Review

Stem Cells, Colorectal Cancer and Cancer Stem Cell Markers Correlations

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ABSTRACT: The idea of stem cells as being progenitors of cancer was initially controversial, but later supported by research in the field of leukemia and solid tumors. Afterwards, it was established that genetic abnormalities can affect the stem and progenitor cells, leading to uncontrolled replication and deregulated differentiation. These alterations will cause the changeover to cancerous stem cells (CSC) having two main characteristics: tumor initiation and maintenance. This review will focus on the colorectal cancer stem cell (CR-CSCs) theory which provides a better understanding of different tumor processes: initiation, aggressive growth, recurrence, treatment resistance and metastasis. A search in PubMed/Medline was performed using the following keywords: colorectal cancer stem cells (CR-CSCs), colorectal neoplasms stem cells, colorectal cancer stem cell (CR-CSCs) markers, etc. Electronic searches were supplemented by hand searching reference lists, abstracts and proceedings from meetings. Isolation of CR-CSCs can be achieved by targeting and selecting subpopulation of tumor cells based on expression of one or multiple cell surface markers associated with cancer self-renewal, markers as: CD133, CD166, CD44, CD24, beta1 integrin-CD29, Lgr5, EpCAM (ESA), ALDH-1, Msi-1, DCAMLK1 or EphB receptors. The identification and localization of CR-CSCs through different markers will hopefully lead to a better stratification of prognosis and treatment response, as well as the development of new effective strategies for cancer management.

 KEYWORDS: cancer stem cells (CSC), colorectal cancer (CRC), cancer stem cell (CSC) markers, colorectal cancer stem cells (CR-CSC)

Introduction

Colorectal carcinoma (CRC) represents the third most commonly diagnosed cancer in men and second in women, with more than 1.2 million new cancer cases and 600 000 deaths only in 2008 [1]. Its prevalence is still on the rise in the developing countries due to the ageing population associated with a diet low in fruit and vegetables, but high in red meat, fat and processed food.

One recent study regarding the cancer incidence and mortality patterns in Europe revealed that CRC is the second most common cause of cancer death in both men and women. Also, it showed that the incidence rates of CRC are slightly higher in men than in women. There are high variations regarding the incidence rates across Europe. The lowest rates are registered in some of the Balkan countries. In Romania CRC represents the second leading type of cancer in terms of new cases and deaths in both females and males [2].

Although CRC was and continued to be intensely studied, the problem of treatment failure and tumor relapse remains a touchstone regarding the improvement of patient survival which is still poor. Thus, the survival rate at 5 years of follow-up is approximately 50% [3].

Carcinogenesis

Two possible models of colorectal cancer carcinogenesis are currently described: a stochastic model, where any cell has an equal capacity of cancer initiation and promotion, and a cancer stem cell (CSC) model, where tumors are organized in a certain hierarchical degree and only CR-CSCs have a cancer potential. CRC carcinogenesis is a complex process requiring the accumulation of genetic/epigenetic aberrations [4].

The study of CRC leaded to an early observation: tumor heterogeneity is present in all tumors. Heterogeneity is defined by various phenotypical characteristics which are the consequence of the interaction between genomic instability of tumors and micro-environmental factors [5]. According to this model, cancer cells represent the outcome of multiple mutations which will form a population of continuously diversifying cells. This cellular heterogeneity allows different clones to survive and grow under specific conditions, thus being the cause of tumor relapse, metastasis or radio-chemotherapy resistance. On the other hand, the new CSC theory revolutionizes the traditional way of understanding cancer by suggesting that only the pluripotent CSCs can self-renew and promote tumor growth. This theory represents in

fact a modern interpretation of the 'embryonal rest theory' developed by Julius Cohnheim in 1867, stating that fragments of embryonic tissue persist in adult organs and changes of the environment will cause cell proliferation and fetal tissue resembling masses [6].

