. Zhai Z, Yu X, Yang B, et al. Colorectal cancer heterogeneity and targeted therapy: clinical implications, challenges and solutions for treatment resistance. Paper presented at: Semin Cell Dev Biol; 2017;64:107–115. doi:10.1016/j.semcdb.2016.08.033
5. Bailey JR, Aggarwal A, Imperiale TF. Colorectal cancer screening: stool DNA and other noninvasive modalities. Gut Liver. 2016;10 (2):204–211. doi:10.5009/gnl15420
39. Cohen SJ, Alpaugh RK, Gross S, et al. Isolation and characterization of circulating tumor cells in patients with metastatic colorectal cancer. Clin Colorectal Cancer. 2006;6(2):125–132. doi:10.3816/CCC.2006.n.029

40. Gogoi P, Sepehri S, Zhou Y, et al. Development of an automated and sensitive microfluidic device for capturing and characterizing Circulating Tumor Cells (CTCs) from clinical blood samples. PLoS One. 2016;11(1):e0147400. doi:10.1371/journal.pone.0147400

41. Mourtzikou A, Kroupis C, Pourmpouridou N, et al. Molecular detection of circulating tumor cells in peripheral blood of colon cancer patients. J Gastroenterol Hepatol Res. 2012;1(5):74–79.

42. van Zijl F, Krupitzga M, Mikulits W. Initial steps of metastasis formation: cell invasion and endothelial transmigration. Mutat Res. 2011;728(1–2):23–34. doi:10.1016/j.mrr.2011.05.002

43. Nagrah S, Sequist LV, Maheswaran S, et al. Isolation of rare circulating tumor cells in cancer patients by microchip technology. Nature. 2007;450(7173):1235–1239. doi:10.1038/nature06385

44. Raeisossadadi R, Farshchian M, Ganji A, et al. Quantitative analysis of TEM-8 and CEA tumor markers indicating free tumor cells in the peripheral blood of colon cancer patients. Int J Colorectal Dis. 2011;26(10):1265–1270. doi:10.1007/s00384-011-1230-8

45. Wong LS, Cantrill JE, Odogwu S, Morris AG, Fraser IA. Detection of circulating tumour cells and nodal metastasis by reverse transcriptase-polymerase chain reaction technique. Br J Surg. 1997;84(6):834–839.

46. Fava TA, Desnoyers R, Schulz S, et al. Ectopic expression of guanylyl cyclase C in CD34+ progenitor cells in peripheral blood. J Clin Oncol. 2001;19(19):3951–3959. doi:10.1200/JCO.2001.19.19.3951

47. Tien YW, Chang KJ, Jeng YM, et al. Tumor angiogenesis and its possible role in intravasation of colorectal epithelial cells. Clin Cancer Res. 2001;7(6):1627–1632.

48. Tien YW, Jeng YM, Hu RH, Chang KJ, Hsu SM, Lee PH. Intravasation-related metastatic factors in colorectal cancer. Tumour Biol. 2004;25(1–2):48–55. doi:10.1159/000077723

49. Solmi R, De Sanctis P, Zucchinì C, et al. Search for epithelial-specific mRNAs in peripheral blood of patients with colon cancer by RT-PCR. Int J Oncol. 2004;25(4):1049–1056. doi:10.3822/ijo.25.4.1049

50. Yeh CS, Wang JY, Wu CH, et al. Molecular detection of circulating cancer cells in the peripheral blood of patients with colorectal cancer by using membrane array with a multiple mRNA marker panel. Int J Oncol. 2006;28(2):411–420. doi:10.3822/ijo.28.2.411

51. Solmi R, Ugolini G, Rosati G, et al. Microarray-based identification and RT-PCR test screening for epithelial-specific mRNAs in peripheral blood of patients with colon cancer. BMC Cancer. 2006;6:250. doi:10.1186/1471-2407-6-250

