Abstract

Regardless of significant advances over the last years, cancer is still an aggressive and life-threatening disease with a negative impact on the patient’s quality of life, socioeconomic status and lifestyle. Finding a cure for cancer has become a major challenge for all researchers and medical professionals worldwide and enormous efforts have been made to find more effective prophylactic and therapeutic approaches. Nowadays, cancer treatment can be aggressive and it is not always well targeted. Therefore, more specific therapies should be focused on tumor cells and pathogenic mechanisms implicated in carcinogenesis. In this regard, our paper aims at discussing the interrelation between cancer stem cells (CSCs) and Advanced glycation end products (AGEs) with possible future research perspectives. CSCs are stem cells with tumorigenic properties that are capable to give rise to all the cell types found in a tumor. Development of innovative therapies targeted on CSCs could improve the survival rate and the quality of life for cancer patients. AGEs are a heterogeneous group of compounds formed by the non-enzymatic reaction between the reducing sugars and proteins, lipids or amino acids. Multiple sources of AGEs have been described, as well as AGEs involvement in numerous pathogenic pathways, carcinogenesis being one of them. In order to better understand the pathogenic mechanisms implicated in carcinogenesis and to develop efficient prophylactic and therapeutic approaches, the interrelation between CSCs and AGEs should be taken into consideration.

Keywords

Cancer stem cells; Advanced glycation end products; Dietary advanced glycation end products; Cancer treatment; Carcinogenesis; Targeted therapies

Introduction

Cancer is one of the most difficult to cure diseases and also one of the most frequent causes of death worldwide (considered the dominant cause of death in the United States citizens in 2016). Despite the progress in cancer death prevention over the last 20 years, the statistics still show extremely increased mortality rates due to cancer, which demand continuous efforts for researchers and medical professionals against oncogenesis and its consequences [1]. Although there are numerous methods for treating cancer including surgery, chemotherapy, and radiotherapy [2], which are sometimes combined, cancer prevention and treatment requires more specific methods, based on advanced investigations [3-6].

Literature Review

What are cancer stem cells?

Cancer Stem Cells (CSCs) can be defined as the stem cells of a tumor. Some authors don’t consider CSCs as a separate cell lineage; others describe CSCs as a different type of cells. There is evidence demonstrating the differences between CSCs and common stem cells (SCs). Some authors even state that stem cells are CSCs precursors.

There are some characteristics that distinguish CSCs from other types of cells. One significant property of CSCs is generation of cancer cells, with inexhaustible replication rate. Multiple literature sources agree that CSCs could play an
important role in the development of metastases distant from the initial tumor and could also contribute to the recurrence of a tumor after treatment [4,7,8].

Identification and characterization of CSCs vs SCs

The methods for CSCs isolation use the specific surface markers: magnetic cell sorting and flow cytometry [9]. The specific markers are different depending on the type of tumor e.g. CD105 and CD133 for renal cell carcinoma [9].

CD44 positivity and CD24 negativity cells pattern were used for identifying the breast CSCs, but there were some CSCs that could not be identified with this set of markers [10]. CD44 and aldehyde dehydrogenase (ALDH) were found to be positive for head and neck CSCs [11]. ALDH1 is a member of aldehyde dehydrogenase enzymes family useful for differentiating a normal SC from CSCs. Stem cells with high levels of CD44 expression and ALDH1 activity have greater tumorigenic capacity compared with stem cells with low levels [12].

The presence of CD44 marker in head and neck squamous cell carcinoma is related with high tumorigenicty, anti-apoptotic function and clonogenic ability, aspects that meet the CSCs criteria. Moreover, CSCs can exhibit features of mesenchymal or epithelial type, according to the expression of CD44 and epithelial specific antigen (ESA). The mesenchymal phenotype CD44\textsuperscript{high}/ESA\textsuperscript{low} of CSCs is associated with migratory function whereas the epithelial phenotype CD44\textsuperscript{high}/ESA\textsuperscript{high} have proliferative activities. Glycogen synthase kinase 3b (GSK3b) is responsible for regulating the self-renewal capacity [12].

