

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Stem Cell Markers in Colon Cancer

Miana Gabriela Pop

Abstract

Colon cancer incidence is increasing in young people. Even if, so far, colon cancer had a maximum incidence in the sixth and seventh decades of life, lately its incidence in people age 50 and younger is increasing. Thus, colon cancer still represents a major health problem despite constant research made in the field. Early detection of colon cancer is mandatory for an appropriate treatment of the disease and to attain increased overall survival. Even if various stem cell markers have been studied in order to evaluate their prognostic value in colon cancer cases, results in literature are heterogeneous, and no clear consensus has been drafted so far. This paper aims to review the most important stem cell markers identified in colon cancer and to establish their role in both cancer diagnosis and progression.

Keywords: colon, cancer, stem cell markers, CD133, CD44, CD166, EpCAM

1. Introduction

Colon cancer is a frequent neoplastic disease which is ranked second in female after breast cancer and third in men after prostate and lung and cancer [1]. Despite constant research in the field of colon cancer, its incidence continues to be high worldwide. Moreover, the number of people age 50 or younger diagnosed with colon cancer is dramatically increasing in last years. This finding upholds the idea that colon cancer is not a disease considered to be under control at this time, and efforts should be made in order to better understand its pathogenic mechanism.

Five-year overall survival in colon cancer ranges from 90% in early stages to less than 10% in advanced, metastatic cases [2]. It is thus important to try to diagnose the disease in early stage, so an appropriate treatment can be applied. Achieving this condition can be difficult, considering the fact that a large number of colon cancer patients present with late stage, often inoperable tumors.

Even if important progress has been made in terms of imaging diagnosis of colon cancer, early detection is still difficult to achieve. An important role in detecting early colon cancer cases is assigned to screening programs that have to be applied nationally, and population should be well informed of their importance. More than detecting incipient cases, early detection of advanced cases is also of crucial importance, and efforts should continue in this direction by further research groups.

Colon cancer stem cells (CCSCs) are multipotent neoplastic cells that have the ability to differentiate and initiate the carcinogenesis process [3]. Due to their increased viability, CCSCs are responsible for both tumor growth and tumor recurrence [4, 5]. According to a recent study, the presence of CCSCs is also responsible for resistance to chemotherapeutic treatments, which is observed in some cases [5]. A new treatment concept linked to CCSCs is based on their early detection, before the onset of the tumor, which would allow them to target with apoptotic substances.

Detection of cancer stem cells (CSCs) in various digestive and extra-digestive cancers has been a topic of great interest in the literature of recent years and was frequently done using cluster of differentiation (CD) markers. In colon cancer, various biomarkers have been identified at the surface of CSCs, and their role in colon cancer is currently being tested: EpCAM, CD133, CD29, CD24, CD44, CD166, ALDH1A1, and ALDH1B1 [3, 4].

The aim of this paper is to review the most important biomarkers which have been identified in colon cancer, to expose current information regarding their role in colon cancer development and progression and to identify possible predictive biomarkers for advanced stages of the disease.

2. CD133/prominin-1

CD133 was first described in 1997 by Yin et al. on the cellular surface of hematopoietic cells [6]. Also called prominin-1, CD133 is a 5-transmembrane glycoprotein of 120 kDa which can be found in two isoforms: CD133-1 and CD133-2 [5–7]. CD133 is found on the short arm of chromosome 4 [5]. Its cellular function is unclear [5–7], but its involvement in cell-cell and cell-matrix interactions was described [5]. According to some recent studies, CD133 expression is an important tool in cancer stem cells (CSCs) identification and characterization [7]. CD133 was found to be expressed in various digestive (pancreatic, liver, colorectal) and non-digestive tumors (brain, kidney, prostate, ovary cancer) [7–9]. CD133 expression promotes cancer cell proliferation through activation of Wnt/beta-catenin pathway [10, 11]. Moreover, in highly expression CD133 cancer stem cells, the development of solid tumor mass is assured by the anti-apoptotic factors BCL-2, BCL-XL, and MCL-1 that are stimulated through PI3K pathway, with subsequent activation of Akt [11]. Even if various studies focused on targeting CSCs and especially CD133 due to its overexpression, most of the results arise from in vitro research and not from clinical experience. Targeted therapy was tested using Anti-CD133 scFv immunotoxins by Waldron et al. that found an interruption of the protein synthesis secondary to this process [12].

CD133 expression in colon cancer was confirmed 10 years after its initial description in 2007 [13, 14], when Obrien et al. proved that neoplastic cells expressing CD133 have the ability to form solid colon cancer masses in immunodeficient mice. From that point, many studies focused on CD133 expression in colon cancer carcinogenesis. Various studies analyzed CD133 expression in relation to clinical and pathological characteristics of the neoplastic patients, but result were inconsistent. CD133 expression correlates with the degree of tumor wall involvement (T) [15], with distant metastasis formation (M) [5, 16], with venous (V) and lymphatic (L) invasion [15]. A relation between CD133 expression and tumor recurrence was also noticed in one study [5], while other research groups found a significant association between CD133 expression and tumor size [7]. CD133 expression was correlated in some studies with a poor degree of tumor differentiation (G) [7], but the result was not confirmed by other studies where CD133 expression was found more frequent in moderate (G2) and well differentiated (G1) colon tumor tissues [17].