A recent study endorsed the idea that CRC pathogenesis might be induced by transformed CSCs that have the ability to self-renew and to aberrantly differentiate, associating also the interaction between the microenvironment and CR-CSCs [7]. Tumor micro-environment plays an important role in the progression of CRC through the following steps of invasion and metastatic dissemination [8]. Metastasis are a consequence of uncontrolled proliferation of cancer cells group which includes the subpopulation of CR-CSCs [9]. This proliferation process represents the effect of a disequilibrium between the positive and negative angiogenic factors (released by both tumor and host cells), disequilibrium generated by the cancer-stromal cell interaction (Fig.1) [10].
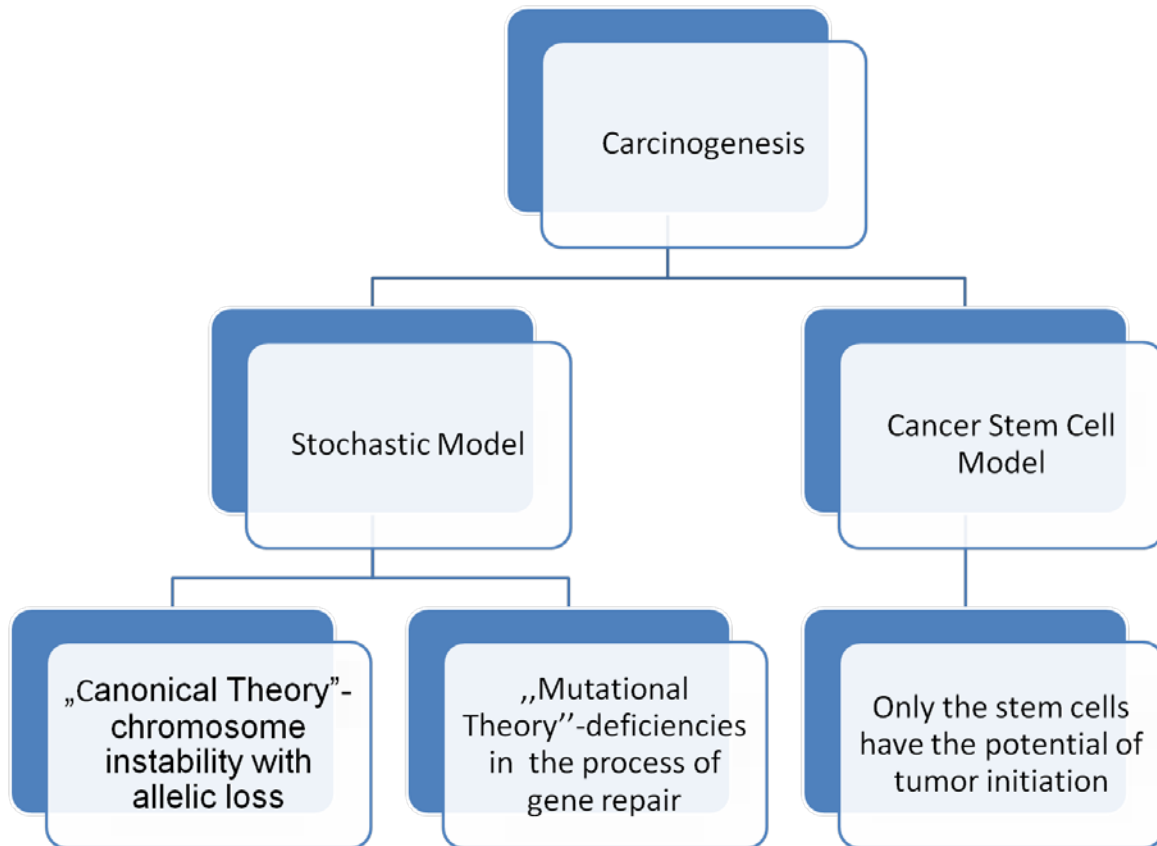

Fig.1 Colorectal Cancer Carcinogenesis Models (modified after [10])

The Path of Normal Stem Cells to CSCs

The wall of the colon is a layering of four distinct anatomical structures: mucosa, submucosa, muscularis propria and serosa. The mucosa is a combination of glandular epithelium, lamina propria and muscularis mucosae. The epithelial cells are arranged in a single layer which is folded into finger-like invaginations and enclosed in the submucosal connective tissue forming the crypts of Lieberkuhn, the functional units of the colon. The stem cells, pluripotent cells, are located at the base of these crypts, among other cells [11, 12]. While residing at the base of the crypt, the stem cells differentiate (proliferating cells, differentiating cells, apoptotic cells) while migrating upwards to the top of the crypt.

