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Personalized medicine: Stem cells in colorectal cancer treatment

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ABSTRACT

Treatment failure in primary as well as metastatic cancer patients, caused by chemo and radioresistance, has reinforced the research for the applicability of personalized medicine. The use of stem cells (SCs) and cancer stem cells (CSCs) in such a treatment approach will be reviewed in this study. Colorectal cancer (CRC) SCs prove to be a promising asset for CRC treatment optimization both by serving as biomarkers for the current therapy modalities, by means of treatment personalization and patient/tumor stratification, as well as in the development of targeted therapies, selective for the stem cell population. Similar conclusions are drawn, regarding mesenchymal stromal cells (MSCs) and their effect in CRC therapy; while resident stromal cells (RSCs) of tumor microenvironment (TME) seem to promote the tumorigenic and metastatic processes in addition to conferring to the chemo- and radioresistance, under certain conditions they are able to improve the treatment outcome of CRC chemotherapy, e.g. by targeted enzyme/prodrug treatment of CRC cells. This review, points out the dynamic potential of CSCs and other SCs types in CRC treatment personalization as well as, in the improvement of current treatment approaches, opting to a higher therapeutic rate, improved prognosis, survival and quality of life for CRC patients.

1. Introduction

Cancer is a group of diseases, characterized by uncontrolled cellular proliferation, tissue invasion and metastasis. Since it constitutes one of the major causes of fatality in our era, scientific research has successfully developed a very wide armoury of antineoplastic drugs, with a variety of pharmacological properties, including the novel forms of targeted anticancer therapy [1].

Cancer, however, is enormously complex and heterogeneous: phenomena such as chemo- or radiotherapy failure and subsequent tumor relapse and metastasis, as well as inter-individual variabilities towards anticancer treatment efficacy, decrease the overall survival rates and indicate the need of new, personalized and targeted anticancer treatment modalities, which shall "dodge the bullets" of treatment failure as well as treatment response variability, among patients of different, or quite frequently the same types of neoplasia. The "vision" of personalized medicine involves measurement of some "key" tumor characteristics, which assist the optimum therapy regimen to be tailored for each patient and successfully treat their cancer, preventing relapse [2] (Fig. 1). The use of novel molecular analyses, has resulted in an enormous data collection in the field of cancer research. In this article, we opt to review systematically, the most recent advances in the field of personalized medicine towards a very common and unfortunately, lethal disease, namely CRC, with a focus on the most up to date evidence in the role of SCs and CSCs in this field.

1.1. CRC: overview of the disease

1.1.1. Statistics

CRC is the third leading cause of cancer death worldwide, with the 5-

Abbreviations: 5-FC, 5-Fluorocytosine; 5-FU, 5-Fluorouracil; ABC transporters, ATP-binding cassette transporters; APC gene, Adenomatous polyposis coli gene; CSCs, cancer stem cells; CAFs, cancer-associated fibroblasts; CXCR4, chemokine receptor type 4; CRC-SCs, colorectal cancer stem cells; CRC, Colorectal cancer; DEAB, diethylaminobenzaldehyde; FAP, Familial adenomatous polyposis; IBD, inflammatory bowel disease; MSCs, mesenchymal stromal cells; mCRC, metastatic CRC; mAbs, monoclonal antibodies; MCP-1, monocyte chemotactic protein −1; MDR, multidrug resistance; NF-κB, nuclear factor kappa B; RA, retinoid acid; SCs, stem cells; SDF-1, stromal cell derived factor-1; TME, tumor microenvironment; VCAM-1, vascular cell adhesion molecule −1.

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year relative survival rate being only 8%, despite many diagnostic and therapeutic advances [3]. Regardless of the existence of excellent screening and preventive strategies, (CRC) remains a major public health problem in Western countries. About 72% of new CRCs arise in the colon, and the remaining 28% arise in the rectum [4].

1.1.2. Histopathology and genetics of CRC

The main histopathological classification of CRC includes: 90–95% of adenocarcinomas, malignant tumors derived from cuboidal or columnar epithelial cells; 10% of mucinous adenocarcinoma, more commonly seen in younger patients. Around 1% are signet-ring cell carcinoma and other tumor types, such as, squamous cell carcinomas, small-cell carcinomas, carcinoid tumors, adeno-squamous and undifferentiated carcinomas which can be found in the colon and rectum. Sarcomas and lymphomas as non-epithelial tumors, are very unusual [4].

Tumorigenesis has the following phases: 1. independent growth signaling, 2. loss of sensitivity to the growth inhibitory signal, 3. escape from apoptosis, 4. uncontrolled replication, 5. aberrant angiogenesis, and 6. tissue invasion. Primary genetic defects cause DNA instability, and this results in defects of tumor suppressor genes [5].

Screening of CRC cells with DNA probes specific for known proto-oncogenes and some tumor suppressor genes revealed that 50% of these malignant tumors have acquired an activating point mutation of K-Ras oncogene and 75% of these cancers had an inactivating mutation on the p53 tumor suppressor gene. Moreover, studies of multiple cases of Familial adenomatous polyposis (FAP), have shown that more than 70% of colon cancers derived from intestinal polyps have a mutation in the Adenomatous polyposis coli (APC) gene. Indeed, it now seems clear that mutations inactivating the APC gene represent an early stage [6]. This mutation causes the upregulation of pathways connected with carcinogenesis namely the retinoid acid (RA) signalling pathway [7].

Colorectal carcinogenesis begins with the loss of polarity of epithelial cells and their separation from the basement membrane. In the next phase, come changes in the interactions between the cells and the extracellular matrix. Subsequently, signalling leading to changes in SCs is triggered. Taken together, this causes tumor SCs to form. In this way, tumor cells can subsequently be disseminated to various tissues by the lymphatic or vascular system [5].

1.2. Current CRC treatment options overview

CRC treatment consists of local or systemic therapy. Local therapies consist of surgery, radiation therapy and interventional radiology. Systemic therapy consists of chemotherapy and immunotherapy, since these drugs attack the neoplastic cells after entering the systemic circulation [8].