Chemoresistance was also found to be influenced by CD133 expression in colon cancer especially due to upregulation of FLICE-like inhibitory protein (FLIP), a ligand that inhibits tumor necrosis factors (TNF)-mediated apoptosis [11]. According to some studies, tumors expressing CD133 are more likely to be resistant to chemotherapy [5, 7, 18]. Moreover, tumors expressing high CD133 and CD44 biomarkers on the cellular surface are expected to be unresponsive to chemotherapy when compared to tumors where the expression of the two molecules is low or absent [16].

Results are contradictory in terms of CD133 expression in liver metastases secondary to colon cancer. While CD133 expression in liver metastases was thought to predict a better overall survival (OS) in colon cancer patients [19], Spelt et al. found, in a recent study, different results [4]. According to them, CD133 expression in liver metastases is associated with worse overall survival (OS). Results in favor of a worse prognostic impact of CD133 expression in liver metastases are suggested also by Narita et al. which demonstrated an increased CD133 expression in cases of early recurrence of liver metastases compared with a low CD133 expression in late recurrent liver metastases [20].

In terms of survival, overexpression of CD133 was associated with worse overall survival in some studies [16, 21–23] and also with low disease-free survival interval [23], but the relation was not found by others [4, 5, 17, 24, 25]. According to two recent meta-analyses, CD133 expression represents a negative prognostic factor in colon cancer patients [23, 26].

Heterogeneous results exist in literature considering CD133 role in colon cancer. Its involvement in tumor progression and metastasis formation is suggested, but its precise role remains unclear. CD133 represents a useful tool for CSCs identification and characterization in colon cancer samples. Various studies analyzed the correlation between CD133 expression and clinical and pathological characteristic of the patient, but a direct association between its degree of expression and advanced tumor stages was not confirmed. Moreover, its prognostic role regarding overall survival in colon cancer is still debated, and further studies are needed for a better characterization of the molecule in relation to colon cancer patients.

3. CD44 in colon cancer

CD44 is a type 1, 85–200 kDa transmembrane glycoprotein expressed in both normal and tumor tissues [16, 27, 28]. Discovered initially as a receptor for hyaluronic acid, the molecule has retained its affinity for it and for other components like collagens, osteopontin, or type I metalloproteinase [3, 27]. Supplementary, an adhesion function was highlighted for CD44 that was found to intervene in both cell-cell and cell-matrix interactions [4, 16]. From a structural point of view, CD44 has three main domains: an extracellular one, a transmembrane, and, respectively, an intracellular domain [27]. CD44 has the capacity to present in various isoform, depending on the exons that attach to the extracellular part (CD44v) [27]. Its encoded gene is located on the short arm of chromosome 13 [29].

CD44 is expressed ubiquitarily in normal tissue and participates, through lymphocytes activation, in various inflammatory processes [3, 27]; its involvement in wound healing processes was also described by some authors [3]. In neoplastic lesions, CD44 is expressed, in different isoforms, in pancreatic (CD44v8–10) and colon cancer (CD44v6) [27], in prostatic tumors (CD44s—standard isoform), in breast cancer [27], and also in epithelial ovarian cancers [30]. Through its adhesiveness properties, CD44 was found to intervene in tumor growth [16, 17]. Additionally, tumor cells expressing CD44 present with invasiveness properties and are also characterized by the capacity to initiate the metastatic process [28, 31] intervening thus in cell differentiation, proliferation, and migration [32]. The mechanisms by which the molecule intervenes in these processes remain, however, unknown, and further studies have to be performed.

Assessment of the prognostic value of CD44 was analyzed in recent papers that highlighted an association between CD44 expression and both advanced tumor stages and liver metastasis formation [27, 31]. Overexpression of CD44 in colon cancer samples was found to negatively influence overall survival of colon cancer patients [33, 34]; one study group found a negative association between CD44

expression and poor overall survival only for a specific variant of CD44 and, respectively, Cd44v2 [35]. The association between upregulation of CD44 in colon cancer and worse overall survival was not confirmed by other study groups [24, 36], but the analysis was completed based on standard isoform of CD44 (CD44s). CD44 usage as an independent prognostic factor in colon cancer patients is not currently recommended [17], but further studies need to concentrate on specific isoforms, like the one abovementioned, in order to correctly identify its value as a prognostic marker.

