

Serum and tissue markers in colorectal cancer: State of art

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ABSTRACT

Colorectal cancer (CRC) represents one of the most commonly diagnosed cancers worldwide. It is the second leading cause of cancer death in Western Countries. In the last decade, the survival of patients with metastatic CRC has improved dramatically. Due to the advent of new drugs (irinotecan and oxaliplatin) and target therapies (i.e. bevacizumab, cetuximab, panitumab, aflibercept and regorafenib), the median overall survival has risen from about 12 mo in the mid nineties to 30 mo recently. Molecular studies have recently widened the opportunity for testing new possible markers, but actually, only few markers can be recommended for practical use in clinic. In the next future, the hope is to have a complete panel of clinical biomarkers to use in every setting of CRC disease, and at the same time: 1) to receive information about prognostic significance by their expression and 2) to be oriented in the choice of the adequate treatment. Moreover, molecular analyses have shown that the natural history of all CRCs is not the same. Individual patients with same stage tumors may have different long-term prognosis and response to therapy. In addition, some prognostic variables are likely to be more important than others. Here we review the role of serum and tissue markers according to the recently published English literature. This paper is an extension of the article "Biological and clinical markers in colorectal cancer: state of art" by Cappellani A published in Jan 2010.

1. Introduction

CRC is one of the most commonly diagnosed cancers in the world and remains the second leading cause of cancer death in Western countries. Approximately 50% of patients with CRC present, at diagnosis, distant metastases. From the late 1990s the median overall survival (OS) for patients with mCRC has increased from about 12 mo, for those treated with a 5-fluorouracil (5-FU)-based chemotherapeutic regimens, to approximately 18 mo with the addition of irinotecan and oxaliplatin (Jemal et al., 2011; Dušek and Mužík, 2017; Ferlay et al., 2010a,b, 2007). The availability of targeted biologics, in fact, along with the results obtained with chemotherapy alone, has increased the OS of mCRC to more than 24 mo, median. The use of monoclonal antibodies such as cetuximab, panitumumab and bevacizumab has improved the treatment options and the OS, but, on the other hand, has made the planning of treatment strategies increasingly articulated and complex. Furthermore, it is understood that the natural history of mCRC is not always the same; patients with mCRC may have various long-term prognosis and respond differently to the same treatment. All this justifies the frantic search for biological, prognostic and pre-

dictive markers able to implement the knowledge on the biology of the tumor and drive the clinician in an increasingly personalized decision-making process.

Therefore the information on the biology of CRC, together with the identification of serum and tissue markers with prognostic and predictive value play today a crucial role in the management of advanced disease and in the treatment of early-stage forms, offering new tools to estimate the possibility of cure and, more generally, the overall outcome of patient. It seems clear that the promise of personalized medicine in the treatment of mCRC is becoming a reality thanks to new knowledge of genetics that have allowed, at times, changing clinical practice.

2. Tissue markers

2.1. Diagnostic tissue markers

Immunohistochemistry (IHC) is commonly used in the diagnosis of gastrointestinal neoplasms to facilitate accurate tumor classifi-

cation. There are two practical goals: one is to confirm a tumor diagnosis by excluding morphologic mimickers or to identify the most reasonable tissue or organ of origin in cases of metastatic carcinoma of unknown primary. The other is to provide meaningful prognostic information and even predict responsiveness to standard chemotherapy or novel molecular targeted therapy (see paragraphs below). Diagnostic tissue biomarkers therefore provide additional and fundamental information that complement clinical colonoscopy findings.

2.2. Cytokeratins

The cytokeratins (CKs), members of the family of intermediate filaments along with vimentin, desmin, neurofilament, and glial filament, are proteins expressed by epithelial cells. Nonneoplastic colonic mucosa proximal to the rectum exhibits a CK7-/CK20+ phenotype, as do 90% of CRCs (Chu et al., 2000). The only other neoplasms to demonstrate this CK7-/CK20+ phenotype in a significant proportion of cases are Merkel cell carcinoma and a fraction of gastric adenocarcinomas (GAs) (Chu et al., 2000). CK7 staining, when present in CRC, is often patchy and less intense than tumors from other sites that exhibit CK7 positivity (Wauters et al., 1995; Vang et al., 2006). The inclusion of CK17 in the diagnostic workup has also been shown to be beneficial because less than 10% of CRCs express CK17, whereas pancreatic ductal carcinomas are consistently positive and a number of carcinomas from other sites, including stomach, endometrium, and bladder may show CK17 expression (Miettinen et al., 1997).

2.3. CDX2

CDX2 is a transcription factor encoded by CDX2, a member of the caudal subgroup of homeobox genes. It is essential for embryonic and lifelong maintenance of a cellular intestinal phenotype (Silberg et al., 2000). CDX2 is strongly expressed in epithelial cells of the normal small intestine, appendix, colon, and rectum as well as the centroacinar and interacinar ductal cells of the pancreas (Moskaluk et al., 2003). CRCs (with the exception of those exhibiting MSI) consistently express CDX2 (Werling et al., 2003). The distribution of CDX2 expression is far more diverse in neoplastic tissues, with positive staining seen in a variety of adenocarcinomas that exhibit intestinal-type differentiation, including adenocarcinomas of the gastroesophageal junction, bladder, urachus, small bowel, pancreas, appendix, and ovary (Werling et al., 2003).

2.4. Villin

Villin is a microfilament-associated, actin-binding cytoskeletal protein normally expressed in cells with highly specialized, brush border-type microvilli such as enterocytes (Bretscher and Villin, 1979). The specificity of villin as a marker of intestinal origin is limited because, like CDX2, expression may be seen in adenocarcinomas with intestinal differentiation arising from a variety of sites including the stomach, lung, and ovary as well as, rarely, in malignancies of other sites such as the endocervix and liver (Bacchi and Gown, 1991).

2.5. Beta-catenin

β -Catenin is a multifunctional protein involved in both cell adhesion and intracellular signaling, the latter function being accomplished through β -catenin's actions in the Wnt signaling pathway (Willert and Nusse, 1998). Activation of the Wnt signaling pathway results in an increase in the cytoplasmic pool of free β -catenin and, to a lesser degree, within the nucleus where it induces proliferation. In the absence of functional adenomatous

polyposis coli (APC), as is commonly seen in CRC, nuclear β -catenin can be identified immunohistochemically (Clevvers, 2006). Although nuclear expression of β -catenin is not unique to CRC, it can be useful as part of a panel in selected settings.