Depending on the stage of the cancer and other factors (age, pathophysiological status, gender, types of mutations, etc.), different types of treatment may be preferred, combined at the same time or used after one another: early stages of CRC (~95% of stage I and ~70% of stage II) commonly receive surgery, with or without adjuvant chemotherapy; Advanced stages (III and IV), commonly receive combination therapies, including chemotherapy radiation and surgery [9].

1.2.1. CRC chemotherapy

In CRC, chemotherapy is usually given after surgery of stage III tumors, where the carcinoma cells have spread to lymph nodes (adjuvant chemotherapy). The scope of chemotherapy is to reduce the cancer recurrence and mortality. In some cases chemotherapy may be administered before surgery, for tumor volume minimizing [10]. This is more common in rectal cancer than in colon cancer. Chemotherapy can be furthermore deployed as palliative, i.e., symptom-treating chemotherapy in metastatic patients. Finally, patients presenting with advanced (unresectable) metastatic CRC (mCRC) receive chemotherapy as a treatment of choice, which has highly improved the prognosis and increased the median survival rate of such patients [11].

First line chemotherapeutic agent, 5-fluorouracil (5-FU), is the mainstay chemotherapeutic agent in most CRC cases [8]. The development of additional agents, however, including platinum-containing agents, irinotecan, oral fluoropyrimidines and raltitrexed has expanded the chemotherapeutic “arsenal” for CRC patients. The choice

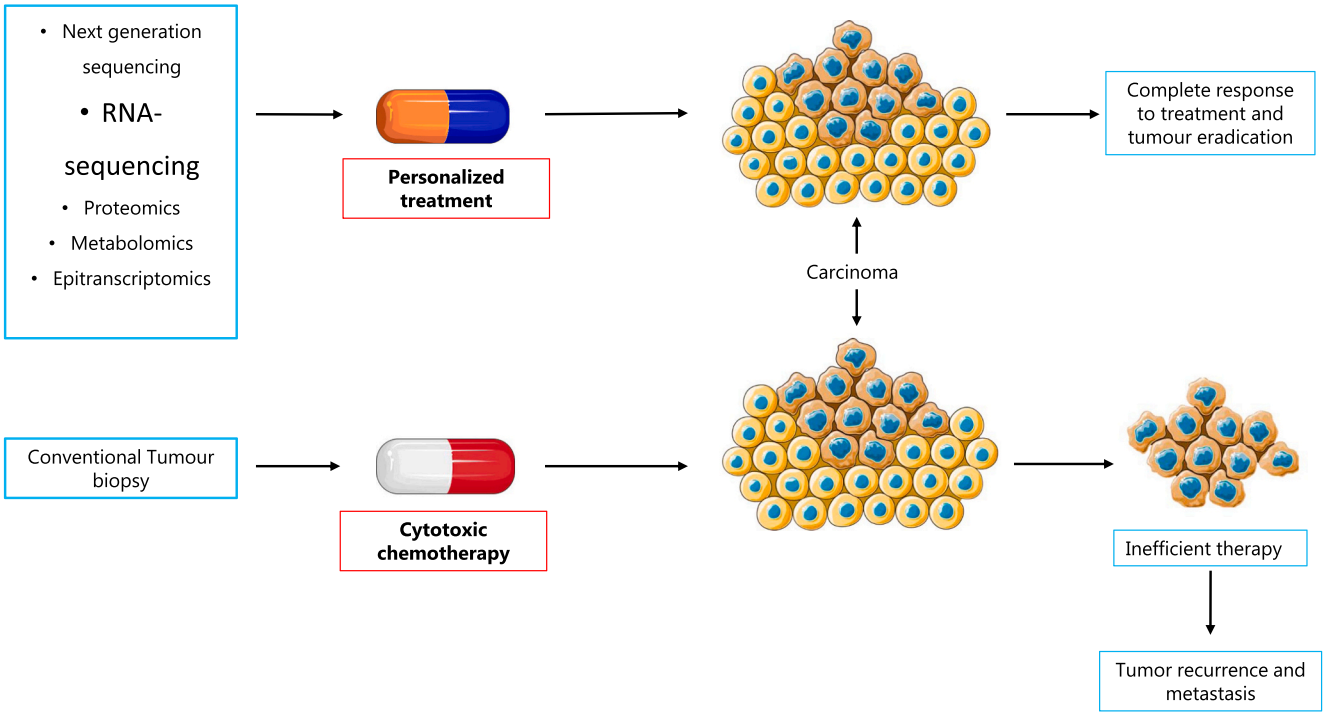

Fig. 1. Personalized medicine. An analysis of “key” tumor characteristics, which assist the optimum therapy for each patient. Conventional therapy, very often insufficient.

of combination chemotherapy is available also, mainly for stage IV (advanced) mCRC, with a profound synergistic toxicity advantage; the same benefit is nowadays evident for combinations in adjuvant (post-resection) chemotherapy of CRC [12,13].

1.2.2. Current CRC immunotherapy overview

The increased cases of progressed CRC despite both surgical interventions and traditional chemotherapy, resulted in an immense necessity for the development of novel therapeutic approaches, including immunotherapy [14]. Immunotherapy is an active therapeutic approach designed to trigger the immune system to respond to tumor-specific antigens and attack tumor cells. Immunotherapy approaches can use different types of peptides, which can be derived from: 1. tumor-associated antigens, 2. whole tumor cells, 3. in vitro-generated dendritic cells, or 4. viral vector-based cancer vaccines [15].