CD44 targeting is currently being tested in various digestive (stomach, colon cancer) [31, 37] and non-digestive cancer (lung, breast cancer) [38]. The results in terms of cancer stem cell apoptosis for in vitro and preclinical animal models are promising. In pancreatic cancer the anti-CD44 antibody tested against CD44v6 isoforms with promising antitumor results was bivatuzumab [37], while the first humanized antibody directed toward solid tumors expressing CD44 approved for clinical research is RO5429083 (NCT01358903), and the publication of results is in progress.

4. CD24 in colon cancer

CD24 is a glycoprotein located on the external surface of the cellular membrane [16]. It is formed of 27 amino acids, and it has a molecular weight of 24–70 kDa [5, 26]. Its expression was confirmed in normal nervous tissue [16] and in cancers of the colon [5], pancreas [24], breast, and prostate [26]. CD24 is involved in cellular signaling processes, in cellular differentiation, and in proliferation and is being considered a significant marker of cancer stem cells (CSCs) [4, 16, 39]. The mechanism by which CD24 participates in signaling processes seems to be related to mitogen-activated protein kinase (MAPK) and serine/threonine pathway [26].

In colon cancer, CD24 was found to be expressed in a percentage of 50–68% [24, 40]. CD44 is involved in first steps of carcinogenesis and plays an important role in liver metastasis formation [4, 9, 41–43]. Yeo et al. found CD24 a useful diagnostic marker of early colon cancer [39], whereas its expression was higher in malignant polyps than CD24 expression in colon adenomatous lesions.

No correlation was found between CD24 expression in colon cancer and tumor type or degree of differentiation (G) [5, 44]; other authors have highlighted, however, an inverse relation between CD24 expression and tumor size, poor differentiated cancers, and advanced TNM stages [39]. Regarding lymph node involvement and CD24 expression, as association between high CD24 expression and a larger number of lymph nodes involved was reported in some research papers [45] but not in others [5, 24]. In terms of overall survival, CD24 expression was in general associated with worse survival rates [16, 26]; results were not confirmed by other recent research papers [5, 24, 44]. Resistance to chemotherapeutic treatment was also objective by Nosrati et al. [5] probably due to their capacity to induce the epithelial-mesenchymal transition (EMT) mechanism [46]. Moreover, colon cancer stem cells expressing both CD133 and CD24 markers were found to be resistant to chemotherapeutic regimens based on 5-FU [47].

CD24 was highly studied in colon cancer samples, but consistent results have failed to establish its precise role in colon cancer, considering the heterogeneous results observed.

5. Epithelial cell adhesion molecule (EpCAM)

Epithelial cell adhesion molecule (EpCAM) is a Ca^{2+} independent, type I transmembrane glycoprotein with a molecular weight of 40 kDa [48] located on

the basolateral surface of epithelial tissues [49]. EpCAM expression was not seen in mesenchymal or lymphoid tissues [50]. EpCAM presents with two main domains: EpICD, an intracellular domain, and EpEx, an extracellular domain of 26, respectively, and 242 amino acids [48, 51].

EpCAM was found to be overexpressed in various digestive (stomach, colon, pancreas, and esophagus) and non-digestive (prostate, ovary, breast) cancers [49]. EpCAM is principally involved in adhesion processes, but its role in cellular differentiation and progression was also confirmed [50].

A high percentage of colon cancer cases (79–99.7%) is characterized by overexpression of EpCAM molecule at tumor level [52, 53]. Moreover, EpCAM was found to be expressed also in liver metastases secondary to colon cancer, a situation that confirmed its involvement in cancer progression as well [50, 52]. Normal liver parenchyma does not express EpCAM [54].

Overexpression of EpCAM in colon cancer correlates in some studies with advanced stages of the disease [50, 55, 56], with a higher risk of metastases [55, 56], with poor differentiated (G3) patterns [54–57], with the number of lymph nodes involved (N) [48, 54], and with perilymphatic (L) and perivenous (V) invasion [54, 57] but also with worse overall survival [55, 56]. The results were not, however, confirmed by other study groups, so the predictive value of EpCAM in colon cancer patients was difficult to establish [58].

EpCAM is also involved in epithelial-mesenchymal transition (EMT) process [56]. During EMT, neoplastic cell detaches from the primary tumor (due to loss of EpCAM expression and less intercellular adhesions) to enter the lymphatic and vascular system and initiate the carcinogenesis process [56]. Detached cells, also called circulating tumor cells (CTCs), can be identified from blood samples through “liquid biopsy” technique that is based also on EpCAM detection using specific anti-EpCAM antibodies [59, 60].

In order to achieve distant metastasis formation, circulating tumor cells have to undergo a second, reversed process called mesenchymal-epithelial transition (MET) during which an upregulation of EpCAM expression at the cellular surface has been observed [59]. Secondary to it, cells acquire adhesion properties that allow them to form a solid metastatic mass [59].