2.6. Carcinoembryonic antigen (CEA)

The gene product members of the CEA subgroup are membrane-associated glycoproteins with variable roles in cell adhesion or signal transduction (Sheahan et al., 1990). Monoclonal CEA (mCEA) may be expressed in a wide variety of adenocarcinomas, including those arising from the colon (Zhou et al., 2002). Although this lack of specificity limits its overall utility as a diagnostic marker of CRC, it remains a useful component of a panel in selected cases.

2.7. Alpha-methylacyl-CoA racemase

α -Methylacyl-CoA racemase (AMACR/p504s) is a peroxisomal and mitochondrial enzyme involved in β -oxidation of branched-chain fatty acids. Among malignancies, AMACR protein expression is frequently seen in adenocarcinomas of the prostate and colon (Cao et al., 1997). Although lacking specificity, AMACR has been shown to be very useful as a panel component in selected cases, particularly in the differentiation of CRC from ovarian carcinomas. Reduced sensitivity of AMACR expression has been identified in more poorly differentiated CRCs as well as decreased expression has been found in CRCs compared with precursor adenomatous lesions (Hanski et al., 1997).

2.8. Mucins

Within the central portions of the polypeptide backbones of these glycoprotein molecules are heavily O-glycosylated, tandemly repeated sequences of amino acids rich in threonine and serine moieties: the various combinations of these repeats are unique for each mucin type. The colon normally contains a mixture of neutral, sialomucin, and sulphomucin with MUC2 being the most prominent, primarily in goblet cells. MUC4 is also abundant in the colon and is expressed in both goblet and columnar cells, whereas MUC3 appears to be expressed primarily within enterocytes (Hanski et al., 1997). MUC1, MUC5AC, and MUC6 are not normally expressed in the colonic mucosa (Hanski et al., 1997). MUC2 is frequently expressed in mucinous CRC as well as mucinous carcinomas of the ovary, breast, and pancreas (Hanski et al., 1997). Gastric mucins may also be expressed in CRC. MUC5AC expression is associated with mucinous differentiation and MSI, with most mucinous carcinomas exhibiting a MUC2+/MUC5AC+ phenotype (Park et al., 2006).

2.9. Cadherin 17

Cadherin 17 (CDH17) is also known as liver-intestine cadherin because it was originally discovered as a novel calcium-dependent cell adhesion molecule expressed in the liver and intestine of rats (Dantzig et al., 1994). In humans its distribution is actually limited to the duodenum, jejunum, ileum, colon, and part of the pancreatic duct. It is believed to function as an intestinal peptide transporter (Su et al., 2008). Its clinical utility in diagnosing GI tumors was only recognized recently (Panarelli et al., 2012; Zongming and Fan, 2015). Recent data indicate that positive CDH17 immunoreactivity is most commonly seen in colorectal adenocarcinomas up to 96% and a significant portion of gastric, pancreatic, and biliary adenocarcinomas (25%–50%). It is rarely found in adenocarcinomas from outside of GI tract (1%–10%). Interestingly, although CDH17 is transcriptionally regulated by CDX2, some authors found it to be slightly

more sensitive and specific than CDX2 in identifying colorectal adenocarcinomas (Panarelli et al., 2012; Zongming and Fan, 2015). CDH17 also demonstrated its usefulness in diagnosing CRC variant with poorly differentiated or undifferentiated morphology, such as medullary carcinoma, which characteristically lacks expression of conventional intestinal differential markers, such as CK20 and CDX2 (Panarelli et al., 2012).

2.10. Special AT-rich sequence binding protein 2

Special AT-rich sequence binding protein 2 (SATB2) belongs to a family of nuclear matrix-associated transcription factors that function as epigenetic regulators of gene expression in a tissue-specific manner (Magnusson et al., 2011). Studies have shown that SATB2 carries out a wide spectrum of biologic functions. However, the role of SATB2 in the GI tract is still elusive. Recently, Magnusson et al. (2011) found that SATB2 immunoreactivity was restricted to the glandular lining cells of the human lower GI tract, including appendix, colon, and rectum. SATB2 is a highly sensitive and specific marker for adenocarcinomas of the colon and rectum, with a diagnostic sensitivity of 97% (121 of 125 cases) in CRCs (Magnusson et al., 2011) and of 81% in CRC metastases (Magnusson et al., 2011).

The expression of all above listed markers in the main differential diagnoses of CRC is summarized in Table 1.

2.11. Prognostic tissue markers

Beside their diagnostic potential, mucins, SATB2 protein and CK20/CDX2 expression are also related with important prognostic information in CRC patients. Studies addressing the potential implications of MUC2 expression in the prognostic/predictive arena have also yielded mixed results (Perez et al., 2008; Lugli et al., 2007a). Although the significance of MUC2 expression may be, in part, related to its association with MSI, Lugli et al. (2007a) identified loss of MUC2 expression as a poor prognostic indicator in both mismatch repair protein (MMR)-proficient and MLH1-negative CRC. Moreover, a recent study on mucins immunohistochemical expression in 381 CRCs, using tissue microarray, proved that loss of MUC2 expression was a predictor of adverse outcome (Betge et al., 2016). The role of MUC1 seems controversial, either unrelated to CRC patients' outcome (Betge et al., 2016), or related to tumor grade (Kesari et al., 2015).

High SATB2 expression was associated with good prognosis in colon cancer and might modulate sensitivity to chemotherapy and radiation, whereas reduced expression of SATB2 in colorectal adenocarcinomas was found to be associated with poor prognosis, including tumor invasion, lymph node metastasis, and distant metastasis (Wang et al., 2009; Eberhard et al., 2012). Moreover, loss of CDX2 expression is associated with proximal location, infiltrative growth, advanced T, N, M, overall stage and is an independent poor prognostic factor of overall survival and progression-free survival (Bae et al., 2015).

2.12. MSI-H status and expression profile of MMR proteins

MSI status in CRCs can be determined by DNA testing using microsatellite markers, and five microsatellite markers recommended by the National Cancer Institute (NCI) workshop have been officially used for MSI analysis: BAT25, BAT26, D2S123, D5S346 and D17S250 (Jenkins et al., 2007). In DNA analysis using these NCI markers, instability observed in two or more of the five markers corresponds to MSI-H (high level of MSI instability). MSI-H CRC is known to have distinct clinicopathological and molecular features, including preferential localization in the proximal colon, a less advanced cancer stage, extracellular mucin production, medullary carcinoma and poorly differentiated carcinoma, tumor infiltrating

lymphocytes, a Crohn's-like lymphoid reaction, and a BRAF V600E mutation (Ogino et al., 2009). Normal DNA MMR function is executed by MMR protein complexes composed of heterodimers of MutL homologues (the MLH or PMS series) or MutS homologues (the MSH series). Therefore, loss of expression of MMR proteins can serve as a molecular hallmark of the MSI-H status in tumors. IHC for MMR proteins is a simple and valuable tool for investigating the underlying molecular alteration and hereditary/sporadic status of MSI-H CRCs. The immunohistochemical profile of four MMR proteins in MSI-H CRCs can be summarized as four expression phenotypes: MLH1-negative/PMS2-negative, PMS2-negative only, MSH2-negative/MSH6-negative, and MSH6-negative only (Pino and Chung, 2011; Geiersbach and Samowitz, 2011). These four phenotypes most likely represent inactivation of MLH1, PMS2, MSH2, and MSH6, respectively. The majority of MSI-H CRCs are induced by inactivation of MLH1 or MSH2, whereas inactivation of PMS2 or MSH6 causes only a minor portion of MSI-H CRCs.