52. Lauriola M, Ugolini G, Rosati G, et al. Identification by a Digital Gene Expression Display (DGED) and test by RT-PCR analysis of new mRNA candidate markers for colorectal cancer in peripheral blood. Int J Oncol. 2010;37(2):519–525. doi:10.3822/ijo.0000701

53. Barbazan J, Alonso-Alconada L, Muñoz-Romay L, et al. Molecular characterization of circulating tumor cells in human metastatic colorectal cancer. PLoS One. 2012;7(7):e40476. doi:10.1371/journal.pone.0040476

54. Barbazan J, Vieito M, Abalo A, et al. A logistic model for the detection of circulating tumour cells in human metastatic colorectal cancer. J Cell Mol Med. 2012;16(10):2342–2349. doi:10.1111/j.1582-4934.2012.01544.x

55. Alonso-Alconada L, Barbazan J, Candamio S, et al. PrediCTC, liquid biopsy in precision oncology: a technology transfer experience in the Spanish health system. Clin Transl Oncol. 2018;20(5):630–638. doi:10.1007/s12994-017-1760-9

56. Barbazan J, Muñoz-Romay L, Vieito M, et al. A biomarker panel for circulating tumor cells detection predicts patient outcome and therapy response in metastatic colorectal cancer. Int J Cancer. 2014;135(11):2633–2643. doi:10.1002/ijc.28910

57. Finkenstock P, Rockel M, Nees M, et al. Systematic identification and validation of candidate genes for detection of circulating tumor cells in peripheral blood specimens of colorectal cancer patients. Int J Oncol. 2008;33(5):1001–1010. doi:10.3892/ijo.0000088

58. Vaiopoulos AG, Kostakis ID, Gkioka E, et al. Detection of circulating tumor cells in colorectal and gastric cancer using a multiplex PCR assay. Anticancer Res. 2014;34(6):3083–3092.

59. Teama SH, Agwa SHA. Detection of circulating tumor cells by nested RT-PCR targeting EFGR/CEA/CK20mRNAs in colorectal carcinoma patients. Egypt J Med Human Genet. 2010;11(2):173–180. doi:10.1016/j.ejmhg.2009.10.001

60. Lankiewicz S, Rother E, Zimmermann S, Hoffmann C, Korangy F, Greten TF. Tumour-associated transcripts and EGFR deletion variants in colorectal cancer in primary tumour, metastases and circulating tumour cells. Cell Oncol. 2008;30(6):463–471.

61. Chen CJ, Sung WJ, Chen HC, et al. Early assessment of colorectal cancer by quantifying circulating tumor cells in peripheral blood: ECT2 in diagnosis of colorectal cancer. Int J Mol Sci. 2017;18(4):743. doi:10.3390/ijms18040743

62. Cui CH, Chen RH, Zhao Y, Li X, Qian J, Yu JL. Detection of FAM172A expressed in circulating tumor cells is a feasible method to predict high-risk subgroups of colorectal cancer. Tumour Biol. 2017;39(6):101428317699126. doi:10.1177/1028338416654431

63. El-Nasr MAM, El-Faham A, Ahamad EA, et al. Microarray-based identification of a panel of two mRNAs as blood biomarkers for colorectal cancer detection. OncoTarget. 2016;7(21):3029–3036. doi:10.18632/oncotarget.8108

64. Ogata-Kawata H, Izumiya M, Kurioka D, et al. Circulating exosomal microRNAs as biomarkers of colon cancer. PLoS One. 2014;9(4). doi:10.1371/journal.pone.0092921

65. Zhang J, Raju GS, Chang DW, Lin SH, Chen Z, Wu X. Global and targeted circulating microRNA profiling of colorectal adenoma and colorectal cancer. Cancer. 2018;124(4):785–796. doi:10.1002/cncr.31062

66. Ostenfeld MS, Jensen SG, Jeppesen DK, et al. miRNA profiling of circulating EpCAM+ extracellular vesicles: promising biomarkers of colorectal cancer. J Extracell Vesicles. 2016;5(1). doi:10.3402/jev.v5.31488

