Chapter

Stem Cell Markers in Colon Cancer

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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-transmembranaire 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 ubiquitary 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 regiments 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 β1, 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.

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