Putative prognostic biomarkers for CRC in relation to its molecular classification

Dorard et al. (Dorard et al., 2011) recently reported that the expression level of mutant HSP110 (heat shock protein 110 kDa) is significantly associated with prognosis and chemotherapy response in MSI-H CRCs. MSI-H CRC patients with a high mRNA expression level of HSP110 Δ E9 survived longer, and this improved survival was maintained in both stage III and adjuvant chemotherapy-treated subgroups (Dorard et al., 2011). The expression status of wild-type HSP110 (HSP110 wt) was evaluated by IHC in MSI-H CRCs (Kim et al., 2014): reduced expression of HSP110 wt was correlated with a large deletion in the HSP110 T17 repeat and favorable prognosis in MSI-H CRCs, which is reasonable because the HSP110 wt expression level is expected to be inversely correlated with the HSP110 Δ E9 expression level. Mutation of HSP110 and variation in HSP110 expression are representative of the molecular heterogeneity associated with the prognostic heterogeneity of MSI-H CRCs. According to recent investigations, coding microsatellite mutations in the beta2-microglobulin gene occur in approximately 30% of MSI-H CRCs and are significantly associated with a low risk of disease relapse and a low frequency of distant metastasis in MSI-H CRCs (Tikidzhieva et al., 2012). A recent study by Mazzolini et al. (2012) reported that the brush border protein myosin 1a (MYO1A) could act as a tumor suppressor in the intestine, and frameshift mutations in the MYO1A gene were detected in 32% of MSI-H CRCs. Interestingly, according to this study, a low expression level of MYO1A was associated with worse survival in patients with MSI-H CRCs, and MYO1A expression was identified as an independent prognostic factor in MSI-H CRCs. Several previous studies identified that a loss of CDX2 and/or CK20 expression in CRCs was associated with MSI-H or CIMP-H status (Lugli et al., 2008; Baba et al., 2009). In a recent investigation, a loss of CDX2/CK20 expression was significantly associated with poor differentiation, CIMP-H status, and an unfavorable prognosis in MSI-H CRCs (Kim et al., 2013). According to this study, CRC patients with simultaneous loss of CDX2 and CK20 expression in tumor tissue constituted a highly aggressive subgroup of MSI-H CRC patients, with early death or recurrence occurring in this subgroup. In a recent investigation by Isaksson-Mettävainio et al. (2012), high SMAD4 (SMAD family member 4) expression was significantly correlated with a favorable prognosis in MSI-H CRCs. Previous studies have also revealed that a loss of SMAD4 expression is associated with advanced stage, metastatic potential and an adverse prognosis in CRCs (Alazzouzi et al., 2005; Tanaka et al., 2008, 2006; Miyaki et al., 1999), regardless of its molecular classification. Ogino et al. (2009) provided contrasting data showing that CIMP-H was associated with a low cancer-specific mortality in CRC patients, regardless of both MSI status and BRAF mutations. In addition, a study by Dahlin et al. (2010) found that the CIMP-L subtype was associated with an

Table 1
Immunohistochemical expression of useful markers in the differentials of CRC.

Carcinoma	CK7	CK20	CK17	CDX2	Villin	CEA	AMACR	MUC2	MUC5A	MUC1	CDH17	SATB2
Anal gland	+	–	NA	–	NA	+	NA	NA	+	NA	NA	NA
Ovary- mucinous	+	+/- ^a	–	-/+ ^a	+/-	+/-	-/+	+	+/-	NA	NA	NA
Ovary- endometrioid	+	–	NA	- ^b	-/+	- ^b	-/+	NA	NA	–	NA	NA
Uterus- endometrioid	+	–	-/+	- ^b	-/+	–	–	NA	NA	+/-	NA	NA
Stomach-intestinal	+/-	+/-	-/+	+/- ^a	+/-	+/-	+/-	+/-	+/-	-/+	+	-/+
Stomach-diffuse	+/-	+/-	–	+/- ^a	–	+	-+	+/-	+/-	+/-	+	-/+
Small bowel	+	+/-	NA	+/-	+/-	NA	–	+/-	+/-	+/-	+	-/+
Pancreas	+	+/-	+	+/- ^a	-/+	+/-	–	–	+/-	NA	+/-	–
Bladder- transitional	+	+/-	+/-	–	–	+/-	-/+	NA	NA	NA	–	–
Bladder- adenocarcinoma	+/-	+/-	NA	+/-	+/-	+/-	+/-	NA	NA	NA	-/+	–
Bladder- urachal	+/-	+	NA	+	NA	NA	NA	+	+	–	NA	NA
Lung- mucinous BAC	+	+/-	NA	–	–	NA	NA	+/-	+/-	+	NA	NA
Lung- enteric	+	-/+	NA	+/-	NA	NA	NA	-/+	-/+	+	–	–
Lung-mucinous	+	-/+	NA	+/-	NA	NA	NA	-/+	-/+	+	NA	NA

Abbreviations: –, negative in most cases (b10% of cases exhibit positive staining); +, positive in most cases (N90% of cases exhibit positive staining); +/-, positive in most of cases (50%–90% of cases exhibit positive staining); -/+, negative in most of cases (10%–50% of cases exhibit positive staining); NA, information unavailable or deemed too limited for routine application, BAC= bronchiolo-alveolar carcinoma.

^a Heterogeneous and weak staining pattern typical.

^b Positivity may be seen in areas of squamous and morular differentiation.

unfavorable prognosis for CRCs, regardless of MSI status. Focusing on the prognostic implication of CIMP for MSI-H CRCs, among MSI-H CRC patients, those with CIMP-H tumors had worse survival than those with CIMP-L/O tumors (Bae et al., 2011). Several recent investigations have revealed that a low long interspersed nucleotide element-1 (LINE-1) methylation level is independently associated with an adverse prognosis for CRCs (Baba et al., 2010; Ogino et al., 2008; Rhee et al., 2012). This prognostic significance of LINE-1 methylation was also maintained in MSI-H CRCs: a low LINE-1 methylation level was an independent factor indicating poor prognosis in MSI-H CRC (Rhee et al., 2012).