67. Rapado-González O, Alvarez-Castro A, Lopez-Lopez R, Iglesias-Canel J, Suarez-Cunqueiro MM, Muñoz-Romay L. Circulating microRNAs as promising biomarkers in colorectal cancer. Cancers. 2019;11(7). doi:10.3390/cancers11070898

68. Hao YX, Li YM, Ye M, et al. KRAS and BRAF mutations in serum exosomes from patients with colorectal cancer in a Chinese population. Oncol Lett. 2017;13(5):3606–3616. doi:10.3892/ol.2017.5889

69. Soldevilla B, Rodríguez M, Millán CS, et al. Tumor-derived exosomes are enriched in ApN73, which promotes oncogenic potential in acceptor cells and correlates with patient survival. Hum Mol Genet. 2014;23(2):467–478. doi:10.1093/hmg/ddt437

70. Chiba M, Kimura M, Asari S. Exosomes secreted from human colorectal cancer cell lines contain miRNAs, microRNAs and natural antisense RNAs, that can transfer into the human hepatoma HepG2 and lung cancer A549 cell lines. Oncol Rep. 2012;28(5):1551–1558. doi:10.3892/or.2012.1767

71. Barbagallo C, Brex D, Caponnetto A, et al. LncRNA UCA1, upregulated in CRC biopsies and downregulated in serum exosomes, controls mRNA expression by RNA-RNA interactions. Mol Ther. 2018;12:229–241. doi:10.1016/j.ymthe.2018.05.009
83. Hinz S, Bockhorst J, Roder C, et al. Disseminated tumor cells in blood as a prognostic factor in patients undergoing surgery for colorectal cancer. Br J Cancer. 2011;104(7):1178–1184. doi:10.1038/bjc.2011.40

84. Funaki NO, Tanaka J, Itami A, et al. Detection of colorectal cancer cells in peripheral blood by reverse transcription-polymerase chain reaction during colorectal cancer resection. Ann Surg. 2000;232(1):58–65. doi:10.1097/00000658-200007000-00009

85. Mathur P, Wharton RQ, Jonas SK, Saini S, Allen-Mersh TG. Relationship between tumour vascularity and circulating cancer cells in patients with colorectal carcinoma. Eur J Surg Oncol. 2001;27(4):354–358. doi:10.1016/s0267-3829(01)00118-8

86. Kust D, Samija I, Kirac I, Radic J, Kovacevic D, Kusic Z. Disseminated positive cells in blood of colorectal cancer patients with benign bowel disease. J Cell Mol Med. 2011;15(10 Suppl):S36. doi:10.1007/bf02062017

87. Piva MG, Navaglia F, Basso D, et al. CEA mRNA identifying free tumor cells in the peripheral blood of colorectal cancer patients during surgery with real-time RT-PCR on a LightCycler. Cancer Lett. 2002;183(2):195–203. doi:10.1016/s0304-3835(02)00157-x

88. Ito S, Nakashita H, Hira T, et al. Quantitative detection of CEA expressing free tumor cells in the peripheral blood of colorectal cancer patients via multiple blood sampling: prognostic significance of detection for early relapse. Br J Cancer. 2011;104(7):1178–1184. doi:10.1038/bjc.2011.40

89. Kanellos I, Zacharakis E, Kanellos D, et al. Significant detection of circulating cancer cells in the blood by reverse transcription-polymerase chain reaction during colorectal cancer resection. Ann Surg. 2000;232(1):58–65. doi:10.1097/00000658-200007000-00009

90. Lu CY, Uen YH, Tsai HL, et al. Molecular detection of persistent postoperative circulating tumour cells in stages II and III colon cancer patients via multiple blood sampling: prognostic significance of detection for early relapse. Br J Cancer. 2011;104(7):1178–1184. doi:10.1038/bjc.2011.40

91. Thorsteinsson M, Soletormos G, Jess P. Low number of detectable circulating tumor cells in non-metastatic colon cancer. Anticancer Res. 2011;31(2):613–617.