Expression of MACC -1 gene is twofold higher in CSCs than in head and neck cancer cells and could be a biomarker for head and neck cancer [13].

Another technique for separating the cell types is MACS (Magnetic-activated Cell Sorting) [9], but the most widely used and the most adjustable method for stem cell identification is flow cytometry [14].

Signaling mechanisms in carcinogenesis involving CSCs

The origin of CSCs has not been yet established [15], even though numerous theories have been described and proposed in the literature. One of the theories sustains that CSCs originate from stem cells [16,17]. This hypothesis is considered accurate due to the common proprieties that the two cell lineages share, such as the ability of self-renewal and differentiation capacity. The difference between CSCs and stem cells is that CSCs have the capacity of developing secondary malignant tumors [16]. Another theory sustains the origin of CSCs from previously differentiated cells which regain their multipotent or pluripotent capacities [16,17]. A third theory suggests that CSCs could originate from the combination of stem cells with cells belonging to other cell lineages [16] (Figure 1).

Some theories imply that the stem cells microenvironment (niche) has a tremendous role in the cells behavior and could maintain a balance regarding the proliferative rate of the cells. The niche that hosts the stem cells produces molecules, such as hh, Wnts, bone morphogenetic proteins (BMP), fibroblast growth factors and Noch, that can influence the cells’ predetermined course [18]. Stem cells may undergo genetic mutations that could block the response to the signals from the microenvironment and, in consequence, the cells separate from the niche’s influence. When the stem cell becomes immune to these control mechanisms, it could undergo unlimited division and even carcinogenesis [18].

There are multiple signaling pathways described in the literature: Wnt, Notch, Hedgehog, TGF-β and tyrosine kinase receptors, all correlated with the guidance of CSCs behavior [16]. For the skin stem cells Wnt signaling has a restraining expansion effect, due to the Wnt inhibitors: Dtt, sFRP and Wif. Wnt can also have a proliferative effect on hematopoietic stem cells, but the mechanism is still unidentified [18]. TGF-β and BMP signaling can also have both proliferating and inhibiting effects on stem cells, but their presence in the microenvironment of the CSCs is uncertain. BMP4 is an exception, since it is found in the niche of the intestinal stem cells adhering to the general rule of the TGF-β signaling, which inhibits cell growth [18]. The effect of the BMP signaling deficit is uncontrollable cell proliferation and, consequently, carcinogenesis [18]. The niche also has the capacity to maintain the cells attached due to adhesion molecules: β-catenin and cadherin [18].

Expression of transcription factors in head and neck cancer

The transcription factors which define the CSCs include: Nanog, Oct 3/4, Sox2, Nestin, and CD34 [19]. aberrant expression of Nanog, Oct 3/4 and Sox2 isoforms and pseudogenes play important roles in tumorigenesis and tumor metastasis, but the mechanisms involved are not completely understood [20].

Etopic expression of Oct 3/4 increases chemoresistance through ABCG6 expression and tumor invasion through Slug expression. According to the higher or lower expression of Oct 3/4 in head and neck squamous carcinoma, the cells express more or less the stemness characteristics. Oct 3/4 enables the distinction between differentiated head and neck squamous carcinoma cells and CSC-like cells [21].

The Nanog transcription factor from CSCs is involved in promoting tumorigenesis, and is also associated with poorer outcome, chemoresistance and epithelial-mesenchymal transition (EMT) which is a crucial event in the metastatic process [22,23]. Other genes expressed in relation with the EMT are: Snail, Slug, Twist, vimentin and fibronectin [23]. ZEB1 and ZEB2 are members of the zinc-finger E-box-binding homeobox factor (ZEB) family. These are transcriptional repressors which induce EMT by suppressing the expression of E-cadherin and thus contribute to the cancer progression.
However, the role of ZEB1 and ZEB2 in mediating CSCs properties in head and neck cancers is still unclear [24].