2.13. Prognostic relevance of epithelial-mesenchymal transition (EMT) in CRC

Three core groups of transcriptional regulators, which are able to suppress E-cadherin transcription directly or indirectly, initially drive the EMT process. The first group is the transcription factors of the Snail zinc-finger family, including SNAI1 and SNAI2 (SLUG) (Cao et al., 2015). The second group is the distantly related zinc-finger E-box-binding home box family proteins ZEB1 and ZEB2 (SIP1) (Kroepil et al., 2013). The last group is the basic helix–loop–helix (bHLH) family of transcription factors, including TWIST1, TWIST2 and E12/E47 (Hoshino et al., 2009). In CRC, 85% of resected specimens have moderate to strong Twist1 expression, which is notably more than either SNAI1 or SLUG (Toiyama et al., 2013). SLUG and ZEB1 expression is significantly correlated with lower expression of E-cadherin (Shioiri et al., 2006) and up-regulation of ZEB1 and ZEB2 at the invasion front both correlate with shorter survival times (Welch-Reardon et al., 2014). The clinical significance of the different expression of these EMT markers is summarized in Table 2 (Findlay et al., 2014; Singh et al., 2011; Spaderna et al., 2006; Kahlert et al., 2011; Kilic et al., 2011; Sarkar et al., 2012; Jackstadt et al., 2013; Celesti et al., 2013; Valdes-Mora et al., 2009; Okada et al., 2010; Mani et al., 2007; Watanabe et al., 2011; Han et al., 2012; Dai et al., 2013; Meng et al., 2010; Lu et al., 2012; Takahashi et al., 2013; Diesch et al., 2014).

2.14. MicroRNAs

MicroRNAs (miRNAs; miR) are evolutionarily conserved small noncoding RNAs that are able to control gene expression at a post-transcriptional level, either by blocking mRNA translation or inducing their degradation. The potential role of some important miRNAs (including miR-21, -29, -34a, -124a, -155, -224) as prog-

nostic (and predictive) biomarkers in CRC is evident in Table 3 (Shibuya et al., 2010; Yamamichi et al., 2009; Xia et al., 2013; Fukushima et al., 2015; Kjaer-Frifeldt et al., 2012; Nana-Sinkam et al., 2010; Huang et al., 2010; Tang et al., 2014; Wang and Gu, 2012; Tazawa et al., 2007; Gao et al., 2015; Hiyoshi et al., 2015; Akao et al., 2011; Shen et al., 2005; Leedham et al., 2009; Ueda et al., 2014; Zhang et al., 2013; Hongliang et al., 2014; Lv et al., 2015; Liao et al., 2013; Ling et al., 2015; Salendo et al., 2013).

2.15. Predictive tissue markers

2.15.1. BRAF V600E IHC

BRAF represents one of the most frequently mutated protein kinase genes in human tumors (Davies et al., 2002). In CRC, the most common mutation is BRAF V600E. Currently, the mutation is tested in CRC mainly for two purposes. BRAF V600E mutation in MSI CRCs can virtually exclude Lynch syndrome, and mutation positive tumors are resistant to anti-epithelial growth factor receptor (EGFR) therapy (Toon et al., 2013; Kuan et al., 2014). Although it has been shown that BRAF V600E mutation predicts a poor prognosis in right-sided microsatellite-stable CRC; this prognostic indication has not been widely explored clinically. Traditionally, BRAF mutation is detected by DNA sequencing or polymerase chain reaction–based mutation detection methods. Recently, antibodies specific to BRAF V600E have been developed, and their use in IHC on formalin-fixed, paraffin-embedded tumor tissue has become popular (Preusser et al., 2013; Tiacci et al., 2011; Sinicrope et al., 2013; Toon et al., 2014). It seems that a new trend of using IHC as screening test for BRAF V600E mutation in CRC has evolved, as some authors have proposed incorporating BRAF V600E IHC into the current algorithm for universal screening of CRC for Lynch syndrome (Preusser et al., 2013; Tiacci et al., 2011; Sinicrope et al., 2013; Toon et al., 2014; Routhier et al., 2013). Most recently published data indicate that BRAF V600E IHC is a sensitive and reliable assay, generating results that correlated well with those from molecular detection methods (Preusser et al., 2013; Tiacci et al., 2011; Sinicrope et al., 2013; Toon et al., 2014; Routhier et al., 2013).

2.15.2. EMT and drug resistance

An increasing number of findings suggest that tumors undergoing EMT resist conventional drug therapy. In clinical, tumor specimens taken from CRC patients who have received preoperative chemotherapy followed by radical surgery display phenotypic changes characteristic of and molecular changes consistent with the EMT. Consistently, loss of E-cadherin promotes drug resistance

Table 2
Transcription factors involved in EMT and CRC progression.

Transcription factor	Molecular family	Clinical significance in CRC	Refs.
SNAI1	Zinc-finger protein	Metastasis, chemoresistance, poor prognosis	Cao et al. (2015), Kroepil et al., (2013), Hoshino et al., (2009)
SLUG	Zinc-finger protein	Metastasis, poor survival, poor prognosis, pathological angiogenesis, drug resistance	Toiyama et al. (2013), Shioiri et al. (2006), Welch-Reardon et al. (2014), Findlay et al. (2014)
ZEB1	Zinc-finger E-box binding homeobox protein	Invasion, metastasis, poor survival	Singh et al. (2011), Spaderna et al. (2006)
ZEB2	Zinc-finger E-box binding homeobox protein	Tumor progression, poor survival	Kahlert et al. (2011)
Brachyury	T-box family of transcription factor	Poor prognosis	Kilic et al. (2011), Sarkar et al. (2012)
AP4	bHLH-zipper factor	Liver metastasis, poor survival	Jackstadt et al. (2013)
TWIST1	bHLH factor	Nodal metastasis, poor prognosis	Celesti et al. (2013), Valdes-Mora et al. (2009), Okada et al. (2010)
FOXC2	Forkhead family of transcription factor	Nodal metastasis	Mani et al. 2007), Watanabe et al. 2011)
SOX2	SRY-related HMG-box (SOX) factor	Liver and lymph node metastasis	Han et al. (2012)
OCT	Octamer-binding transcription factor	Liver metastasis	Dai et al. (2013)
Nanog	Homeobox protein	Nodal metastasis, poor prognosis	Meng et al. (2010)
PROX1	Homeobox protein	Nodal metastasis, advanced tumor stage	Lu et al. (2012)
PRRX1	Homeobox protein	Metastasis and poor prognosis	Takahashi et al. (2013)
Fra-1	Fos family of transcription factor	Higher T-stage, poor recurrence-free survival	Diesch et al. (2014)

Table 3
Functions of different miRNAs in CRC.