92. Yamaguchi T, Takagi Y, Aoki S, Futamura M, Saij S. Significant detection of circulating cancer cells in blood by reverse transcription-polymerase chain reaction during colorectal cancer resection. Ann Surg. 2000;232(1):58–65. doi:10.1097/00000658-200007000-00009

93. Mathur P, Wharton RQ, Jonas SK, Saini S, Allen-Mersh TG. Relationship between tumour vascularity and circulating cancer cells in patients with colorectal carcinoma. Eur J Surg Oncol. 2001;27(4):354–358. doi:10.1016/s0267-3829(01)00118-8

94. Guller U, Zajac P, Schneider A, et al. Disseminated single tumor cells as detected by real-time quantitative polymerase chain reaction represent a prognostic factor in patients undergoing surgery for colorectal cancer. Ann Surg. 2002;236(6):768–775; discussion 775–766. doi:10.1097/00000658-200212000-00009

95. Inuma H, Okinaka K, Egami H, et al. Usefulness and clinical significance of quantitative real-time RT-PCR to detect isolated tumor cells in the peripheral blood and tumor drainage blood of patients with colorectal cancer. Int J Oncol. 2006;28(2):297–306. doi:10.3892/ijo.28.2.297

96. Liu DP, Li LM, Liu XL, Zhang DX. Comparative analysis of tumor markers and evaluation of their predictive value in patients with colorectal cancer. Oncol. 2012;35(3):108–113. doi:10.1159/000338136

97. Huang P, Wang J, Guo Y, Xie W. Molecular detection of disseminated tumor cells in the peripheral blood in patients with gastrointestinal cancer. J Cancer Res Clin Oncol. 2003;129(3):192–198. doi:10.1007/s00432-003-0425-y

98. Nakamori S, Kameyama M, Furukawa H, et al. Genetic detection of colorectal cancer cells in circulation and lymph nodes. Dis Colon Rectum. 1997;40(10 Suppl):S29–S36. doi:10.1007/bf02062017

99. Hardingham JE, Hewett PJ, Sage RE, et al. Molecular detection of blood-borne epithelial cells in colorectal cancer patients and in patients with benign bowel disease. Int J Cancer. 2000;89(1):8–13. doi:10.1002/(sici)1097-0215

100. Shimada R, Inuma H, Akahane T, Watanabe T. Prognostic significance of CTCs and CSCs of tumor drainage vein blood in Dukes’ stage B and C colorectal cancer patients. Oncol Rep. 2012;27(4):947–953. doi:10.3892/or.2012.1649

101. Xi L, Nacisti DG, El-Hefnawy T, Hughes SJ, Luketich JD, Godfrey TE. Optimal markers for real-time quantitative reverse transcription PCR detection of circulating tumor cells from melanoma, breast, colon, esophageal, head and neck, and lung cancers. Clin Chem. 2007;53(7):1206–1215. doi:10.1373/clinchem.2006.081828

102. Winter M, Cai Z, Winkler K, et al. Circulating tumour cell RNA characterisation from colorectal cancer patient blood after inertial microfluidic enrichment. MethodsX. 2019;6:1512–1520. doi:10.1016/j.mex.2019.06.012

103. Liu Y, Cheng G, Qian J, et al. Expression of guanylyl cyclase C in tissue samples and the circulation of rectal cancer patients. Oncotarget. 2017;8(24):38841–38849. doi:10.18632/oncotarget.16406

104. Liu Y, Qian J, Feng JG, et al. Detection of circulating tumor cells in peripheral blood of colorectal cancer patients without distant organ metastases. Cell Oncol. 2013;36(1):43–53. doi:10.3892/cmo.2012-0112-6

105. Chalopin A, Tellez-Gabriel M, Brown HK, et al. Isolation of circulating tumor cells in a preclinical model of osteosarcoma: effect of chemotherapy. J Bone Oncol. 2018;7:83–90. doi:10.1016/j.jbo.2018.07.002
106. Inouma 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(12):1547–1555. doi:10.1200/JCO.2010.30.5151

107. Zieglschmid V, Hollmann C, Mannel J, et al. Tumor-associated gene expression in disseminated tumor cells correlates with disease progression and tumor stage in colorectal cancer. Anticancer Res. 2007;27(4B):1823–1832.