CSCs can be found at the base of the crypt in a relatively limited number and are responsible for the physiologic cellular turnover of the epithelium and regeneration after injury. Increasing stem cell number associated with their migration from the base to the top of the crypt is the signal of stem cell expansion which is believed to be an early event in intestinal tumorigenesis [13]. There are experiments

showing that tumorigenic events can lead to a colon tumor only if they take place in the stem cell region and not the transient-amplifying region [14].

In the literature, two terms are usually used: cancer stem cell and tumor-initiating cells, both being able to create confusion about the cell type to which its related [15]. The term CSCs might suggest those cells which originate from normal stem cells and acquire a number of genetic mutations sufficient to induce malignant transformation. This might be true in several cancers but it does not apply to all tumors. Also it is plausible for some differentiated cells to achieve a potential of self-renewal and stem-like properties after multiple mutagenic events [16]. Otherwise, the term tumor-initiating cell is frequently used to describe putative CSCs, referring to the ability of these cells to initiate tumors when being transplanted in a heterograft model [17].

CSCs share the same properties of self-renewal and differentiation as normal stem cells and share a similar phenotype to adult stem cells which are isolated from the same tissue. It is believed that CSCs can derive from mutation of normal stem cells or it might have different origins. Considering these possibilities, the heterogeneity of tumors is the result of aberrant differentiation of tumor cells into those tissues containing cells from which the tumor originated. Tumors were suggested to contain stem cell-like cells, the cancer stem cells or tumor-initiating cells which are uniquely capable of propagating a tumor much like normal stem cells fuel proliferation and differentiation in normal tissue [18]. It has being suggested that the mutant colon stem cells expand faster than normal correspondents although it does not imply a higher rate of cell cycle in the mutant stem cell. This might be due to the existence of dormant stem cells group which at some point return to an active cycling state [19].

Although CSCs have been implicated in colon carcinogenesis for several years, the complexity of their biology, an undeniable identification and isolation strategy remain topics of debate [20]. Except these matters, cancer stem cells are of great importance regarding their liability of tumor initiation, maintenance and recurrence due to treatment resistance.

Implications of CSCs resistance to therapy

"It's like dandelions in the back yard: You can cut the leaves off all you want, but unless you kill the root, it will keep growing back": John Dick (the first who identified CSCs in certain types of human leukemia).

Actually, CRC treatment was designed and selected for broad cytotoxic activity that might kill the majority of cancer cells within a specific tumor and induce a dramatic regression of a large tumor. Due to the fact that traditional treatment is not targeting the CSCs, the tumor can be eventually regenerated and relapses driven by the spared CSCs. All in all, the failure of chemotherapy might lie in its capacity of targeting the bulk of cancer by interfering with the ability of rapidly growing cells to divide, without affecting stem cells. Sometimes, the occurrence of drug-resistance usually characterizes and complicates the course of the disease. On the other hand, a treatment addressing directly the CSCs might not determine rapid tumor shrinkage but might achieve long term disease eradication by exhausting the tumors self-renewal and growth potential [21-24].

Therefore, the identification of tumors CSCs component is essential in order to open new therapeutic perspectives, based on the selective targeting of this specific population of cells. Ideally, new treatments should specifically target CSCs and also eliminate the tumor non-CSC population. Targeting molecules or pathways known to be active in CSC might reduce the possible side effects of treatment over normal stem cells [25]. Directing monoclonal antibodies against cell surface molecules, such as CD133, CD166, CD44, etc., might lead to the decrease of tumor size, metastatic potential and resistance to chemo- or radiotherapy [26, 27].