MiRNA	Prognostic relevance	Predictive role	Refs.
MiR-21	Increased MiR-21 correlates with CRC cell proliferation, invasion, lymph node metastases; advanced clinical stage, poor OS and DFS in different Duke stages	Decreased MiR-21 sensitized CRC cells to 5-FU treatment	Shibuya et al. (2010), Yamamichi et al. (2009), Xia et al. (2013), Fukushima et al. (2015), Kjaer-Frifeldt et al. (2012), Nana-Sinkam et al. (2010)
MiR-29	Elevated miR-29a is significantly correlated with metastases, especially liver metastases and could be used to predict survival.	–	Huang et al. (2010), Tang et al. (2014), Wang and Gu (2012)
MiR-34	Downregulation of miR-34a is associated with CRC development; miR-34a predicates recurrence in CRC; Increased miR-34b/c in more advanced tumors and associated with poor prognosis; it could predict recurrence of stage II and III CRC patients	Increased MiR-34sensitized CRC cells to 5-FU treatment	Tazawa et al. (2007), Gao et al. (2015), Hiyoshi et al. 2015, Akao et al. (2011)
MiR-124	High frequency of methylation of miR-124a in chronic inflammation and CRC; useful biomarker for evaluating carcinogenetic risk in Ulcerative colitis patients	–	Shen et al. (2005), Leedham et al. (2009), Ueda et al. (2014)
MiR-155	High expression correlates with advanced stage and metastasis; increased postoperative expression correlates with recurrence and metastasis; miR-155 could be considered a prognostic marker for OS and DFS of CRC patients	–	Shibuya et al. (2010), Zhang et al. (2013), Hongliang et al. (2014), Lv et al. (2015))
MiR-224	Increased expression associated with tumor growth and metastasis; it is a predictor of short-time relapse and shorter metastasis-free survival	Suppression of miR-224 sensitizes CRC to chemotherapy	Liao et al. (2013), Ling et al. (2015), Salendo et al. (2013)

in CRC patients (Chen et al., 2012) and the recurrent tumors have a significantly EMT-like signature (Kawamoto et al., 2012). SNAI1-expressing cells are also associated with resistance to dendritic-cell immunotherapy (Kudo-Saito et al., 2009), implying that inhibition of SNAI1-induced EMT simultaneously suppresses both metastasis and immunosuppression in cancer patients. Besides the evidence of drug resistance to chemotherapy and immunotherapy, recent studies have also suggested a role of the EMT in drug resistance to targeted therapies. Pancreatic and CRC cell lines insensitive to EGFR inhibition often express proteins associated with an EMT, such as vimentin, ZEB1 and SNAI1. In turn, epithelial cells show an average

7-fold greater sensitivity than mesenchymal-like cells (Kudo-Saito et al., 2009), implying a strong correlation between EMT activation and drug resistance.

2.15.3. EGFR expression (IHC and in situ hybridization-ISH) and EGFR targeted therapy predictive markers

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein with an intracellular tyrosine kinase domain. Binding of ligands to the EGFR promotes tumor growth and progression by controlling transcription, cell-cycle progression, apoptosis and differentiation through the EGFR downstream signal pathway.

EGFR monoclonal antibodies [e.g. cetuximab and panitumumab] are active drugs in combined chemotherapy or as monotherapy for metastatic CRC (Buck et al., 2007; Cunningham et al., 2004; Van Cutsem et al., 2007; Jonker et al., 2007; Sobrero et al., 2008; Douillard et al., 2010). Gene mutation of KRAS codon 12/13 is recognized as a strong predictive factor for no-benefit in anti-EGFR antibody treatment (De Roock et al., 2008; Lievre et al., 2008; Karapetis et al., 2008a). EGFR overexpression by IHC was reported to be associated with poor prognosis in advanced CRC (Galizia et al., 2006; Rego et al., 2010). In the metastatic state, different studies showed opposing results in the efficacy of EGFR as a predictor of response to anti-EGFR treatment (Saltz et al., 2004; Hebbar et al., 2006; Chung et al., 2005; Takahashi et al., 2014). Recently, Takahashi et al. proved on formalin-fixed paraffin-embedded tissue samples, that combined evaluation of EGFR IHC and dual color in situ hybridization (DISH) and genomic analysis of downstream effectors of EGFR by direct sequencing are useful to predict the response to anti-EGFR antibodies treatment in metastatic CRC patients (Takahashi et al., 2014). However, a meaningful proportion of patients with metastatic CRC have tumors bearing mutations in RAS genes other than KRAS codon 12/13. Recent studies have demonstrated that evaluation of an extended panel of RAS mutations—including mutations in KRAS exon 2, 3, and 4 and NRAS exons 2, 3, and 4—can better define the patient population that is unlikely to benefit from anti-EGFR therapy, with concomitant improvements in outcomes in the more highly selected RAS wild-type group (Hecht et al., 2015). Distinct from the RAS/RAF pathway, phosphoinositide-3-kinase (PI3K), AKT, and PTEN also are downstream effectors of EGFR signaling. Mutations in these genes may also affect anti-EGFR therapy responsiveness. In retrospective studies, patients with CRCs that have PIK3CA exon 20 (kinase domain) mutations have much worse outcomes with cetuximab compared to patients with PIK3CA-wildtype CRCs (De Roock et al., 2010). PIK3CA exon 9 (helical domain) mutations do not seem to serve as a predictive marker for anti-EGFR therapy, which highlights the complexity of the effects of the specific mutations on the functions of the altered kinases. Of interest, PIK3CA exon 9 mutations are often seen with KRAS mutations and KRAS remains the stronger predictive biomarker. Collectively, approximately 70–80% of cases unresponsive to EGFR targeted therapy appear to be secondary to mutations in KRAS, NRAS, PIK3CA and other proteins relevant to EGFR signaling (De Roock et al., 2010; Bardelli et al., 2013; Bertotti et al., 2011).

PIK3CA mutations may also be beneficial in guiding secondary prevention options. Large studies have demonstrated that aspirin reduces adenoma and CRC formation (Chan et al., 2009). More recent observational data suggests that the aspirin benefit is limited to individuals with PIK3CA-mutant tumors (Liao et al., 2012). The use of aspirin in secondary prevention remains a complex issue because of the risks of aspirin use and further study is required.