Despite constant research in the field of cancer stem cell biomarkers in colon cancer, specific factors or local conditions that initiate and promote EMT or MET are insufficiently known, and further research have to be performed.

6. CD166 or ALCAM

CD166, also called activated leukocyte cell adhesion molecule (ALCAM), is a 110 kDa, transmembrane type-1 glycoprotein used for colon cancer stem cell (CCSC) identification [3, 61, 62]. Providing the leukocyte receptor function, CD166 expression was identified in both normal and colonic tissue, in the latter cases the expression being superior [3, 63]. CD166 expression in colon cancer varies between 58.6 and 76% [64, 65] and is higher in colonic adenomas [66], suggesting its involvement in colon carcinogenesis. Due to its adhesive properties, CD166 is considered to be involved in colon cancer tumor growth [62]. CD166 expression was also confirmed in pancreatic, esophageal and gastric, prostate, melanoma, and breast cancers [63].

Expression of CD166 in colon cancer was studied in relation to tumor stage [61, 64, 65], lymph node involvement [61, 64], or degree of cellular differentiation (G) [61], but even if overexpression was confirmed, no statistic significant correlation was found. Regarding overall survival of colon cancer patients,

overexpression of CD166 failed to predict its outcome. Some literature studies found a worse overall survival in colon cancer cases characterized by high CD166 expression [64]. Levin et al. found that even the survival was reduced by 15 months for patients who presented colon tumors characterized by high CD166 expression compared with tumors with low or absent CD166 expression [67]. Other studies could not establish the prognostic relation of CD166 in colon cancer patients [65].

Limited number of studies analyzed CD166 expression in colon cancer patients, and existing results are inconclusive. Therefore, the role of CD166 in colon cancer remains unclear.

7. CD29

Through CD29 molecule, also known as integrin $\beta 1$, cells adhere to extracellular compartment proteins and facilitate intracellular transmission of the cellular signal [68]. CD29 presents with 3 structural domains, the extracellular one being best represented [69].

Expression of CD29 was observed in normal and tumor colonic tissues, and a presumptive role in cellular differentiation was attributed to it, due to the activation of Erk signaling pathway [68, 69]. In normal colonic mucosa, CD29 is expressed in the lower part of the intestinal crypt [69] and is considered to be involved in intestinal proliferation [68]. However, its precise role in colon cancer is unknown [68].

At present, CD29 expression in colon cancer is only used as diagnostic marker for CSCs. Further studies are needed to evaluate its involvement in cancer progression and metastasis.

8. Lgr5

Leucine-rich repeat-containing G-protein coupled receptor 5 or Gpr49 is a receptor formed by eight main domains [69]. Lgr5 was identified on the cellular surface of intestinal and colonic stem cells and is being considered thus a biomarker of them [70]. Lgr5 overexpression was also confirmed in esophageal and colon cancer, in hepatocellular carcinoma, and in ovarian cancer [70].

Lgr5 is expressed in both normal and tumor colonic tissues [69]. In normal colon tissue, Lgr5 is expressed in a small area of the intestinal crypts. Its expression area increases with cell transformation in adenoma and is most elevated in colon adenocarcinoma [69]. The percentage of colon cancer patients expressing Lgr5 is, according to literature studies, around 80% [70, 71].

Overexpression of Lgr5 in colon cancer correlates with advanced stages of the disease [70, 71], with lymph node involvement (L) [70, 71] and perineural invasion [71] and distant metastases (M) [70]. Lgr5 involvement in cellular proliferation is also suggested due to the correlation found between Lgr5 expression and Ki-67 expression [70].

Lgr5 is thus considered to have a role in colon cancer development and progression and possibly in liver metastases formation as well [69]. Moreover, Lgr5 is considered to have a clinical role in predicting advanced pathological stages of colon cancer tumors [72].

9. ALDH1

Aldehyde dehydrogenase 1 is a detoxifying enzyme involved in colon cancer proliferation [73]. Expressed in low percentage in normal colonic mucosa, ALDH1

was found to be overexpressed in colon adenocarcinoma [73, 74]. A number of 75.5–76.5% of colon cancer cases express ALDH1 at tumoral level [73, 74].

ALDH1 expression is associated with colon cancer location [73], with advanced stages of the disease [75], with number of lymph nodes involved (N) [73, 75, 76], and with perivenous invasion (V) [73] but also with local tumor recurrence [75]. The association between ALDH1 expression and lymph node involvement was not seen by Zhou et al. [74].

Recently, ALDH1 expression was found to be involved in epithelial-mesenchymal transition (EMT) and could play, thus, a role in cancer progression and distant metastases formation [75–77].

Moreover, ALDH1 associates with resistance to chemotherapy [75] and poor overall survival [75, 76, 78].

In conclusion, ALDH1 could represent a promising prognostic marker in colon cancer patients that associate with advanced colon cancer stages and worse overall prognosis.