108. Sato N, Hayashi N, Imamura Y, et al. Usefulness of transcription-reverse transcription concerted reaction method for detecting circulating tumor cells in patients with colorectal cancer. Ann Surg Oncol. 2012;19(6):2060–2065. doi:10.1245/s10434-011-1889-7

109. Mirzaei A, Tavosiddiana G, Rad AA, et al. A new insight into cancer stem cell markers: could local and circulating cancer stem cell markers correlate in colorectal cancer? Tumor Biol. 2016;37(2):2405–2414. doi:10.1371/journal.pone.0123976

110. Shen C, Hu L, Xiao L, Li Y. Quantitative real-time RT-PCR detection for survivin, CK20 and CEA in peripheral blood of colorectal cancer patients. Jpn J Clin Oncol. 2008;38(11):770–776. doi:10.1093/jjco/hyn105

111. Schuster R, Max N, Mann B, et al. Quantitative real-time RT-PCR for detection of disseminated tumor cells in peripheral blood of patients with colorectal cancer using different mRNA markers. Int J Cancer. 2004;108(2):219–227. doi:10.1002/ijc.11547

112. Wang JY, Wu CH, Lu CY, et al. Molecular detection of circulating tumor cells in the peripheral blood of patients with colorectal cancer using RT-PCR: significance of the prediction of postoperative metastasis. World J Surg. 2006;30(6):1007–1013. doi:10.1007/s00268-005-0485-z

113. Bessa X, Elizalde JI, Boix L, et al. Lack of prognostic in human oxysterol-binding protein-homologue: a potential general molecular marker for blood dissemination of solid tumors. Cancer Res. 1999;59(15):3748–3753.

114. De Luca A, Pignata S, Casamassimi A, et al. Detection of circulating tumor cells in carcinoma patients by a novel epidermal growth factor receptor reverse transcription-PCR assay. Clin Cancer Res. 2000;6(4):1439–1444.

115. Clarke LE, Leitzel K, Smith J, Ali SM, Lipton A. Epidermal growth factor receptor mRNA in peripheral blood of patients with pancreatic, lung, and colon carcinomas detected by RT-PCR. J Oncol. 2003;22(2):425–430. doi:10.3922/joo.2003-22.2.425

116. Fournier MV, Guimarães Da Costa F, Paschoal ME, Ronco LV, Carvalho MG, Pardee AB. Identification of a gene encoding a human oxysterol-binding protein-homologue: a potential general molecular marker for blood dissemination of solid tumors. Cancer Res. 1999;59(15):3748–3753.

117. Gazzaniga P, Nofroni I, Gandini O, et al. Tenascin C and epidermal growth factor receptor as markers of circulating tumoral cells in bladder and colon cancer. Oncol Rep. 2005;14(5):1199–1202. doi:10.3892/or.14.5.1199

118. Yokobori T, Inouma H, Shimamura T, et al. Plastin3 is a novel marker for circulating tumor cells undergoing the epithelial-mesenchymal transition and is associated with colorectal cancer prognosis. Cancer Res. 2013;73(7):2059–2069. doi:10.1158/0008-5472.CAN-12-0326

119. Sugimachi K, Yokobori T, Inouma H, et al. aberrant expression of Plastin-3 via copy number gain induces the epithelial-mesenchymal transition in circulating colorectal cancer cells. Ann Surg Oncol. 2015;22(11):3680–3690. doi:10.1245/s10434-013-3366-y

120. Valladares-Ayerbes M, Díaz-Prado S, Rebolero M, et al. Bioinformatics approach to mRNA markers discovery for detection of circulating tumor cells in patients with gastrointestinal cancer. Cancer Detect Prev. 2008;32(3):236–250. doi:10.1016/j.cdpre.2008.08.002