These discoveries have changed the practice of oncology and have the potential to spare patients from exposure to ineffective therapy.

2.15.4. CD133 as a potential marker for traditional and targeted treatment

Vascular endothelial growth factor (VEGF) and its receptors VEGFR-1, VEGFR-2, and VEGFR-3 are intimately involved in cell migration and proliferation and promote endothelial cell survival and protection against endothelial cell apoptosis and senescence. Bevacizumab is a recombinant humanized monoclonal IgG1 antibody against VEGF-A that decreases the availability of free circulating VEGF-A, preventing receptor activation and that significantly improved overall survival in patients with metastatic CRC (Hurwitz et al., 2004). CD133, a surface protein widely used for the isolation of colon cancer stem cells, is associated with

tumor angiogenesis and recurrence. Pohl et al. (2013) and Lu et al. (2008) showed that patients with high gene expression levels of CD133 (>7.76) showed a significantly greater tumor response than patients with low expression levels ($p=0.003$), independent of the expression of VEGF or its receptor. Moreover, Ong et al. (2010) and Lu et al. (2007) demonstrated that positive IHC expression of CD133 was associated with significantly worse survival in patients treated by surgery alone ($P=0.023$) and in patients treated with 5-fluorouracil-based chemotherapy ($P=0.001$). Stage III patients with negative CD133 expression showed an apparent survival benefit from 5-fluorouracil treatment ($P=0.002$), but not those with positive CD133 expression. Positive expression of CD133 was also associated with poorer clinical response to chemotherapy in stage IV patients ($P=0.006$).

3. Serum markers

Serum biomarkers are striking potential tools for surveillance and early diagnosis of colorectal cancer (Hurwitz et al., 2004). Aside from tests on feces, many tests have been recently developed aimed to use humoral immunity against tumor associated antigens as possible biomarkers in early diagnosis of cancer. Unfortunately, only few genes are available for this kind of search.

3.1. P53

Encoded by TP53 gene, located on short arm of chromosome 17, p53 is a transcription factor and one of more investigated oncosuppressor gene. Many functions have been recognized for p53: repair of damaged DNA, block of cellular cycle until repair has been ultimated and promoting of apoptosis. Its alterations are frequent at early stage in natural history of many cancers. This is its force and its weakness since determines its lack of specificity.

3.2. Others

P53 is not the only gene able to raise an immunological reaction and against which serum antibodies have been found in serum: carcinoembryonic antigen, Ras, topoisomerase II-alpha, histone deacetylase 3 and 5, ubiquitin C-terminal hydrolase L3, tropomyosin and cyclin B1 have been studied an observed in serum of CRC patients. Though none of these genes is able alone to detect all cases of cancer, since each antibody is present only in a limited proportion of patients (usually <40%), so appreciable results could be achieved only developing a panel comprising all these genes (signature) (Pohl et al., 2013; Ong et al., 2010; Lu et al., 2012).

3.3. SELDI-TOF-MS

Proteomic technology based on a particular type of laser, Surface enhanced laser desorption/ionization-time of flight-mass spectrometry (SELDI-TOF-MS) and Protein-Chip arrays, can provide high-throughput protein profiling and has showed the ability to discriminate the serum of patients with a cancer and even the stage of cancer with high sensitivity and specificity and a total accuracy of over 75% up to 85% (Xu et al., 2006).

3.4. Alpha defensin

The alpha defensin 1, 2 and 3, produced in granulocytes, macrophages and Paneth cell of the small intestine share with the other members of family the antimicrobial effects but further enhance phagocytosis and increase the production of Tumor Necrosis Factor. The increase in serum of patients with different type of cancers has been related either with invasion of tumor by granulocyte, either with a direct production by tumor cells and this late

opinion is actually predominant. Proteomic techniques can identify the alpha-defensin in serum of CRC patients with 100% of sensitivity and 69% of specificity (Yamashita and Watanabe., 2009; Albrethsen et al., 2005).

3.5. C3A anaphylatoxin

Complement-derived anaphylatoxin peptides, expressed in bronchial epithelium and smooth muscle cell in sepsis and asthma, have been demonstrated in serum of CRC patients with 97% sensitivity and 96% specificity, and in 86% of patients with colorectal adenomas. Although still not sufficiently validated, this marker appears very promising (Yamashita and Watanabe., 2009; Habermann et al., 2006).

3.6. Hereditary cancers setting

Approximately 15% of CRC are thought to be due to an inherited or familial predisposition (Rowley, 2005). The most common hereditary conditions giving rise to an increased risk of CRC are hereditary non-polyposis CRC (HNPCC) and familial adenomatous polyposis (FAP). In this particular high risk, setting it is important to identify specific screening exams.

3.7. Fap

FAP is an autosomal dominant condition characterized by hundreds to thousands of adenomas in the colon and rectum. It has an incidence of approximately 1 per 8000–1 per 14,000 of the population and accounts for about 0.5% of all CRC (Fearnhead et al., 2002). CRC in an almost inevitable consequence of classical FAP, if untreated, with an average of onset of about 39 years of age. An attenuated form of this syndrome (AFAP) is characterized by fewer adenomas (<100) Subjects with this attenuated form of FAP also have a high risk of developing CRC, i.e. approximately 80% by the age of 70 years. A further variant of the FAP syndrome results from biallelic inherited mutations in the BER. (base excision repair) gene, known as *MutYH* or *MYH* (Sampson et al., 2005). This syndrome, which is now referred to as MAP or MYH-associated polyposis, is often indistinguishable in its clinical manifestation from classic or attenuated forms of FAP. Approximately 70–80% of patients with classical FAP harbor germline mutations in the APC gene. Screening for FAP should commence with a detailed family history. For individuals with suspected FAP, genetic testing can be used both to confirm diagnosis in a suspected proband and to assess risk in pre-symptomatic family members. Provided the mutation responsible for FAP within a family is known, testing for APC mutations can be considered for at risk family members (National Comprehensive Cancer, 2006). Most expert panels recommend that for families with classic FAP, APC gene testing should be considered at 10–12 years of age (National Comprehensive Cancer, 2006; Giardiello et al., 2001; American Gastroenterological Association, 2001; Vasen et al., 1991).