Colorectal cancer stem cells (CR-CSCs) markers

Identification and localization of CR-CSCs through different markers is difficult, as well as highlighting different pathways disrupted in CRC. This topic remains highly controversial due to the lack of widely accepted specific molecular markers [4]. Consequently, the study of CR-CSCs is quite challenging and involves a variety of state-of-the-art techniques, including immunohistochemistry and fluorescent microscopy, laser micro dissection (including single cell), quantitative real-time polymerase

chain reaction (qRT-PCR) and confocal laser endomicroscopy (CLE). CLE is a recent imaging technique that allows microscopic analysis of the gastrointestinal (GI) tract mucosa, allowing early recognition of mucosa and submucosa, including micro-vascular changes. This later technique assumes the use of fluorescent contrast agents that have been developed for targeting disease-specific biomarkers, which specifically bound colonic dysplasia, with high sensitivity and specificity [28].

Target detection in CRC has been previously studied in mice models, proving its feasibility [29-32]. A question which remains to be addressed regards the best candidate molecule or cell to target. Stem cells seem to be a good target due to their quality of being the only long living cells in a rapidly proliferating tissue such as the intestinal tract. This makes them good candidates for accumulation of successive mutations required for the tumor evolution, a reason for which CRC is proposed to be a disease of the stem cell [33]. Isolation of CR-CSCs can be achieved by targeting and selecting subpopulation of tumor cells based on expression of one or multiple cell surface markers associated with cancer self-renewal, markers as: CD133, CD166, CD44, CD24, beta1 integrin-CD29, Lgr5, EpCAM (ESA), ALDH-1, Msi-1, DCAMLK1 or EphB receptors [4, 34].

CD133, CD166 and CD44 are three main markers that have recently been associated with CR-CSCs. An important matter is understanding which of these markers has the greatest impact on patient prognosis. A landmark study compared expression and prognostic significance of these three markers, demonstrating that expression of CD133 correlates with that of CD166, while both do not correlate with CD44 [35]. The authors showed that although some of the results regarding these three markers are controversial, their combined analysis may be superior in identification of low, intermediate and high-risk cases of colorectal cancer.

CD133

CD133 was identified for the first time in 1997 on normal human hematopoietic stem cells [36, 37]. Known also as prominin-1 in rodents or AC133 in humans, CD133 is a transmembrane and cell surface protein with a molecular weight of 120 kDa, localized mainly at the level of membrane protrusions. It has been shown to characterize normal and CSCs in several human tissues, including the colonic mucosa [28, 36-41].

Regarding the expression of CD133, several studies were performed using immunohistochemical methods showing that the CD133 antigen was located exclusively on the cell membrane at the luminal surface of cancer glands [35, 42-49], while others demonstrated that CD133 could be detected both on membrane and cytoplasm in CRC (Fig. 2 and 3) [49-53]. Different locations of CD133 were associated with different clinical significance: membranous CD133 expression correlated with patient survival, recurrence-free survival and chemo-resistance, while cytoplasmic expression was not an independent marker for patient survival and recurrence. The changeover of CD133 localization from cytoplasmic to membranous is correlated to the transition of epithelial cells to a more invasive phenotype [54]. Except imunohistochemical staining, another method of revealing CD133 is represented by the use of reverse transcriptase-polymerase chain reaction (RT-PCR) which examines CD133 gene or mRNA expression. Based on this technique it has been suggested that high CD133 gene level may anticipate further recurrence and an overall poor prognosis for the patients with CRC [53-55].