1.2.3. Monoclonal antibodies in CRC treatment

Monoclonal antibodies (mAbs) targeting surface antigens on tumor cells (Fig. 2) are successfully used in the CRC treatment [16]. Three mAbs (Cetuximab, Bevacizumab and Panitumumab) are approved for the treatment of CRC in the United States, and many other mAbs are being tested in clinical trials. Bevacizumab is effective in KRAS wild-type CRC patients. It is a recombinant humanized monoclonal antibody that selectively binds to human VEGF [17]. Patients with KRAS wild-type CRC have clinical benefits from treatment with anti-EGFR, Cetuximab and Panitumumab [18]. A different approach of using monoclonal antibodies mainly in mCRC is the use of antibodies against target, such as: programmed cell death 1 (PD-1) - ipilimumab or cytotoxic T lymphocyte antigen 4 (CTLA4) - pembrolizumab and nivolumab) or programmed cell death 1 ligand 1 PDL-1 - atezolizumab and durvalumab [19].

1.3. Resistance of CRC to current treatment options

The main complication in cancer treatment is resistance to radiation and chemotherapeutic drugs. Tumors are composed from a diverse population of malignant cells, some drug-sensitive, and some drug-resistant. Cancer cells are characterized by intrinsic multidrug resistance (MDR). They show resistance to chemotherapy when first exposed to an anticancer drug. After treatment with chemotherapy the elimination of the drug-sensitive cells is coupled to an unfortunate persistence of the resistant ones. There are also cases of neoplastic tumors which were initially sensitive to therapy and subsequently become insensitive to similar drugs, post-chemotherapeutically (Fig. 3). Such tumors are known to display acquired resistance [20]. After an initial chemotherapeutic treatment, the tumor begins to grow again and develops resistance to chemotherapeutic agents. Cancer cells employ several mechanisms for resistance to chemotherapy and radiotherapy (Table 1) [21].

Radioresistance of cancer cells, moreover limits the benefit of radiotherapy. The intrinsic and extrinsic properties of cancer cells determine the degree of radiosensitivity [22].

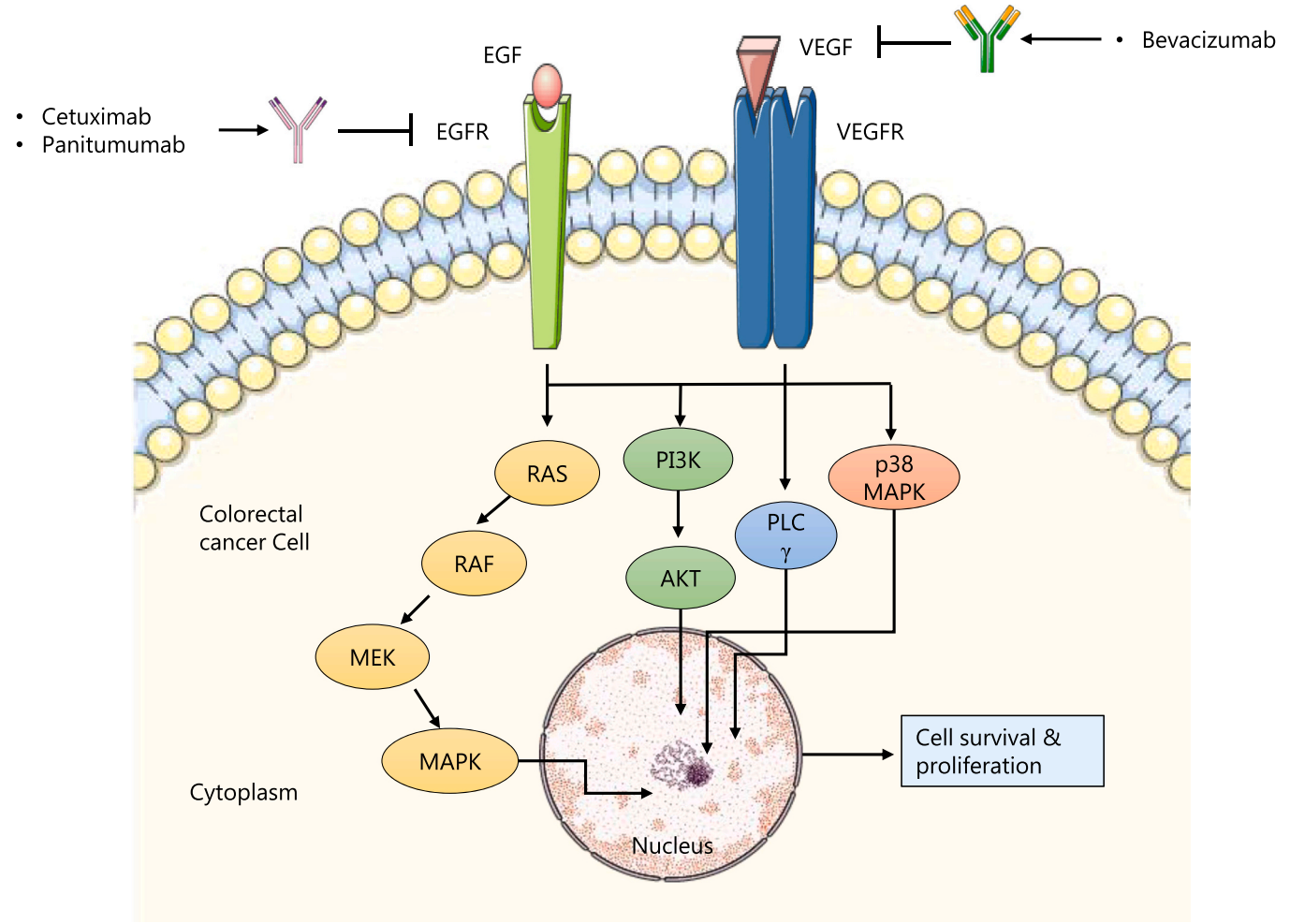

Fig. 2. Monoclonal antibodies (mAbs) mechanism of action. The mAbs targeting surface antigens on tumor cells. Bevacizumab binds to VEGF and Panitumumab, as well as Cetuximab, both recognise EGFR on the cancer cells.