3.8. Hnpcc

HNPCC is clinically defined by the fulfillment of the Amsterdam Criteria (American Gastroenterological Association, 2001) HNPCC includes affected families with disease causing mutations in DNA mismatch repair (MMR) genes displaying an MSI-H phenotype in their corresponding tumors (a subgroup also called Lynch syndrome) and families with MSS tumors and no mutations in DNA MMR genes. The genetic pathogenesis of the latter group is currently unclear. Above screening families with diagnosis of HNPCC, the Bethesda guidelines revised criteria recommended a panel of

5 MS markers: 2 mononucleotides (BAT 25 and BAT 26) and 3 dinucleotides (D2S123, D5S346 and D17S250) (Vasen et al., 1991). Tumours with no instability in any of these markers are considered to be MS stable (MSS). On the other hand, if one marker is mutated, the tumor is regarded to have low MSI (MSI-L) and if 2 or more markers are mutated, the tumor is regarded to have high MSI (MSI-H). The test should be performed on tumor tissue when HNPCC is suspected and, when confirmed, in the serum of consanguineous of the cancer subject. For diagnostic purposes, immunohistochemical tissue analyses with antibodies directed against MLH1, MSH2, MSH6, PMS2 and other MMR gene proteins may provide useful related information on MS status (Rowley, 2005; Fearnhead et al., 2002; Sampson et al., 2005; National Comprehensive Cancer, 2006; Giardiello et al., 2001; American Gastroenterological Association, 2001; Vasen et al., 1991; Locker et al., 2006).

3.9. Evident cancer disease setting (serum tumor markers)

Serum CEA and Ca 19.9 are commonly used as classical tumor markers in CRC patients. Ideal diagnostic markers must be characterized by both high sensitivity and high specificity.

3.10. Cea

3.10.1. Preoperative setting

CEA may be performed preoperatively in patients with CRC if it would assist in staging and surgical treatment planning. Although elevated preoperative CEA (>5 ng/mL) may correlate with poorer prognosis, data are insufficient to support the use of CEA to determine whether to treat a patient with adjuvant therapy. Published data supports the utility of pre-operative CEA levels as prognostic factors (Chen et al., 2005; Kim et al., 2004; Weissenberger et al., 2005). Specifically, a study of 2230 patients demonstrated that preoperative CEA was an important independent prognostic variable in predicting outcome (Park et al., 1999a); and another study of 1146 CRC patients using a multivariate analysis confirmed that preoperative CEA levels was still a highly significant prognostic covariate even after stage and grade were included in the model (Park et al., 1999b). Preoperative levels of CEA are rarely elevated in patients with early cancer (0.5–10%) of patients with TNM-stage 0–I and the frequency of positivity for this marker rises with the stage up to 78% in stage IV High preoperative levels of CEA in III and IV stage CRC have been considered a strong independent risk factor for local recurrence and even for short DFS and OS (Wu et al., 2008; Wang et al., 2000; Takagawa et al., 2008). Furthermore, determination of CEA before resection aids in assessing its utility for postoperative surveillance. An elevated preoperative CEA suggests that the marker would be useful for surveillance.

3.10.2. Postoperative setting

In the post-operative setting, serum CEA testing should be performed every 3 months in patients with stage II or III disease for at least 3 years after diagnosis if the patient is a candidate for surgery or systemic therapy. Elevated CEA levels warrant further evaluation for metastatic disease, but do not justify the institution or adjuvant therapy or systemic therapy for presumed metastatic disease. Moreover, CEA is the most frequent indicator for recurrence in asymptomatic patients (Locker et al., 2006), is more cost-effective than radiology for the detection of potential curable recurrence (Locker et al., 2006), and is the most sensitive detector for liver metastases (Locker et al., 2006).

3.10.3. Metastatic setting

In metastatic CRC setting, CEA is the marker of choice for monitoring the disease during systemic therapy. CEA should be measured at baseline for metastatic disease and every 1–3 months

during antitubercular treatment. Persistently rising values above baseline should prompt restaging, but suggests progressive disease even in the absence of corroborating radiographs. Caution should be used when interpreting a rising CEA level during the first 4–6 weeks of a new antitubercular treatment, since spurious early rises may occur especially after Oxaliplatin use (Sorbye and Dahl, 2003; Nomura and Katunuma, 2005). Moreover, it is important to emphasize that measured levels of CEA may be different between laboratories and countries.

3.11. Ca 19-9

CA 19-9, which is called sialyl Lewis a (sLa), is another alternative marker for CRC. The increase of CA 19-9 has demonstrated a significantly higher frequency of metastasis and distinctly lower survival rate, making it an adverse prognostic factor for CRC patients.

To date there are insufficient data to recommend the use of CA 19.9 in the management of all steps of CRC (screening, diagnosis, staging, surveillance, or monitoring treatment of patients with CRC) (Locker et al., 2006).

4. Molecular studies

Many possibilities have been explored aimed to identify patient at highest risk of recurrence and primarily of developing liver metastases. Actually, even after many molecular and genetic studies that have shown various relationship between genes and cancer behavior only few of these markers involved in tumor growth and metastatic processes have been validated as diagnostic or prognostic tool for use in clinical practice. The genes and proteins can be divided based on their function.

4.1. Adhesion

Adhesion is the mechanism that takes linked together the cells. When adhesion is broken, tumor cells are free to scatter and leave the site. On the other side, adhesion permits cells to bind to other proteins and colonize other sites.

4.1.1. Cadherin/catenin

Among these proteins, E-cadherin and α -catenin have been demonstrated to be down-regulated in highly aggressive tumors. The mechanism by which the complex is responsible for the formation of stable cellular junctions includes the link between cadherin and a submembranal beta catenin, between this last and α -catenin and to actin cytoskeleton. Free β -catenin translocates in nucleus where it acts as a transcription factor for proliferation genes.

4.1.2. Mucins

Mucins and especially MUC1 has been showed related with CRC. The mechanism seems include interaction and competition with binding of β -catenin by E-cadherin. This MUC1 binding to β -catenin is regulated by various proteins including glycogen synthase kinase-3 β , csrc tyrosine kinase, protein kinase C, and epidermal growth factor receptor (Linhares et al., 2015). Mucins have been related with poor prognosis in sporadic CRCs, but not in hereditary cancers. In other series the better outcome of HNPCCs compared to sporadic CRCs seemed related to a lower prevalence of Mucins in the first group.

4.1.3. Integrins

Integrins are a family of transmembrane glycoproteins that bind cells to extracellular matrix proteins of the basement membrane or to ligands on other cells as to laminin, collagen, fibronectin and

vitronectin, conferring a particular aggressiveness to CRC in which they are highly expressed (Linhares et al., 2015).

4.1.4. Osteopontine (OPN)

Osteopontine or Secreted phosphoprotein 1 (SPP1), is a glycoprotein identified first in bone and successively in many tissues, i.e. brain, kidney, placenta, and in immune cells. OPN shows an anti-apoptotic role and interacts with integrins. OPN is over expressed not only in CRC but even in lung, breast, gastric, ovarian cancers, melanoma and mesothelioma (Ding et al., 2002).