Despite constant research efforts, the molecular mechanisms and signaling pathways that regulate the behavior of CD133-expressing cells or their intrinsic biological functions remain mysterious [17]. However it has been demonstrated that CD133+ cells are able to preserve themselves as well as to differentiate and restore tumor heterogeneity upon serial transplantation in vivo [28,36]. Tumorigenic cells in colon cancer are included in the high-density CD133+ population, which account for approximately 2.5% of the tumor cells [11], while it had been suggested that CD133+ tumor cells might be more resistant to radio-chemotherapy than CD133- cells in CRC [12,13]. Although some studies failed to demonstrate an association between the presence of CD133+ cells resistant to radio-chemotherapy and a poor clinical outcome of CRC [55, 56], a recent meta-analysis showed that CD133 is significantly associated with a worse 5-year survival rate in CRC patients. Besides, CD133-high expression was also related with more frequent T3/4 category, N positive and vascular invasion. Nevertheless, one of the main study conclusions was that CSCs in CRC cannot be identified by CD133 expression, alone [54].
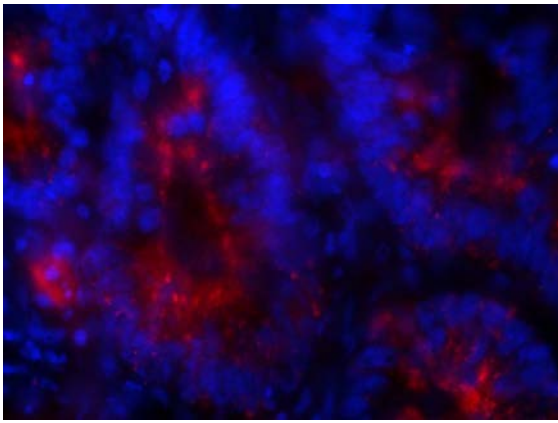
Fig.2.On a well differentiated adenocarcinoma, CD133 is mainly expressed in the supranuclear cytoplasm of the tumor cells, as well as on their apical membranes (scale bars represent 50 μm).
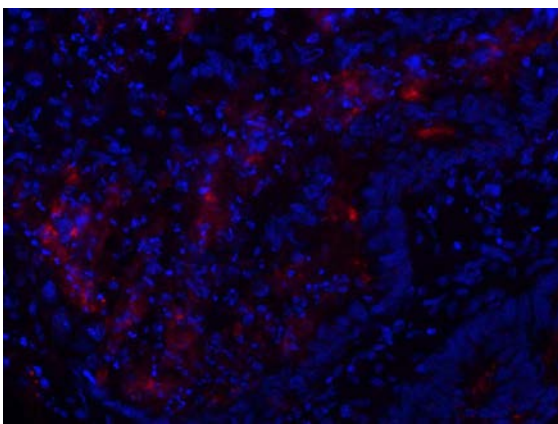

Fig.3.On a poor differentiated adenocarcinoma, CD133 expression is scattered in both the cytoplasm and the membranes of the tumor cells, with some expression being also present scarcely in the inflammatory stromal cells (scale bars represent 50 μm).

CD166

The activated leucocyte cell adhesion molecule (ALCAM) or CD166 is a cell adhesion molecule, a type-1 glycoprotein that belongs to the immunoglobulin superfamily with a weight of 110-kDa, which was associated with tumor initiation in xenografts, colony formation and further enrichment, while it correlated with prognosis and survival of CRC patients [4, 35].

CD 166 is described as having different cellular location, being predominantly expressed at the cell membrane, with a lower expression at the cytoplasmic level (Fig. 4 and 5) [57]). It has a distinct prognostic value, with positive cytoplasmic expression being associated with a poor clinical outcome. CD166 expression usually indicates advanced T category and N-positive status in CRC. The heterogeneous expression of CD166 in CRC, associated with the correlation between marker expression and

poor survival, were the main reasons for choosing CD166 as a prognostic marker for CRC [58].
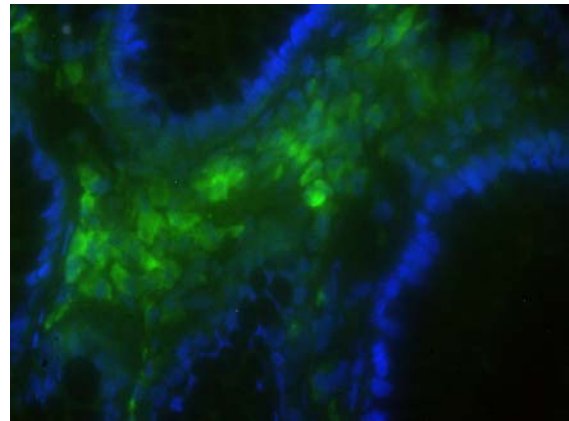