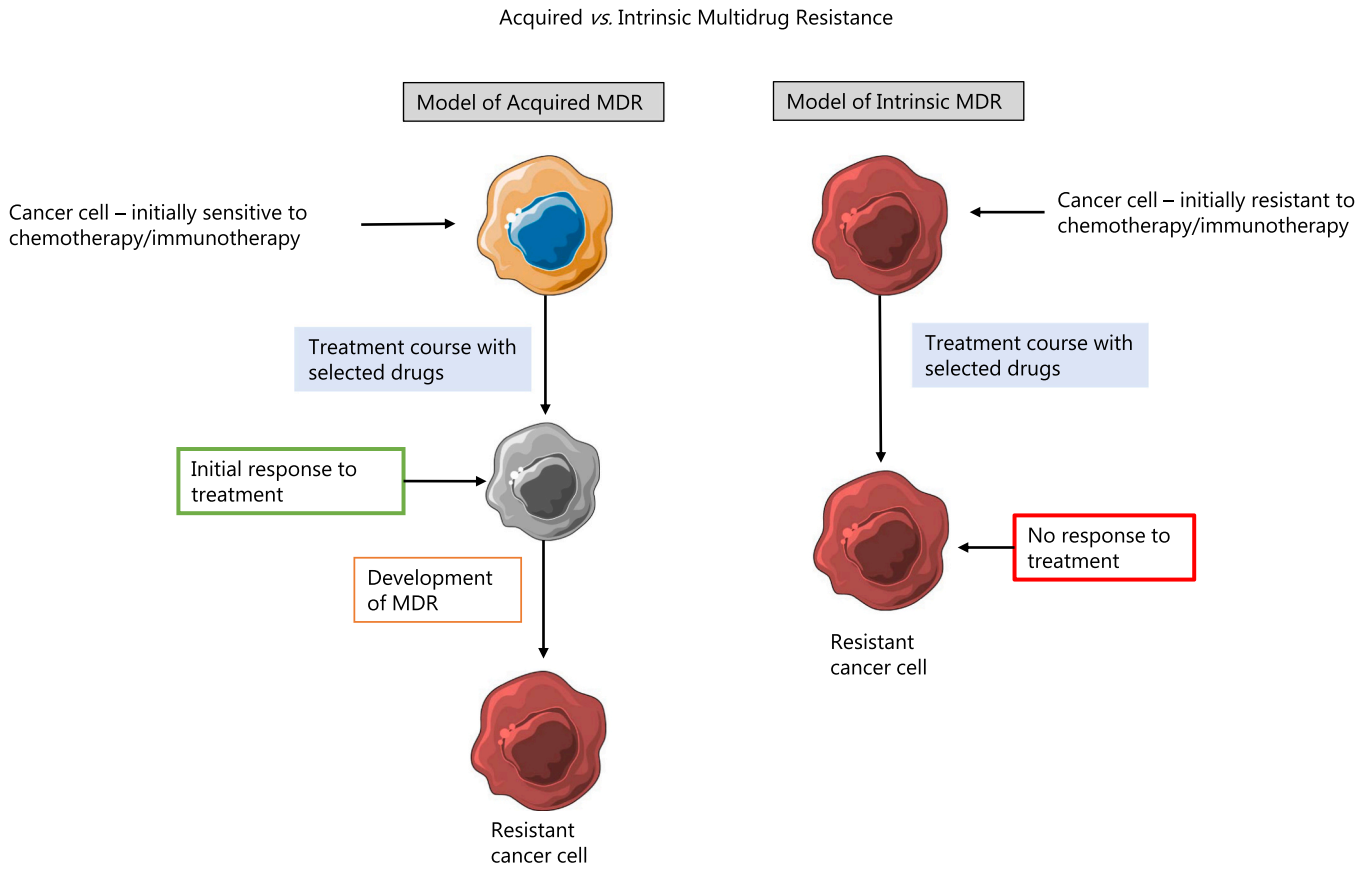

Fig. 3. Differences between intrinsic and acquired MDR.

Table 1
Mechanisms of chemo- and radioresistance of cancer cells.

Radioresistance	Chemoresistance
Tumor hypoxic conditions	Increased drug efflux
Increased production of cellular antioxidants	Decreased or limited drug uptake
Activation of certain protooncogenes	Evasion/Inactivation of apoptosis
Stromal interactions	Increased Drug metabolism and drug compartmentalization
Amplification of DNA repair (caretaker) genes	Increased efficiency of DNA repair mechanisms
Use of epigenetic mechanisms that promote cell survival	Increased expression or expression of altered drug targets (e.g. enzymes, structural proteins, etc.)
Alterations in "checkpoints" of cell cycle	Survival signals favored by transcription factors

2. The emerging role of SCs in CRC treatment

The novel cell-based therapies of cancer were developed under the contemporary notion of adult SCs biology. Conventional therapies, such as tumor resection techniques, chemotherapy regimens, and radiation therapy are evidently insufficient for the treatment of aggressive and recurrent CRC types. Multiple SCs exhibit an inherent propensity to migrate to the tumor site. Recent studies, unravel the potential of using various engineered SCs as therapeutic agents to attack cancer cells, in cases of brain tumors for example.

2.1. The effect of MSC on human CRC cells

The TME is a very important factor affecting growth and metastasis of CRC. The TME consists of many cell types including tumor, stromal, endothelial and immune cell populations. It is well known that cells present in the TME acquire special phenotypes that promote tumor progression. One such cell type is the MSCs. These MSCs exert effects in the TME of CRC cells, promoting angiogenesis, invasion and metastasis [23].