10. Conclusions

Colon cancer stem cells (CCSCs) could be responsible for tumor metastases, resistance to chemotherapy, and recurrence, and their identification is thus of major importance. However, the amount of biomarkers identified at the cellular surface of CCSC failed to become valuable prognostic markers, and further studies are necessary to evaluate their role in cancer progression and distant metastases formation.

Conflict of interest


The author declares that she has no conflict of interest.

Author details

Miana Gabriela Pop
Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

*Address all correspondence to: miana_my@yahoo.com

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in Globocan. *International Journal of Cancer*. 2012;**136**(5):E359-E386
- [2] Coopede F, Lopomo A, Spisni R, Migliore L. Genetic and epigenetic biomarkers for diagnosis, prognosis and treatment of colorectal cancer. *World Journal of Gastroenterology*. 2014;**20**:943-956
- [3] Ribeiro KB, da Silva ZJ, Ribeiro-Silva A, Rapatoni L, de Oliveira HF, da Cunha Tirapelli DP, et al. KRAS mutation associated with CD44/CD166 immunoexpression as predictors of worse outcome in metastatic colon cancer. *Cancer Biomarkers*. 2016;**16**(4):513-521
- [4] Spelt L, Sasor A, Ansari D, Hilmersson KS, Andersson R. The prognostic role of cancer stem cell markers for long-term outcome after resection of colonic liver metastases. *Anticancer Research*. 2018;**38**(1):313-320
- [5] Nosrati A, Naghshvar F, Maleki I, Salehi F. Cancer stem cells CD133 and CD24 in colorectal cancers in Northern Iran. *Gastroenterology and Hepatology from Bed to Bench*. 2016;**9**(2):132-139
- [6] Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood*. 1997;**90**:5002-5012
- [7] Kazama S, Kishikawa J, Kiyomatsu T, Kawai K, Nozawa H, Ishihara S, et al. Expression of the stem cell marker CD133 is related to tumor development in colorectal carcinogenesis. *Asian Journal of Surgery*. 2018;**41**(3):274-278
- [8] Pop MG, Fit AM, Bartos D, Vesa SC, Puia IC, Al-Hajjar N, et al. CD133 expression in colon cancer. An immunohistochemical analysis of 72 cases. *Medicine and Pharmacy Reports*. 2019;**92**(supplement 1):S60
- [9] Ren F, Sheng WQ, Du X. CD133: A cancer stem cell marker, is used in colorectal cancers. *World Journal of Gastroenterology*. 2013;**19**:2603-2611
- [10] Schmohl JU, Vallera DA. CD133, selectively targeting the root of Cancer. *Toxins (Basel)*. 2016;**8**(6):165
- [11] Barzegar Behrooz A, Syahir A, Ahmad S. CD133: Beyond a cancer stem cell biomarker. *Journal of Drug Targeting*. 2018;**17**:1-13
- [12] Waldron NN. Development and characterization of CD133 positive cancer stem cell targeted toxins for use in carcinoma therapy [thesis]. Daniel A Vallera, University of Minnesota; 2013
- [13] Obrien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature*. 2007;**445**:106-110
- [14] Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature*. 2007;**445**:111-115
- [15] Huang R, Mo D, Wu J, Ai H, Lu Y. CD133 expression correlates with clinicopathologic features and poor prognosis of colorectal cancer patients: An updated meta-analysis of 37 studies. *Medicine*. 2018;**97**(23):e10446
- [16] Sahlberg SH, Spielberg D, Glimelius B, Stenerlow B, Nestor M. Evaluation of cancer stem cell markers CD133, CD44, CD24: Association with AKT isoforms and radiation resistance in colon cancer cells. *PLoS One*. 2014;**23**(9):e94621
- [17] Pitule P, Miroslava C, Daum O, Vojtisek J, Vycital O, Hosek P, et al.

Immunohistochemical detection of cancer stem cell related markers CD44 and CD 133 in metastatic colorectal cancer patients. *BioMed Research International*. 2014;**2014**:7. Article ID: 432139. <https://doi.org/10.1155/2014/432139>

[18] Ong CW, Kim LG, Kong HH, Low LY, Iacopetta B, Soong R, et al. CD133 expression predicts for non-response to chemotherapy in colorectal cancer. *Modern Pathology*. 2010;**23**:450-457

[19] Yamamoto S, Tanaka K, Takeda K, Akiyama H, Ichikawa Y, Nagashima Y, et al. Patients with CD133-negative colorectal liver metastasis have a poor prognosis after hepatectomy. *Annals of Surgical Oncology*. 2014;**21**:1853-1861

[20] Narita M, Oussoultzoglou E, Chenard MP, Fuchshuber P, Yammamoto T, Addeo P, et al. Predicting early intrahepatic recurrence after curative resection of colorectal liver metastases with molecular markers. *World Journal of Surgery*. 2015;**39**:1167-1176