4.1.5. CD 44

CD44 is glycoprotein. A cell surface glycoprotein binding hyaluronic acid and able to interact with OPN, collagen and matrix metalloproteinases. CD 44 is present in CRC and has also been found in prostate cancer, breast cancer and ovarian cancer. Cell adhesion molecules may also mediate the selection of the host organ for the development of distant colorectal metastases (Seo et al., 2015).

4.1.6. Invasion

Invasion is the most typical activity of tumor cells related with metastatization and is principally due to the action of some enzymes.

4.1.7. Metalloproteases (MMPs)

These zinc-dependent endopeptidases are thought to play a primary role in almost all cellular activities: proliferation, differentiation, dispersion, angiogenesis and apoptosis. MMP2 has been related with liver metastatization.

4.1.8. Cathepsines

Cathepsin A (serine protease) and Cathepsin B (Cysteine protease), members of a numerous family of proteases, play a role in cellular turnover by breakdown of polypeptides, and have been related with progression of cancer (Kuester et al., 2008).

4.2. UPA and PAI (Plasminogen activators (urokinase) its inhibitor)

UPA is a component of the plasminogen activation system and, due to the extracellular matrix degradation following the activation of the proteolytic cascade, facilitates the invasion of tissues by cancer cells being a reliable prognostic factor (Forsti et al., 2007). Further, UPA is a target for therapies since its inhibitors can act as targeted drugs.

4.3. Angiogenesis

4.3.1. VEGF

VEGF is the Vascular Endothelial Growth Factor, mainly derived from tumor cells, that determines blood vessel growth and indirectly promotes tumor growth and metastases by favoring tumor neo-vascularization. (VEGF) is a marker of poor prognosis in CRC (Moehler et al., 2008; Hawinkels et al., 2008; Maltese et al., 2009).

4.3.2. Thymidine phosphorilases

Thymidine phosphorilases a glycosilpeptidase involved in nucleotides metabolism, is a factor related with liver metastases (Haraguchi et al., 2008).

4.3.3. Inhibitors of angiogenesis

Inhibitors of angiogenesis such as angiostatin, endostatin and thrombospondin-1 (TSP-1) can contribute to regulate the growth of liver metastases. These factors can be produced by primary or secondary tumors. Primary CRC producing such inhibitors, after its removal, can be followed by rapid growth of new vessels and

then of metastases. On the other hands, in absence of inhibitors, synchronous metastases may be more frequent.

4.4. Cell growth

4.4.1. Epidermal growth factor receptors (EGFR)

Epidermal growth factor receptors (EGFR), member of the family of Erb- B receptors, are related with growth of cancer and, other than in breast cancer, have been reported to be highly expressed at protein level and more or less associate with gene amplification or mutation in 72%–82% of metastatic CRC tissue samples (Italiano et al., 2005; de Castro-Carpeño et al., 2008). This marker is related to poor prognosis but can predict response to specific targeted therapy.

4.4.2. K-ras

Activation, by single amino-acid change, of this proto-oncogene is one of the former steps in adenomacarcinoma sequence and acts via activation of EGFR pathway independently from EGFR. This is why actually K-ras is important in evaluating therapies since a mutant K-ras is a predictor of failure in response to anti-EGFR and should be routinely tested together with EGFR in all colorectal tumors (Karapetis et al., 2008b; Allegra et al., 2009).

4.4.3. APC

APC is an oncosuppressor gene, which encodes a protein binding the free b-catenin permitting its degradation and preventing translocation in nucleus. More than 800 possible mutations of this gene are already known and some are responsible not only of FAP but even of many sporadic CRC. The test is useful in screening in individuals from family suspected to harboring the mutation. No prognostic value has been described for sporadic CRC (Lugli et al., 2007b).

4.5. Cell survival

Cell survival is linked to a balance between apoptotic and anti-apoptotic factor. Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is expressed by hepatic NK cells. Apoptosis can be triggered by the contact between TRAIL and its corresponding ligand as well as between tumor necrosis factor receptor FAS and its ligand FASL. The down regulation of the first and up regulation of the second can promote cancer progression and has been observed in liver metastases of CRC compared to primary tumors (Mohr et al., 2008; Rudmik and Magliocco, 2005; Xu et al., 2003). It is possible that tumor cell escape to immunological aggression through desensitization to FAS/TRAIL killing. This mechanism can be enhanced by integrins and Src genes. This last gene codifies a tyrosine kinase and is highly activated in CRC.

5. Circulating tumor DNA

In recent years Circulating Tumor Cells, (CTCs) and circulating tumor DNA (ctDNA) have become very important for early diagnosis, therapeutic selection, monitoring of metastasis, monitoring response and resistance to treatment thanks to their noninvasiveness. CTCs and ctDNA are released from different tumor types at different stages and contribute to give complementary information for clinical decision. Unluckily although big enhancements have been taken in technology development for its detection and characterization, they are not widely adopted and validated for routine cancer patient care (Tan et al., 2016). Nowadays the use of ctDNA goes from diagnostic stage to the phase of therapeutic drug monitoring. A paper by Tie J et al. have evaluated patients with stage II colon cancer after resection to identify an highest risk of recurrence

group to help adjuvant treatment decisions. They used sequencing-based assays to detect minimal residual disease in plasma samples (Tie et al., 2016).

In a study by Zhou J et al., is been analyzed the molecular genetic characteristics of CRC and described a multimodality profile of ctDNA of CRC patients during the course of clinical multimodality treatment. The authors described a good correlation between patients' ctDNA level and their clinical disease status. They therefore proposed its potential use in clinical practice as a new monitoring strategy, which may potentially provide better sensitivity and specificity than the traditionally used biomarkers (Zhou et al., 2016).

6. Conclusions

Our review focused on CCR-specific tissue and serum biomarkers that may help in the early diagnosis of cancer or guide therapeutic decisions in the case of inoperable malignancy. A non-invasive serologic screening test with a high sensitivity or multi-marker panels could be very advantageous for patients and very useful in clinical practice. Despite the more invasive nature of tissue markers, high-risk patients would benefit from their high specificity. Further validations of novel biomarkers and multicenter international studies are needed.

Conflict-of-interest statement

No Authors have financial or personal relationships with other people or organizations that could potentially and inappropriately influence (bias) this work and conclusions.

Authorship

1) All authors contributed equally to conception and design of the review; 2) drafting the article or making critical revisions related to important intellectual content of the manuscript; 3) final approval of the article by all Authors.

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