The role of MSCs in tumorigenesis is controversial. There is huge evidence about a tumor-promoting role of MSCs, opposed by studies pointing to an anti-tumor effect which is specific to the very early stages of tumor development. It is in connection with the course of a chronic inflammatory condition like inflammatory bowel disease (IBD), where the epithelium becomes inflamed and damaged, leading thus to the over-expression of transcription factors such as nuclear factor kappa B (NF-κB), signal transducers and activators of transcription STAT 3 and STAT6, all of which are potentially tumorigenic [24]. Administration of MSCs at this very early stage can have a tumor inhibiting effect by decreasing interleukin IL-6 and phosphoSTAT3 signaling and reducing DNA damage. After this very early stage, MSCs are recruited to the tumor by factors such as NF-κB, chemokine receptor type 4 (CXCR4), stromal cell derived factor (SDF)−1, monocyte chemotactic protein (MCP)−1 and vascular cell adhesion molecule (VCAM)−1. Such factors, synergistically promote tumorigenesis via differentiation of cancer-associated fibroblasts (CAFs), promoting tumor growth, invasion, metastasis and angiogenesis and the dampening of anti-tumor immunity [24–27].

2.2. The effect of MSCs in chemo- and radioresistance of CRC cells

In addition, CRC-MSC interactions begin, to bear a potential impact on CRC treatment. Recent research has proven the ability of MSCs to increase the chemoresistance of both hematological and solid tumors. In CRC, 5-FU is a first-line treatment choice. However, it has now been discovered that a bone marrow-derived type of MSCs exhibits both

immunosuppressive and 5-FU chemoresistance properties, a grave coupling in the context of CRC chemotherapy. Similar complications can arise in the case of radiotherapy, as well: MSCs have been proven to be radio-resistant and retaining their malignant phenotype and potentially contributing to cancer relapse. Because of this, genetically modified MSCs have been constructed which are used in an enzyme/prodrug therapy. This therapy can be targeted selectively, against the cancer cells. These cells then continuously express a transgene for the enzyme which converts the, non-toxic compound (prodrug) into a cytotoxic drug (suicide gene) (Fig. 4). There are several enzyme/prodrug combinations in use:

1. herpes simplex virus-thymidine kinase, combined with the antiviral prodrug ganciclovir (HSVtk/GCV) [28,29],
2. yeast or bacterial cytosine deaminase (CD), either alone or fused with uracil phosphoribosyltransferase (CD::UPRT) converting 5-fluorocytosine (5-FC) to toxic 5-FU [30,31],
3. prokaryotic purine nucleotide phosphorylase, with the anticancer agent fludarabine (PNP/Fara) [32].

Genetically modified MSCs were successfully used in the treatment of colon cancer.

2.3. CSCs and CRC

Experimental results have highlighted the existence of a small sub-population of tumor cells, notoriously called CSCs, posing them capable of propagating cancer in a highly efficient manner. This malignant cell group constitutes about 0.1–10% of total tumor cells of which only some have the ability of tumorigenicity [5]. Compared to normal SCs, CSCs are attributed an unlimited multiplication potential (i.e., proliferation); moreover, their slow cell-cycle could also play a role in resistance to treatment approaches (chemotherapy and radiotherapy) and tumor relapse. Also, the inherent ability of CSCs to form new tumors, may be of critical importance for metastatic progression of CRC [33].

In the CRC model, malignant mutations are suggested to take place either at the stem cell level or at the precursor cell level. Precursor cells are a type of partially differentiated SCs which have the potential to differentiate into only one cell type (unipotent SCs). Epigenetic alterations, including hyper-methylation, can result in silencing of genes, such as, p16, SFRPs, GATA-4/−5 and APC in stem or precursor cells in cell renewal systems, enabling these cells to enter stem-like states of abnormal clonal expansion. The stem/precursor cells are thence mutated into pre-invasive CSCs. Subsequently, pre-invasive CSCs transform into “mature” CSCs ultimately turning into cancer cells by the gradual accumulation of further epigenetic and genetic alterations [34].

Many recent studies have proven that CSCs sub-population, may be identified within a diverse group of human neoplastic lesions, with CRC being one of the most studied. Human colorectal CSCs were first isolated, by means of CD133 expression levels and moreover proven to initiate neoplastic lesions in mice, highly resembling the original malignancy [35,36]. Research for different surface markers of colorectal CSCs aimed in developing a CRC-SCs-specific biomarker, which should greatly facilitate the development of novel prognostic and therapeutic tools. While various CRC-SCs phenotypes have been described, the surface markers identified so far are expressed also by normal SCs, complicating hence, their potential use as treatment targets. Many molecules have been anointed as CRC-SCs markers, including CD133, CD44, CD24, CD166, Lgr-5, and Aldehyde dehydrogenase 1 (ALDH1), with CD133, a pentaspan transmembrane glycoprotein, being first colorectal CSCs marker to be identified [37].

A cell surface glycoprotein, known as cell adhesion molecule CD44 has been identified as in several types of CSCs. CD44 + cells displayed CSCs properties; single cells formed spheres in vitro, in addition to xenograft tumors resembling the original neoplasm, in vivo. Overexpression of CD44 in CRC has been linked with tumor volume and lymph node invasion and is therefore, accounted as a marker for prediction of overall survival [38].

In addition, activities of several signaling/metabolic pathways and various enzymes may also account as markers of cell stemness. For instance, resident colonic SCs may be isolated based on the activity of ALDH1, a detoxifying enzyme, that oxidizes intracellular aldehydes [39].

Other CSCc markers include, epithelial cell adhesion molecules CD29, CD24, CD166, CD26 along with molecules such as, Msi-1, Lgr-5, and β-catenin. The occurrence and levels of expression of these molecules has been associated with SCs features, both in vitro and in vivo. These markers were also used to prove/mitigate the tumorigenicity of isolated CSCs [40]. The transcription factors Sox2 and Oct4 are also regarded as potential CSCs markers, considering their participation in cell renewal mechanisms. Oct-4 and Sox2 levels have been proven to be augmented in CRC and to correlate with increased CRC-SCs proliferation and overall poor prognosis [41,42]. Other stemness genes, namely Nanog and c-myc, are investigated as CRC-SCs markers, appearing to facilitate a “shift” towards an “undifferentiated” cellular state [43].