RhoC is a member of Rho family of GTPases involved in: intracellular signaling, cytoskeletal organization, cell proliferation and regulation of gene expression. RhoC belongs to the Ras superfamily in which some members have been identified with oncogenic activities. RhoC expression was associated with metastasis and genetic mutations in RhoC genes were over-express in head and neck squamous cell carcinoma [25].

Although interleukin 6 (IL-6) contributes, in normal conditions, to host defense in the acute phase responses to injury and infections, several studies demonstrated that IL-6 contributed to the tumorigenic behavior of CSCs [26,27]. Cross talk between endothelial cells and CSC which reside in perivascular niches is crucial for CSCs survival and self-renewal. IL-6 interacts with the IL-6 receptors (IL-6R) and activates Janus kinase (JAK) and STAT3 transcription factor. In head and neck squamous cell carcinoma, IL-6R expression is higher in CSCs than in SCs and the serum levels of IL-6 are correlated with poor patients’ survival [28].

Keratins in head and neck cancer

Keratins are a type of intermediate filament proteins in the cytoskeleton of epithelial cells, which are encoded by 54 functional genes. They contribute to several internal cell processes, such as signaling [29], cell proliferation [30] and stress [31-33]. Keratins’ mutations can lead to keratinopathies; additionally, keratins can be diagnostic markers for proliferative or non-proliferative diseases [34]. Several studies showed that keratins can also be found in the head and neck area (HNA). Their presence has been validated in healthy tissues and also in the soft tissue tumors of the oral cavity. In the HNA, 19 types of keratin (K1 – K19) have been reported (bibliografie - proteomic profile of keratins) [35-39]. Regarding the diagnosis value of keratins, Fulzele et al. showed that K4, K13, K14, K16 and K18 can be used to identify whether a tumor is present or not [33]. Their study was able to establish that K4 and K13 were mainly present in the normal tissue whereas K14, K16 and K17 were detected in the tumor tissue. Futures studies are needed to clarify if CSCs keratins are involved in carcinogenesis.

Integrins in head and neck cancer

Integrins also play an important role in the innovative procedures of fighting against cancer. They are defined as cell surface receptors, involved in the extracellular matrix (ECM) adherence, both in other normal and pathological processes, and this characteristic makes them suitable to be taken into consideration as a potential therapeutic approach in cancer [40,41]. Integrins are involved in cells’ adherence to the ECM, fact that could be incriminated for the resistance to cancer therapy. Through their signals, integrins stimulate the cancer evolution [41]. It was established that through a particular signaling sequence, which includes FAK (Focal Adhesion Kinase) and JNK (c-Jun N-terminal Kinase), beta1-integrins have repercussions on the radioresistance and DNA-protein-kinase dependent non-homologous end joining [41-43]. Koppenhagen et al., in a study on head and neck cancer cells, demonstrated that c-Abl was a significant factor involved in radioresistance of cells derived from solid tumors [41]. Further studies on the communication between CSCs in tumors and with the surrounding tissues through cell surface receptors like integrins (CSCs – CSCs, CSCs – tumor cells, CSCs - normal surrounding cells and CSCs - normal surrounding cells), could lead to a better understanding of the cancer spreading mechanisms and local invasion.

Are advanced glycation end products involved in cancer development?