Fig.4.On a control fragment, CD166 is heavily expressed in both the cytoplasm and the membranes of stromal cells with no expression in the epithelial glandular cells (scale bars represent 50 μm).
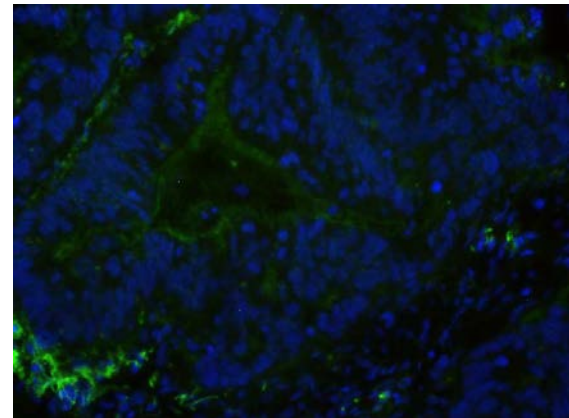

Fig.5.On a moderately differentiated adenocarcinoma, CD166 begins also to be present in the supranuclear cytoplasm of the tumor cells, as well as on their apical membranes (scale bars represent 50 μm).

CD44

The cell adhesion molecule CD44 is a hyaluronic acid receptor which was proposed as alternative CSC marker. CD44 is known to be involved in cell growth, differentiation and survival. As an important adhesion molecule, CD44 plays a major role in cancer cell migration being associated with tumor initiation in xenografts and colony formation, as well as tumor stage, lymph node infiltration, prognosis and survival [26, 35]. CD44 cells display CSC properties meaning that a single cell could self-renew, differentiate and form a xenograft tumor featuring the original lesion. CRC cells sorted for CD44+ displayed high tumorigenicity, especially in combination with CD133+ cells,

whereas CD44- cells could not form new tumors [4]. Furthermore, CD44 can also be used together with the stem cell marker CD166. A study performed on immunodeficient mice showed that CD44+CD166+ colon cancer cells present a higher ability to form tumors compared to CD44+CD166-, CD44-CD166+ or CD44-CD166- cells. Revealing this useful markers combination plays an important role in the identification of colon CSCs [59,60].
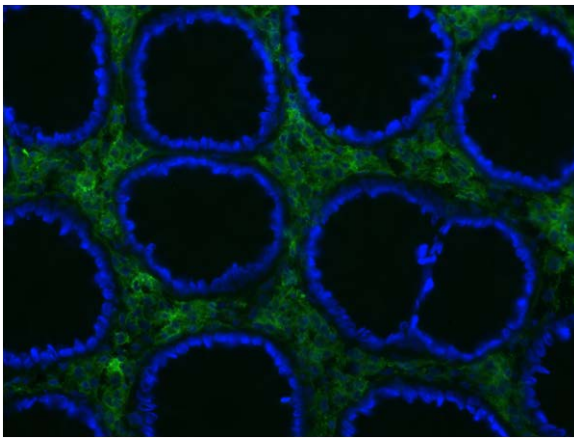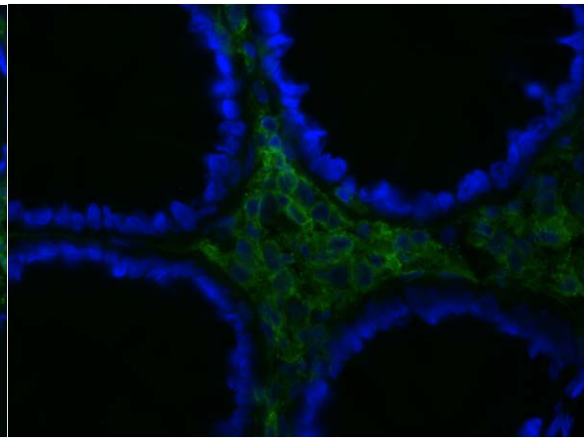