3. CRC stem cells and CRC – treatment personalization

The notion of the CRC stem cells (CRC-SCs) model draws the glances at their potential role in guiding CRC treatment. The current approaches, generally target mature cancer cells and not the promising CRC-SCs. Although these treatments can reduce the tumor volume, they cannot completely eliminate CSCs that have higher proliferative potential in addition to inherent resistance to anticancer agents; they can “escape” the effects of chemotherapy and thence differentiate into

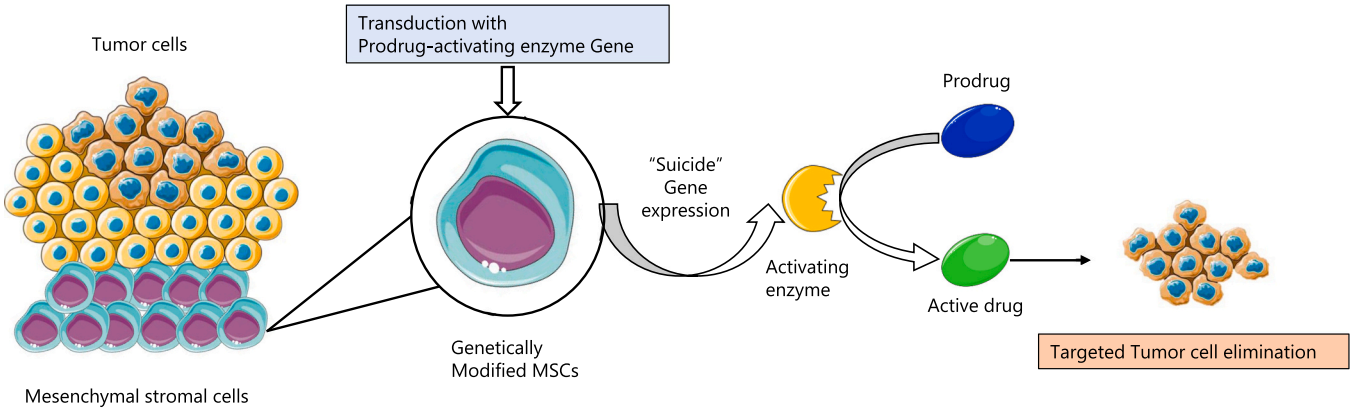

Fig. 4. Scheme of action of genetically modified MSCs which are used in the enzyme/prodrug therapy.

"mature cancer cells" when treatment course is completed, resulting in cancer recurrence and metastasis. Therefore, development of novel treatment tools, targeting exclusively CRC-SCs, has significant potential to achieve better treatment to suppress cancer growth and metastasis.

3.1. The role of CRC-SCs in CRC chemotherapy and chemoresistance

Two models can refer to the origin of CSCs MDR in CRCs displaying increased resistance to mainstay chemotherapeutic regimens. The first model proposing that after exposure to the anticancer agents, only the CRC-SCs expressing ATP-binding cassette transporters (ABC transporters) are able to "repopulate the tumor by asymmetrical cell division" with new CRC-SCs and/or partly differentiated precursor cells. The second model claims that post-chemotherapeutically, only CSCs survive and, those that acquire chemoresistance, develop new and even more aggressive/chemoresistant cell phenotypes, after accumulating mutations [44].

Conventional anticancer therapies, including chemotherapy and radiotherapy, depend on the rapid cell cycle and target specific phases, including CRC treatment tools like 5-FU, the targeting enzymes of the S-stage of mitosis, and Oxaliplatin, a platinum anticancer drug [43]. Nevertheless, there is evidence that intrinsic chemoresistance in CRC SCs may be caused by their slow growth rates in the G0 stage. A large clinical research conducted in 501 CRC patients concluded that, CRC cells with CD133 overexpression showed higher resistance to 5-FU treatment and the expression of CD133 was linked to poor prognosis and decreased survival [45]. In oxaliplatin treated SW620 and LoVo CRC cells, overexpression of CD133 was also seen [46].

Recently, it was shown that human HT-29 CRC cells, treated with high concentrations of 5-FU and/or oxaliplatin treated exhibited increased levels of CD133 + and CD44 + CRC-SCs and decreased in vitro proliferation [47]. Long term cultivation in the 5-FU changed the properties of parental HT-29 CRC cell lines. Not only they became MDR, but they also started to express different surface markers. They started to express the surface marker CD271 which is connected with tumorigenicity and stem-like properties [48]. Moreover, increased expression levels of insulin-like growth factor receptor I (IGF-1R), was seen in chemoresistant HT-29 CRC cells, while treatment with an IGF-1R inhibitor (AVE-1642), reduced the volume of an in vivo tumor xenograft. Other researchers report that, human CD133 + CRC-SCs can "regulate death receptors and inhibit chemotherapy-induced cell apoptosis through specifically expressed interleukin-4 (IL-4)". Treatment of CD133 + CRC-SCs, with anti-IL-4 antibody increased the effectiveness of 5-FU or oxaliplatin-based therapy [49].

A recent analysis, reported an additional mechanism of resistance of CD133 + CRC-SCs towards the anticancer agents oxaliplatin and 5-FU: Aurora-A kinase, a pro-mitotic protein kinase generally affecting the process of cell cycle, is found to be overexpressed in CRC-SCs; Aurora-A silencing experiments resulted in decreased growth, reduced anti-apoptotic protein expression levels and increased sensitivity towards anticancer chemotherapeutics, which then, additively eliminate CRC-SCs [50].

Furthermore, microRNAs (miRNAs) were found to take part in chemoresistance of CRC-SCs by regulating specific molecular pathways. For example, "miRNA-140 inhibits the proliferation of CD133 +high /CD44 +high human CRC cells through regulating histone deacetylase 4, leading to resistance to methotrexate and 5-FU" [51]. Bioinformatic analyses, identified nine miRNA which have significant effects on CRC development. The experimental studies are required to confirm these results [52]. It was confirmed though, that the miRNA144 can abolish invasion and migration of CRC [53].