In tumor pathophysiology, numerous demographics are involved. Exogenous and endogenous factors contribute to DNA damage, DNA repair deficiency, somatic mutations and the subsequent progression towards tumor development. Besides the known causes, there is a new approach regarding AGEs (Advanced glycation end products) implication in the evolution and progression of tumors. AGEs are a heterogeneous group of compounds formed by the non-enzymatic reaction between reducing sugars and a free amino or ketone group, found in proteins or lipids. AGEs may have exogenous or endogenous sources and accumulate in time- and dose-dependent manners, leading to a chronic subclinical inflammatory response. Because of the variety in what concerns the receptors, mediators and ligands, AGEs modulate multiple pathogenic mechanisms, including carcinogenesis (Figure 1). Of the AGEs family, Van Heijst found CML and argpyrimidine in human cancer tissues [44]. AGEs accumulation in collagen fibers leads to a deleterious effect, depending on the type of soft or hard structure. Studies have shown a strong correlation between salivary AGEs and bone metastasis in patients with multiple myeloma [45,46]. Vikova’ and co. evaluated in 16 premalignant lesions (leukoplakia, erythroplakia and lichen planus) ROS salivary markers and found 80% higher AGEs levels compared with healthy patients. The authors supposed the results might be due to the interaction between the carbonyl and oxidative stress [47]. AGEs receptor family includes surface receptors: RAGE, proinflammatory mediators, such as S100/calgranulin family (S100A12 and S100B), amyloid, high-mobility group box 1 (HMGB1), Mac-1. Sanders et al. evaluated the influence of RAGE mediated inflammation of gingival carcinoma cells caused by tobacco exposure and found significant increase of RAGE, IL-6 and IL-8 after 6 hours exposure to tobacco [48]. Moreover, they tested the reverse effect of semi-synthetic glycosaminoglycans ethers – SAGEs, which reduced glycation compounds in tested samples. Chapman et al. used oral squamous carcinoma cells (OSCC) to evaluate the cellular response induced by RAGE, during cigar smoke. Their findings showed that smoke exposure increased RAGE expression and tumor invasion; removal of exposure factor with the inhibition of RAGE, increased SAGEs in OSCC cells, and reduced smoke-RAGE related invasion [49].
AGE-RAGE interaction leads to mutual molecular increase, reactive oxygen species (ROS) and a continuous inflammatory response [50]. The authors found that AGE-RAGE connection increased vascular inflammation, angiogenesis and thrombogenesis. They also showed that AGE2, AGE3 (glyceraldehyde- and glycolaldehyde derived AGE) were found in human G361 and A375 melanoma cells, and they promoted growth and migration of tumor cells. Bhawal and co. evaluated the role of RAGE in OSCC progression [51] by using primary and metastatic site cells cultures. Their results showed that RAGE mRNA was detected in all primary and metastatic tumors, while RAGE was overall expressed in metastatic tumors. The authors also used invasion assays, to find that after 12 hours, RAGE was present in a higher amount in type IV collagen cell invasion. Tateno et al. analyzed the expression of RAGE in 216 esophageal carcinoma specimens [52].

They found 50% RAGE-positive tumor cells, in cell membrane and cytoplasm. The RAGE levels were negatively correlated with the depth and venous invasion. Landesberg and coworkers evaluated RAGE expression in 38 oral squamous cell carcinoma samples. They found positive correlation regarding the histological differentiation, the RAGE expression being more reduced with the degree of differentiation [53]. Sasahira’s research investigated RAGE expression in 74 OSCC specimens, and their correlation with clinical and pathological parameters. They found a highly positive connection between RAGE and the depth of tumor invasion, but inversely with the degree of histological differentiation [54]. The same authors analyzed RAGE implication in tumor OSCC angiogenesis [55]. They correlated RAGE with microvessel density (MVD) and vascular endothelial growth factor (VEGF) with the tumor progression. When HMGB1 was added to RAGE, VEGF secretion was elevated as well, the results suggesting RAGE role in tumoral angiogenesis.

Antioxidant effect is modulated by nuclear factor-erythroid 2-related factor 2 (Nrf-2), also involved in p53 apoptotic mediated response. An in vitro study [56] showed that cell exposed to AGES increased ERK (extracellular-signal-regulated kinase) phosphorylation, which lead to Nrf-2 downregulation, suppression of p53 protection gene, which could explain the degree of invasiveness of tumor cells. Another in vitro study [57] analyzed the effect of HMGB1 in progression and invasion of human nasopharyngeal carcinoma cell line (HONE-1). They also used assays to evaluate the cells metastatic capacity. The research team demonstrated that suppression of HMGB1 downregulated RAGE expression and ERK dependent process, which inhibited all malignant HONE-1 cells properties of proliferation, migration and invasion.

A systematic review investigated the HMGB1-RAGE inflammatory interaction in what concerns head and neck melanoma pathology [58]. Intratumoral RAGE and HMGB1 were found to be overexpressed compared to control ones, whereas HMGB1 levels were positively correlated with the aggressiveness of melanoma.