121. Mostert B, Sieuwerts AM, Bolt-de Vries J, et al. mRNA expression profiles in circulating tumor cells of metastatic colorectal cancer patients. Mol Oncol. 2015;9(4):920–932. doi:10.1016/j.molonc.2015.01.001

122. Valladares-Ayerbes M, Blanco-Calvo M, Rebolero M, et al. Evaluation of the adenocarcinoma-associated gene AGR2 and the intestinal stem cell marker LGR5 as biomarkers in colorectal cancer. Int J Mol Sci. 2012;13(4):4367–4387. doi:10.3390/ijms13043867

123. Mirzaei A, Tavosiddiana G, Modarresi MH, et al. Uptregulation of circulating stem cell marker, DCLK1 but not Lgr5 in chemoradiotherapy-treated colorectal cancer patients. Tumour Biol. 2015;36(6):4801–4810. doi:10.1007/s13277-015-3132-9

124. Wang W, Wan L, Wu S, et al. Mesenchymal marker and LGR5 expression levels in circulating tumor cells correlate with colorectal cancer prognosis. Cell Oncol. 2018;45:1–10. doi:10.1007/s13042-018-0386-4

125. Ning Y, Zhang W, Hanna DL, et al. Clinical relevance of EMT and stem-like gene expression in circulating tumor cells of metastatic colorectal cancer patients. Pharmacogenomics J. 2018;18(1):29–34. doi:10.1038/s41373-016-0062

126. Gradiione A, Gazzaniga P, Silvestri I, et al. Detection of CK19, CK20 and EGFR mRNAs in peripheral blood of carcinoïd patients: correlation with clinical stage of disease. Oncol Rep. 2003;10(1):217–222. doi:10.3892/or.10.1.217

127. Shah T, Cui X, Li W, Lin W, Li Y. AQP5: a novel biomarker that predicts poor clinical outcome in colorectal cancer. Oncol Rep. 2014;32(4):1564–1570. doi:10.3892/or.2014.3377

128. Burchill SA, Perebolte L, Johnston C, Top B, Selby P. Comparison of the RNA-amplification based methods RT-PCR and NASBA for the detection of circulating tumour cells. Br J Cancer. 2002;86(1):102–109. doi:10.1038/sj.bjc.6600014

129. Miyashiro I, Kuo C, Huyhn K, et al. Molecular strategy for detecting metastatic cancers with use of multiple tumor-specific MAGE-A genes. Clin Chem. 2001;47(3):505–512.

130. Huang MY, Yen LC, Liu HC, et al. Significant overexpression of DVL1 in Taiwanese colorectal cancer patients with liver metastasis. Int J Mol Sci. 2013;14(10):20492–20507. doi:10.3390/ijms141020492

131. Steinert G, Scholch S, Niemietz T, et al. immune escape and survival mechanisms in circulating tumor cells of colorectal cancer. Cancer Res. 2014;74(6):1694–1704. doi:10.1158/0008-5472.CAN-13-1885

132. Katoh S, Goi T, Naruse T, et al. Cancer stem cell marker in circulating tumor cells: expression of CD44 variant exon 9 is strongly correlated to treatment refractoriness, recurrence and prognosis of human colorectal cancer. Anticancer Res. 2015;35(1):239–244.

133. Bao H, Burke PA, Huang J, et al. circulating tumor cells: application as a biomarker for molecular characterization and predictor of survival in an all-comer solid tissue phase i clinical study. PLoS One. 2013;8(8):e68557. doi:10.1371/journal.pone.0063264

134. Chang YT, Huang MY, Yeh YS, et al. A prospective study of comparing multi-genic biomarker chip and serum carcinoembryonic antigen in the postoperative surveillance for patients with stage I-II colorectal cancer. PLoS One. 2016;11(10):e0163264. doi:10.1371/journal.pone.0163264