In the treatment of CRC and more importantly, mCRC, chemotherapy effectiveness is also negatively impacted in regimes which include several chemotherapeutic anticancer agents of various pharmacologic groups (having different and diverse mechanisms of anticancer action), with this phenomenon being addressed as MDR. Reduction of the chemotherapeutic agent(s) concentration in the CRC cells, is the most studied mechanism of MDR: mostly elucidated by the proven overexpression of ABCB1 transporter, also known as p-glycoprotein which actively assists the CRC cell detoxification at the expense of ATP. In addition, resistance to camptothecin and 5-FU, two commonly used anticancer drugs in CRC treatment, has been observed in relevant experiments with human melanoma cells [54].

CSCs surface markers identified so far, are expressed also by normal SCs, preventing their potential use as targets of a specialized anticancer treatment modality. In contrast to such surface marker molecules, the aldehyde dehydrogenase enzymes as a CRC-SCs marker, stands as an intracellular protein with an enzymatic function of oxidation of both endogenously and exogenously produced aldehydes to their respective carboxylic acids. There are 19 isoforms of ALDH in humans from which only few are connected with CSCs features [55]. The resistance caused by high expression of ALDH1A3 could be connected also with CXCR4 expression. However, this fact needs more experimental studies for clarification of the mechanism of action [55]. High activity of ALDH, "interferes'' with several chemotherapeutics used in the treatment of patients with CRC. Detoxification and drug inactivation represents one of the mechanisms contributing to chemoresistance. Recent data provides the evidence for CRC-type specific ALDH isoform expression: chemosensitivity of tested cell lines with silencing of ALDH1A1 or ALDH1A3 gene were compared to unaffected ones. Samples after silencing were exposed to 5-FU, Capecitabine, Raltitrexed and Irinotecan, commonly deployed anticancer agents for the treatment of primary, as well as mCRC. Silencing of these two ALDH1A isoforms led to different effects on chemosensitivity to tested drugs [56].

Finally, the effect of pharmacological inhibition of several ALDH isozyme activities by the chemical diethylaminobenzaldehyde (DEAB) partially sensitized the tested cell lines to chemotherapeutics: Chemosensitivity test in the presence of a sub-inhibitory concentration of DEAB, confirmed its potential to increase the antiproliferative effect of 5-FU, Irinotecan, Raltitrexed and Cisplatin in CRC-SCs [56].

3.2. The role of CRC-SCs, as biomarkers for CRC personalized medicine

Currently, the majority of clinical trials in process, involve metastatic cancer patients, investigating the potential of temporarily controlling tumor progression. However, unravelling of CRC-SCs biology, brings forth the hope for several, novel therapeutic options. Current therapies do not target the CSCs; this could be the reason behind recurrence of tumor growth or resistance to treatment post therapy [34].

CSCs, have been obtained from colorectal, neuronal, breast, lung, pancreatic and various other types of cancer, either by means of cell surface marker molecules (e.g., CD24 +, CD44 +, CD133 +) or by using dye-efflux sub-population isolation methods combined with cell sorting techniques [57]. The prognostic value of the CSCs markers in the clinic is expanding, given their role as indicators of poor prognosis [58,59]. Studies on CSCs markers for CRC have shown that "tumors harbouring higher levels of both CD44 and CD133 had a higher risk of developing early liver metastases" [60].

CSCs markers are not only important for their identification but can also prove to be useful treatment targets [61]. Moreover, such markers may assist in proper selection of regimen of therapy and current status of treatment, as well as disease: By definition, a predictive biomarker, should enable the accurate determination of the outcome of anticancer treatment approach applied in each case, for both the site of neoplastic lesion and the totality organism, as early as possible in the treatment course to permit the proper regimen modification and selection, of the most suitable therapeutic intervention. According to this definition, thus, the causative link between a biological factor and therapy outcome is brought in the front scene, i.e. detection of changes in specific biomarker expression level can enable the respective modifications in anticancer treatment response. In other words, "a strong association between biomarker and treatment outcome should result from a similar

direct treatment effect on both, biomarker level and endpoint of therapy" [62].

It is evident therefore that, patient stratification on the basis of a biomarker classification system, aiming to treatment individualization, should enable the identification of patient groups, with the possibility of a good response to traditional anticancer treatment regimens and the concomitant compartmentalization of patient groups with poor treatment-outcome prediction percentages (Fig. 1). Such patient groups can then be studied, providing further knowledge on "interpersonal disease variations" and thus increase the knowledge available for personalization of anticancer treatment. However, for patients in-between these two extreme groups, the difficulty of biomarker based classification increases, clearly points to the need for research refinement, in the aspect of biomarkers, placing CSCs in a promising position [63].

Several specific gene-collections, assisting in the identification of tumor types, and furthermore, in the prediction of metastatic potential to distant body tissues, have been well-elucidated in recent years and thus rendered potentially valuable in the future clinical practice of anticancer therapy. Moreover, the characterization of SCs markers, both in vitro and in vivo and the elucidation of the special biological and metabolic characteristics of CSCs in tumor specimens derived from patients with cancer and/or cancer cell lines has also revealed their major role in malignant progression/metastasis, as well as, in chemoresistance development and tumor recurrence.

In a recent study, effectiveness of the anticancer agent Cisplatin coupled to ALDH targeting compounds or JAK2 inhibitors, the potential of ovarian CSCs population targeting was investigated: cells which survived the chemotherapy were isolated and used to form spheroids and model tumor initiation in a "personalized manner". The study concluded that ovarian CSCs spheroids, derived from different patients displayed response variations to chemotherapeutics in distinct patterns, enabling thus a level of treatment personalization. Tumor xenografts were formed, from ovarian CSCs spheroidal origin, even by single-cell spheroid, with distinct treatment responses seen in each of the xenografts, in accordance with the respective observations made on spheroids. Lower ALDH expression coupled with a complete ceasing of CD133, was proven in cells spheroids, resistant to cisplatin/ALDH inhibitor combination treatment while spheroids that were resistant to cisplatin/JAK2 inhibitor combination schemes consisted of increased number of ALDH+ cells [62].