Because of the present methods used in cooking food, the Western diet which has been spread worldwide, habits in bringing microwaves rather than classic heating, dietary AGES (DAGEs) could be as well, as an exogenous source, an etiological factor in carcinogenesis. Depending on the intake, DAGEs exert their continuous negative effect. Studies have been made on oral cavity biofluids and biological ones, to determine alterations associated with different pathologies. Saliva, as an easy to harvest fluid, could be used to provide information regarding AGES, DAGEs and their correlation with the state of tumor progression. A salivary biosensor could be used for screening and as diagnosis device or for real-time AGEs monitoring [59]. The tool might be useful to evaluate salivary AGES in correlation with the cancer relapse after surgical excision, chemotherapy or both, as a non-invasive and easy to use device.

The above studies contribute with new evidence which suggest an AGES contribution in etiology and progression of malignant lesions which should be taken into account. Future treatment strategies could target AGE-RAGE axis for a superior efficiency and disease prognosis. Moreover, studies on RAGE expression in CSCs subpopulation of head and neck cancer tissues could represent a future approach in targeting CSCs for cancer treatment.

**New Challenges in Targeting Cscs and Ages in Cancer Treatment**

While in 2013, there was only an aspiration for identifying stem cells by using their specific markers and there was not enough data proving that CSCs existed in some forms of cancer, the researchers had a hope that in perspective, the metastasis and recurrence of cancer could be prevented only by targeting the CSC populations [18]. Researches acknowledged the importance using CSCs as an objective for trying to treat many types of cancer, among them being the head and neck researchers, who suggested not only targeting

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**Figure 1** Signaling mechanisms in carcinogenesis.
CSCs, but also their microenvironment, stimulating apoptosis by sending extrinsic signals to the cells [16].

Discussion

Futures research is needed in order to assess the involvement of CSCs keratins in carcinogenesis and to clarify if cellular stress induced by keratins is added (even have potentiation or augmentation effect) to AGEs (Advanced glycation end products) cellular damages. Another question would be if by controlling dietary AGEs intake, we could prevent cancer development. Blocking CSCs surface receptors like integrins, probably will enable the prevention of cancer relapses.

Additional questions arise and require further studies: Could AGEs receptor family such as surface receptors – RAGE interact with integrins in cancer cells and/or CSCs? Could RAGE blocking be useful in targeting the treatments toward CSCs? Could blockage of AGEs accumulation in collagen fibers prevent local tumor invasion? Could salivary AGEs assessment be a useful tool in the follow up of cancer patients with relapse and metastasis or in the assessment of the risk in cancer development? Could also be useful the salivary IL-6 and IL-8 evaluation? Could dietary AGEs be involved in carcinogenesis?

These are some of the questions that still need to be addressed in the future research.

Conclusion

In head and neck disorders, various pathogenic mechanisms are involved. Besides stem cells abnormalities, exogenous and endogenous factors are implicated in either the initiation or in the aggravation of tumor process. A therapeutic approach focused on targeting CSCs in head and neck cancers multiple pathogenic mechanisms simultaneously or sequentially, such as receptors (integrins and RAGE) or/and intracellular structures (keratins) and transcriptional factors will probably enable a better management of these diseases.

AGEs, mainly dietary ones, by their time and dose-dependent continuous accumulation, could be an important element in the exacerbation and spreading of cancerous lesions. RAGE-AGEs interaction generates chronic inflammation, promotes ROS formation and activates other deleterious cells and pathways leading uncontrolled cell division. Since the AGEs amount is directly related to the aggressiveness and state of tumor lesions, a salivary biosensor could be an innovative method for the prognosis and monitoring of cancerous cells pathology. Besides classical surgical excision, chemotherapy and radiotherapy, it would represent a non-invasive therapy for completing the protocol in head and neck cancer pathology.

While trying to develop new ways of curing cancer, the pathogenic mechanisms need to be considered. Thus, the treatment will be more targeted and, hopefully, the patients’ survival could be improved.

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Conflict of Interests

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