[21] Kemper K, Versloot M, Cameron K, Colak S, de Sousa e Melo F, de Jong JH, et al. Mutations in the Ras-Raf axis underline the prognostic value of CD133 in colorectal cancer. *Clinical Cancer Research*. 2012;**18**:3132-3141

[22] Saigusa S, Tanaka K, Toiyama Y, Yokoe T, Okugawa Y, Koike Y, et al. Clinical significance of CD133 and hypoxia inducible factor-1 alpha gene expression in rectal cancer after preoperative chemoradiotherapy. *Clinical Oncology*. 2011;**23**:323-332

[23] Chen S, Song X, Chen Z. CD133 expression and the prognosis of colorectal cancer: A systematic review and meta-analysis. *PLoS One*. 2013;**8**:e56380

[24] Choi D, Lee HW, Hur KY, Kim JJ, Park GS, Jang SH, et al. Cancer stem cell markers CD133 and CD24 correlate

with invasiveness and differentiation in colorectal adenocarcinoma. *World Journal of Gastroenterology*. 2009;**15**:2258-2264

[25] Gazzaniga P, Gardilone A, Petracca A, Nicolazzo C, Raimondi C, Iacovelli R, et al. Molecular markers in circulating tumour cells from metastatic colorectal cancer patients. *Journal of Cellular and Molecular Medicine*. 2010;**14**:2073-2077

[26] Wang K, Xu J, Zhang J, Huang J. Prognostic role of CD133 expression in colorectal cancer: A meta-analysis. *BMC Cancer*. 2012;**12**:53

[27] Senbanjo LT, Chellaiah MA. CD44: A multifunctional cell surface adhesion receptor is a regulator of progression and metastasis of cancer cells. *Frontiers in Cell and Development Biology*. 2017;**5**:18. DOI: 10.3389/fcell.2017.00018

[28] Basakran NS. CD44 as a potential diagnostic tumor marker. *Saudi Medical Journal*. 2015;**36**:273-279

[29] Underhill C. CD44: The hyaluronan receptor. *Journal of Cell Science*. 1992;**103**(2):293-298

[30] Sillanpaa S, Anttila MA, Voutilainen K, Tammi RH, Tammi MI, Saarikoski SV, et al. CD44 expression indicated favorable prognosis in epithelial ovarian cancer. *Clinical Cancer Research*. 2003;**9**(14):5318-5324

[31] Todaro M, Gaggianesi M, Catalano V, Benfante A, Iovino F, Biffoni M, et al. Cd44v6 is a marker of constitutive and reprogrammed cancer stem cell driving colon cancer metastasis. *Cell Stem Cell*. 2014;**14**(3):342-356

[32] Yan Y, Zuo X, Wei D. Concise review: Emerging role of CD44 in cancer stem cells: A promising biomarker and therapeutic target. *Stem Cells Translational Medicine*. 2015;**4**(9):1033-1043

- [33] Huh JW, Kim HR, Kim YJ, Lee JH, Park YS, Cho SH, et al. Expression of standard CD44 in human colorectal carcinoma: Association with prognosis. *Pathology International*. 2009;**59**:241-246
- [34] Jing F, Kim HJ, Kim CH, Kim YJ, Lee JH, Kim HR. Colon cancer stem cell markers CD44 and CD133 in patients with colorectal cancer and synchronous hepatic metastases. *International Journal of Oncology*. 2015;**46**(4):1582-1588
- [35] Ozawa M, Ichikawa Y, Zheng Y-W, Oshima T, Miyata H, Nakazawa K, et al. Prognostic significance of CD44 variant 2 upregulation in colorectal cancer. *British Journal of Cancer*. 2014;**111**:365-374
- [36] Rohani P, Noroozinia F, Modarresi P, Abbasi A. CD44 standard isoform; not a good marker for colon cancer. *International Journal of Cancer Management*. 2017;**10**(9):e9166. DOI: 10.5812/ijcm.9166
- [37] Matzke-Ogi A, Jannasch K, Shatirishvili M, Fuchs B, Chiblak S, Morton J, et al. Inhibition of tumor growth and metastasis in pancreatic cancer models by interference with CD44v6 signaling. *Gastroenterology*. 2016;**150**(2):513-525
- [38] Zoller M. CD44: Can a cancer-initiating cell profit from an abundantly expressed molecule? *Nature Reviews. Cancer*. 2011;**11**:254-267
- [39] Yeo M-K, Lee Y-M, Seong I-O, Choi S-Y, Suh K-S, Song KS, et al. Up-regulation of cytoplasmic CD24 expression is associated with malignant transformation but favorable prognosis of colorectal adenocarcinoma. *Anticancer Research*. 2016;**36**(12):6593-6598
- [40] Weichert W, Denkert C, Burkhardt M, Gansukh T, Bellach J, et al. Cytoplasmic CD24 expression in colorectal cancer independently correlates with shortened patient survival. *Clinical Cancer Research*. 2005;**11**:6574-6581
- [41] Marhaba R, Zoller M. CD44 in cancer progression: Adhesion, migration and growth regulation. *Journal of Molecular Histology*. 2004;**35**:211-231
- [42] Du L, Wang H, He L, Zhang J, Ni B, et al. CD44 is of functional importance for colorectal cancer stem cells. *Clinical Cancer Research*. 2008;**14**:6751-6760
- [43] Banky B, Raso-Barnett L, Barbai T, Timar J, Becsagh P, et al. Characteristics of CD44 alternative splice pattern in the course of human colorectal adenocarcinoma progression. *Molecular Cancer*. 2012;**11**:83
- [44] Ahmed MA, Jackson D, Seth R, Robins A, Lobo DN, Tomlinson IP, et al. CD24 is upregulated in inflammatory bowel disease and stimulates cell motility and colony formation. *Inflammatory Bowel Diseases*. 2010;**16**:795-803
- [45] Su N, Peng L, Xia B, Zhao Y, Xu A, Wang J, et al. Lyn is involved in CD24-induced ERK1/2 activation in colorectal cancer. *Molecular Cancer*. 2012;**11**
- [46] Okano M, Konno M, Kano Y, Kim H, Kawamoto K, Ohkuma M, et al. Human colorectal CD24⁺ cancer stem cells are susceptible to epithelial-mesenchymal transition. *International Journal of Oncology*. 2014;**45**:575-580
- [47] Paschall AV, Yang D, Lu C, Redd PS, Choi J-H, Heaton CM, et al. CD133⁺CD24^{lo} defines a 5-fluorouracil-resistant colon cancer stem cell-like phenotype. *Oncotarget*. 2016;**48**:78698-78712
- [48] Seeber A, Untergasser G, Spizzo G, Terracciano L, Luigli A, Kasal A, et al. Predominant expression of truncated