Furthermore, in both published and unpublished results from our own laboratory, we have proven the diversity between responses of several human CRC cells to various anticancer agents, commonly used as a mainstay in early, as well as in advanced mCRC treatment; the differences in expression level of various CRC-SCs molecular markers, including ALDH1 isoforms, ABC transporters, plasma membrane receptors such as, Lgr5 and transcription factors such as, Nanog and Sox2 were correlated to the differences in chemotherapeutic effect of anticancer agents in human CRC cells. With multiple such experiments, we have indicated the increased cytotoxic effect of the drug Raltitrexed on HT-29 and HCT-116 CRC cells compared to the LS-180 CRC cell line- as result associating the different expression profiles of CSCs markers, as a possible explanation of this phenomenon, since all three cell lines belong to the same cancer type, namely colorectal carcinoma. The same patterns account for the drug Irinotecan, while our experiments proved that the drug Capecitabine follows the exact inverse pattern, exhibiting a much greater cytotoxic effect towards LS-180 CRC cells [56].

Finally, we have also proven that CRC cells, rendered resistant to the drug 5-FU, change both their chemosensitivity towards the tested anticancer agents, as well as their levels and patterns of CRC-SCs marker expression, with the above mentioned results pointing further to the potential of use of CSCs and their marker in the personalization of treatment regimens in colorectal neoplasia, stratifying patients according to tumor-CRC-SCs types, with the use of such biomarkers, both pre- and post- chemotherapy initiation [48,56].

4. Discussion

In the prism of the evident need for new approaches in the field of cancer treatment and after due consideration of complications such as, anticancer treatment failure, the use of novel modalities for treatment adjustment and personalization, as well as patient grouping and stratification according to the molecular characteristics and the biology of each, individual tumor, seems indeed, highly promising.In this review, we systematically summarized the most recent and updated experimental results, regarding the role of CSCs, as well as other types of SCs of the TME, in the context of treatment of colorectal neoplasia. After reviewing the current knowledge on the vast importance of this specific type of neoplasia worldwide, as well as its basic molecular and gross-pathological characteristics, we reviewed the mainstays in the CRC treatment nowadays including chemotherapy, surgical interventions and radiotherapy and thence, we analyse the main perplexities of current treatment, including concept of chemo-, radio- and MDR, which account for neoplastic lesion relapse, treatment failure and overall, decreased survival of CRC patients [64]. This phenomenon clearly points the way to the applicability of personalized medicine in this field and in this context, we further analyse the up-to-date research in the SC science field.

Citing results of research for the effects of MSCs and thus, the association of TME aspects on CRC cells, we observe that, evidence pointing to a "tumor-promoting" role of MSCs is being opposed by recent evidence indicating an anti-tumor effect, which, however, appears to be specific for the very early stages of tumor development: stating this, administrating MSCs, at this very early stage, can have a tumor inhibiting effect by reducing inflammatory signalling and DNA damage in human colorectal cells. Furthermore, MSCs studied in the context of chemo- and radioresistance in CRC cells, seem to induce resistance to chemotherapeutics used in CRC, as seen in experiments using 5-FU, the treatment of choice for this cancer type. In addition, MSCs have been proven to be radio-resistant and so, could withstand such therapy and retain their tumor-conditioned phenotype, potentially contributing in this manner, to disease relapse [27]. However, genetically modified MSCs have been tested in the enzyme/prodrug therapy: this therapy can be selective against the neoplastic cells and the results of such studies concluded that, genetically modified MSCs, were successfully used in the treatment of colon cancer [65].

A vast amount of experimental research, moreover, indicated the role of CSCs in novel treatment approaches for CRC, with a predictable extension of this concept in the treatment of many other types of neoplasia. Several molecules have been accounted as colorectal CSCs markers, including CD24, CD44, CD133, CD166 and aldehyde dehydrogenase 1 (ALDH1), CD166, epithelial cell adhesion molecule (EpCAM), CD29, CD26, Msi-1, Lgr-5 receptor, and Wnt activity/β-catenin, among many others [37]. Considering thus, the critical role of CSCs in aspects like tumorigenicity, metastasis, chemo/ radioresistance and in many cases MDR in anticancer treatment, as well as the different molecular biology profiles of CSCs in each individual tumor, research focused on such CSCs associated molecules, serving as both, markers of recognition and separation, as well as targets for their selective treatment opting for decrease of tumor relapse, metastasis and anticancer treatment failure.

The treatment personalization potential of CSCs markers in the clinic is promptly expanding, since it has been proven that CSCs and their associated markers can account for poor patient prognosis. Thus, CSCs markers may not only be important for their identification or targeting but can also prove to be useful treatment personalization "tools"- an asset in proper selection of regimen of therapy and current status of treatment, serving as novel biomarkers in the battle against CRC [2].

According to the relevant, cited results of our research, we can observe the role of CRC-SCs and their molecular markers in conferring chemoresistance to several anticancer chemotherapeutics in certain types of CRCs, with this phenomenon being whatsoever, variable, or

even absent in other CRC cell types. The differences in expression level of various CRC-SCs molecular markers, including ALDH1 isoforms, ABC transporters, plasma membrane receptors such as, Lgr-5 and transcription factors such as, Nanog and SOX2, were correlated to the variabilities in chemotherapeutic/cytotoxic effect of anticancer agents in human CRC cells, indicating their potential for future use as biomarkers in CRC treatment and thus therapeutic regimen individualization.

5. Conclusions

This review summarizes our knowledge of the effects and potential of SCs and CSCs in the field of personalized CRC treatment, serving as an indication of the importance of such research, in the development of novel cancer therapeutic approaches, aiming for improving prognosis, survival and the patient's quality of life. Future perspectives, in the context of taking advantage of these reviewed research results, should opt to further verify the potential of CSCs and their markers as well as SCs in general, regarding CRC treatment personalization.

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Conflict of interest statement

Authors declare no conflict of interest.

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