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The Role of Cancer Stem Cells in Uveal Melanoma

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Summary: Uveal melanoma is the most common type of eye cancer, with primary tumors typically originating in the choroid, though they can also develop in the iris or ciliary body. The condition is associated with high mortality due to metastases, particularly to the liver, which are often resistant to treatment. An important factor in this resistance is the presence of cancer stem cells or progenitor cells. These cells have the ability to self-renew and differentiate into diverse cell types, contributing to the development of resistant and heterogeneous cancer cell populations. There have been numerous efforts to identify cancer stem cell markers across various types of cancer, including uveal melanoma. This paper aims to review stem cell markers, such as CD133 and CD166, common to multiple cancers, alongside markers more specific to melanoma and uveal melanoma, including SOX2 and nestin. Despite extensive research, a definitive characterization of cancer stem cells in uveal melanoma has yet to be achieved.

Key words: uveal melanoma, stemness markers, stem cells, cancer stem cells.

Introduction

As understanding of the causes and mechanisms of cancer development and metastasis advances, increasing attention is being directed toward cancer stem cells (CSCs) [1]. In various types of cancer, research has demonstrated that tumors contain a rare population of cells capable of self-renewal and differentiation, contributing to increased tumor heterogeneity [2]. Moreover, the multidirectional differentiation of cancer stem cells can result in the formation of structures closely resembling normal tissues, such as blood vessels or lymphatic vessels, further complicating the development of effective cancer therapies [3]. Current evidence suggests that cancer stem cells can originate through two distinct pathways. One hypothesis asserts that the differentiation of progenitor cells is halted at an early stage, followed by mutations that trigger uncontrolled differentiation, ultimately leading to a pool of cells with varying levels of maturity. The alternative CSC formation pathway suggests that cancer cells undergo dedifferentiation, acquiring stem cell-like characteristics and enhanced plasticity as a result [4, 5].

Across various cancer types, numerous markers have been identified to characterize stem cells. In some cases, these markers are well-defined. For example, in breast cancer, they include CD44, CD24, and ALDH [6]. In gliomas, markers such as CD133, CD44, SOX2, and nestin have been identified [7]. However, identifying stem cell markers remains challenging in some cancers, with uveal melanoma serving as a good example.

CSC in melanoma

Melanoma is a malignant tumor originating from melanocytes, affecting the skin, mucous membranes, meninges, and uveal layer. According to the GLOBOCAN 2020 report, approximately 325,000 new cases of skin melanoma were recorded, accounting for about 1.7% of all cancer diagnoses [8]. The development of melanoma is influenced by a combination of genetic and epigenetic factors. Genetic contributors include mutations in the *BRAF* gene, a proto-oncogene encoding a serine-threonine kinase critical for cell growth and proliferation, the *PTEN* gene, which is involved in cell cycle regulation, and the *NF1* gene, mutations of which are detected in approximately 15% of cases. Epigenetic factors encompass prolonged exposure to UV radiation, particularly UVB, the presence of naevi, immune system impairment due to UVA and UVB exposure, obesity, and hereditary mutations. The stage at which melanoma is diagnosed plays a crucial role in treatment outcomes. For patients diagnosed at stage 1, the chances of cure and survival exceed 90%, while at stage 3, they range from 27% to 70%. For patients with metastases, survival rates drop below 20% [8–10]. Treatment typically involves surgical methods combined with systemic chemotherapy or immunotherapy.

With growing evidence of CSCs in various cancers, research efforts have expanded to encompass the detection of these cells in melanoma as well. One such marker is CD133, commonly used to identify stem cells, for example in gliomas. Its presence in melanoma is associated with a poorer prognosis [11, 12]. Another noteworthy CSC marker is aldehyde dehydrogenase (ALDH). Studies conducted using a murine model show that ALDH+ cells have a greater tumor-forming potential and exhibit increased resistance to chemotherapeutic agents, likely due to their primary role in detoxification [13]. Additionally, research by Mahamud et al. revealed that the protein EZH2 is present at significantly higher levels in the stabilized melanoma cell line WM266-4, derived from a metastasis, compared to cells from the primary tumor [14].