EpCAM is associated with a more aggressive phenotype and predicts poor overall survival in colorectal cancer. *International Journal of Cancer*. 2016;**139**(3):657-663

[49] Spizzo G, Fong D, Wurm M, Ensinger C, Obrist P, Hofer C, et al. EpCAM expression in primary tumor tissues and metastases: An immunohistochemical analysis. *Journal of Clinical Pathology*. 2011;**64**(5):415-420

[50] Zhou FQ, Qi YM, Xu H, Wang QY, Gao XS, Guo HG. Expression of EpCAM and Wnt/ β -catenin in human colon cancer. *Genetics and Molecular Research*. 2015;**14**(2):4485-4494

[51] Wang A, Ramjeesingh R, Chen CH, Hurlbut D, Hammad N, Mulligan LM, et al. Reduction in membranous immunohistochemical staining for the intracellular domain of epithelial cell adhesion molecule correlates with poor patient outcome in primary colorectal adenocarcinoma. *Current Oncology*. 2016;**23**(3):e171-e178

[52] Manuel Simonab NS, Plückthunb A, Zangemeister-Wittke U. Epithelial cell adhesion molecule-targeted drug delivery for cancer therapy. *Expert Opinion on Drug Delivery*. 2013;**10**(4)

[53] Ulrike Schnell VC, Giepmans BN. EpCAM: Structure and function in health and disease. *Biochimica et Biophysica Acta*. 2013:1989-2001

[54] Yoon SM, Gerasimidou D, Kuwahara R, et al. Epithelial cell adhesion molecule (EpCAM) marks hepatocytes newly derived from stem/progenitor cells in humans. *Hepatology*. 2011;**53**(3):964-973

[55] Han S, Zong S, Hongjia L, Liu S, Yang W, Li W, et al. Is Ep-CAM expression a diagnostic and prognostic biomarker for colorectal cancer? A systematic meta-analysis. *Cancers (Basel)*. 2017;**20**:61-69

[56] Vu T, Datta P. Regulation of EMT in colorectal cancer: A culprit in metastasis. *Cancers (Basel)*. 2017;**9**(12):171

[57] Dolle L, Theise ND, Schmelzer E, et al. EpCAM and the biology of hepatic stem/progenitor cells. *American Journal of Physiology. Gastrointestinal and Liver Physiology*. 2015;**308**:G233-G250

[58] Mokhtari M, Zakerzade Z. EPCAM expression in colon adenocarcinoma and its relationship with TNM staging. *Advanced Biomedical Research*. 2017;**6**:56-63

[59] Joosse SA, Pantel K. Biologic challenges in the detection of circulating tumor cells. *Cancer Research*. 2013;**73**(1):8-11

[60] Wen L, Vivian CJ, Brinker AE, et al. Microenvironmental influences on metastasis suppressor expression and function during a metastatic cell's journey, 2014. *Cancer Microenvironment*. 2014;**7**:117-131