Fig.6

Fig.7

Fig.6,7.CD44 exhibits a clear-cut membrane staining pattern in stromal and inflammatory cells in normal colonic mucosa (scale bars represent 50 μm).
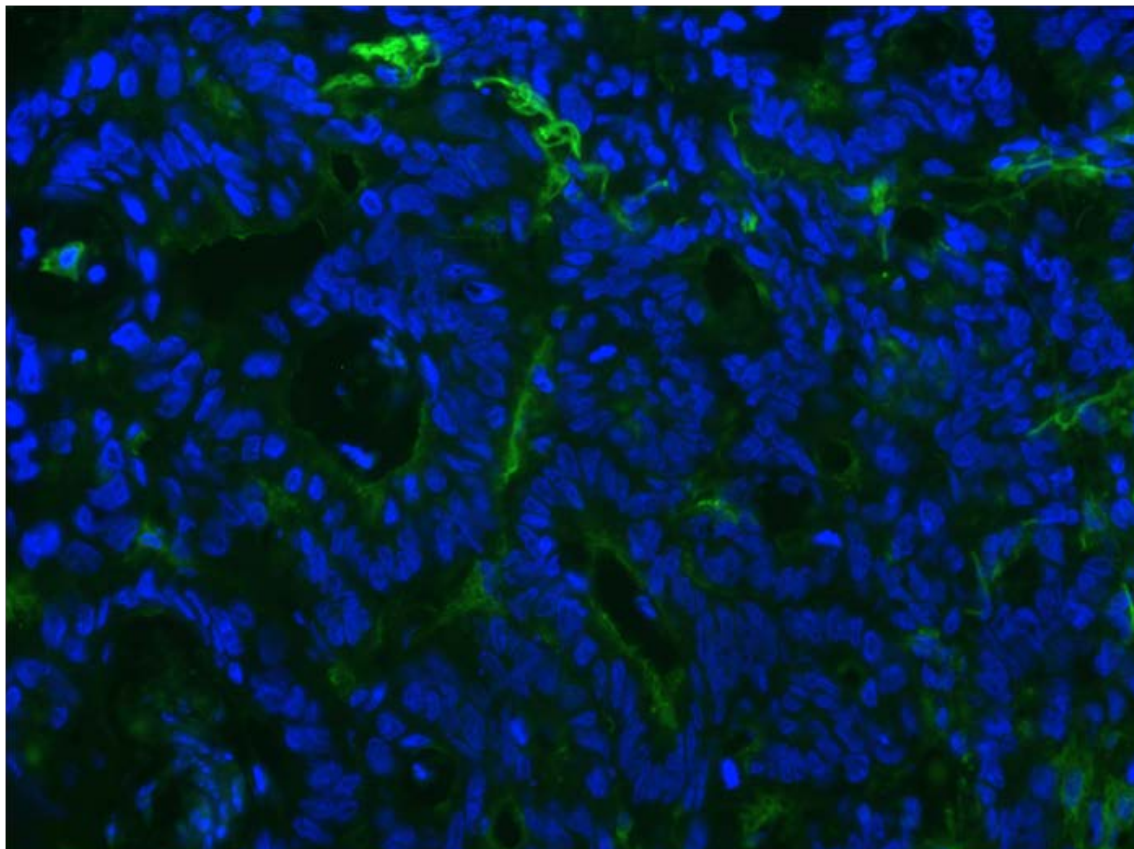

Fig.8.On a moderately differentiated adenocarcinoma, CD44 is also expressed on the apical membranes of the epithelial cells (scale bars represent 50 μm).

Conclusions

Identification of precise cancer stem cells markers will allow early cancer detection, as well as development of more efficient targeted treatment options, which will in turn decrease both local recurrences and metastases, thus improving the prognosis and overall survival of

CRC patients. A large number of studies regarding cancer stem cells and their markers were performed during the past years, but further research is needed for a better understanding of the molecular mechanisms implicated in the cancer stem cells biology. Several markers were proposed to define CRC CSCs, but none of them completely characterizes a distinct population of CSCs. Nevertheless, their implications as early diagnostic, prognostic stratification and therapeutic targets will have to be established by further studies based on innovative translational imaging strategies.

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