[61] Shafaei S, Sharbatdaran M, Kamrani G, Khafri S. The association between CD166 detection rate and clinicopathologic parameters of patients with colorectal cancer. *Caspian Journal of Internal Medicine*. 2013;**4**(4):768-772

[62] Hassan D, Vahid M, Ghanbar M, Habibollah M, Mohammad E, Nagres M. CD166 as a stem cell marker? A potential target for therapy colorectal cancer. *Journal of Stem Cell Research & Therapeutics*. 2016;**I**(6):226-229

[63] Ni C, Zhang Z, Zhu X, Liu Y, Qu D, Wu P, et al. Prognostic value of CD166 expression in cancers of the digestive system: A systematic review and meta-analysis. *PloS one*. 2013;**8**(8):e70958. DOI: 10.1371/journal.pone.0070958

[64] Weichert W, Knosel T, Bellach J, Dietel M, Kristiansen G. ALCAM/CD166 is overexpressed in

colorectal carcinoma and correlates with shortened patient survival. *Journal of Clinical Pathology*. 2004;**57**(11):1160-1164

[65] Tachezy M, Zander H, Gebauer F, Marx A, Kaifi JT, Izbicki JR, et al. Activated leukocyte cell adhesion molecule (CD166)—Its prognostic power for colorectal cancer patients. *The Journal of Surgical Research*. 2012;**177**(1):e15-e20

[66] Han S, Yang W, Zong S, Li H, Liu S, Li W, et al. Clinicopathological, prognostic and predictive value of CD166 expression in colorectal cancer: A meta-analysis. *Oncotarget*. 2017;**8**:64373-64384

[67] Levin TG, Powell AE, Davies PS, et al. Characterization of the intestinal cancer stem cell marker CD166 in the human and mouse gastrointestinal tract. *Gastroenterology*. 2010;**139**(6):2072-2082

[68] Hatano Y, Fukuda S, Hisamatsu K, Hirata A, Hara A, Tomita H. Multifacet interpretation of colon cancer stem cells. *International Journal of Molecular Sciences*. 2017;**18**(7):1446

[69] Fanali C, Luccetti D, Farina M, Corbi M, Cufino V, Cittadini A, et al. Cancer stem cells in colorectal cancer from pathogenesis to therapy: Controversies and perspectives. *World Journal of Gastroenterology*. 2014;**20**(4):923-942

[70] Wu XS, Xi HQ, Chen L. Lgr5 is a potential marker of colorectal carcinoma stem cells that correlates with patient survival. *World Journal of Surgical Oncology*. 2012;**10**:244

[71] Zheng Z, Yu H, Huang Q, Wu H, Fu Y, Shi J, et al. Heterogenous expression of Lgr5 as a risk factor for focal invasion and distant metastasis of colorectal carcinoma. *Oncotarget*. 2018;**9**(53):300025-300033

[72] Wahab R, Islam F, Gopalan V, Kin-yin LA. The identification and clinical implications of cancer stem cells in colorectal cancer. *Clinical Colorectal Cancer*. 2017;**16**(2):93-102

[73] Holah NS, Aiad HAES, Assad NY, Elkhoully EA, Lasheen AG. Evaluation of the role of ALDH1 as cancer stem cell marker in colorectal carcinoma: An immunohistochemical study. *Journal of Clinical and Diagnostic Research*. 2017;**11**(1):EC17-EC23

[74] Zhou F, Mu YD, Liang J, Liu ZX, Chen HS, Zhang JF. Expression and prognostic value of tumor stem cell markers ALDH1 and CD133 in colorectal carcinoma. *Oncology Letters*. 2014;**7**(2):507-512

[75] Mohamed SY, Kaf RM, Ahmed MM, Elwan A, Ashour HR, Ibrahim A. The prognostic value of cancer stem cell markers (Notch1, ALDH1, and CD44) in primary colorectal carcinoma. *Journal of Gastrointestinal Cancer*. 23 August 2018. DOI: 10.1007/s12029-018-0156-6. [Epub ahead of print]

[76] Chen J, Xia Q, Jiang B, Chang W, Yuan W, Ma Z, et al. Prognostic value of cancer stem cell marker ALDH1 expression in colorectal cancer: A systematic review and meta-analysis. *PLoS One*. 2015;**10**(12):e0145164

[77] Ueda K, Ogasawara S, Akiba J, Nakayama M, Todoroki K, Ueda K, et al. Aldehyde dehydrogenase 1 identifies cells with cancer stem cell-like properties in a human renal cell carcinoma cell line. *PLoS One*. 2013;**8**(10):e75463. DOI: 10.1371/journal.pone.0075463

[78] Goossens-Beumer I, Zeestraten E, Benard A, Christen T, Reimers M, Keijzer R, et al. The clinical prognostic value of combined analysis of Aldh1, Survivin, and EpCAM expression in colorectal cancer. *British Journal of Cancer*. 2014;**110**(12):2935-2944