Two other important stemness markers are nestin and SOX2. Nestin is found in the cytoplasm of all neuroepithelial cells, and it plays a role in cell migration during embryonic development and proliferation, particularly in the development of the central nervous system (CNS). Its presence in melanoma tumors is associated with a poorer prognosis. Additionally, nestin-positive cell populations were detected in the peripheral blood samples of patients with advanced melanoma, which also correlated with a poorer prognosis [15, 16]. In turn, SOX2 is a transcription factor responsible for the development of embryonic stem cells. It also

plays a role in maintaining pluripotency and self-renewal of cells, including those derived from the neural crest, such as melanocytes. In melanomas where SOX2-positive cell populations were detected, silencing this gene significantly reduced self-renewal capabilities and induced apoptosis, a finding later confirmed in a murine model [17]. Both nestin and SOX2 can be regarded as stemness markers in the search for CSC populations.

CSC in uveal melanoma

Uveal melanoma (UM) accounts for approximately 5% of all melanomas and around 85% of all eye cancers. Uveal melanoma can arise in any of the uveal structures, including the iris, ciliary body, and choroid. However, UV radiation exposure is only considered a contributing factor in the development of iris melanoma. Several genetic factors are involved in UM development, i.e. chromosome 3 loss, changes in chromosome 8 and 6, as well as mutations in the *BAP1* and the *GNAQ/GNA11*.

In isolated tumor cell lines, three distinct cell types can be identified. In the spindle-shaped type, UM cells are characterized by a slow growth rate. In contrast, the epithelioid type is distinguished by a faster growth rate and more aggressive nature [18].

The treatment of primary intraocular tumors is highly effective (with a success rate of over 90%). However, distant metastases occur in approximately 50% of patients, spreading primarily through blood vessels to the liver (over 90%), and also to the lungs, bones, skin, brain, and other organs. These metastases may become apparent months or even years after treatment, possibly due to the ability of tumor cells to enter a dormant state. The exact reasons why the liver is the primary site of metastasis for uveal melanoma are not completely understood. Despite the introduction of new treatments for metastatic uveal melanoma, they have thus far proven largely ineffective, providing only a modest extension of patients' life. The success of anticancer therapies in UM is known to depend on factors including tumor size and location, histopathological cell type, and the tumor's genetic profile. Recently, the potential role of melanoma CSCs has also been suggested. There is an ongoing debate in the literature about whether the presence of cells with putative stemness markers genuinely impacts cell development and resistance to therapies. In fact, it remains controversial whether these cells can be referred to as cancer stem cells at all. Below are examples of markers that have been successfully identified in UM cells.

One of the most recognizable stem cell markers is the CD133 glycoprotein, which in mature stem cells plays a role in inhibiting cell differentiation. Expression of this protein is observed during embryonic development, including in the formation of neural tissue, the eye, and the liver [19]. A study by Thill et al. demonstrated that this marker is present in UM cell lines derived both from the primary tumor and from metastases. It has been proposed that the presence of the CD133+ CSC population could be associated with UM metastases to the liver, with the observation that the CD133+ population diminishes as metastasis progresses [20]. The origin of melanocytes from the neural crest supports the presence of CD133 and the high plasticity observed in melanomas, particularly uveal melanoma.

Another protein commonly considered a CSC marker is CD166 (ALCAM). Like CD133, CD166 plays a role in the embryonic development of various tissues, including nervous tissue. A 2019 study by Djirackor et al. revealed that the level of CD166 protein in UM cell lines is four times higher than in normal choroidal melanocytes. In patient samples, low BAP1 expression and monosomy of chromosome 3, which is associated with a high risk of metastasis have been correlated with elevated CD166 expression [21]. As this receptor is found in tissues originating from the neural crest and is characteristic exclusively of 3D cultures, it may suggest the plasticity of UM cells rather than the existence of a distinct CSC subpopulation.

The EZH2 protein plays a role in maintaining genes that regulate development and differentiation during embryonic development. Postnatally, it is involved in the processes of proliferation, apoptosis, and cell senescence (a state of permanent cell cycle arrest that occurs in proliferating cells affected by stress). Its overexpression has been observed in breast, stomach, and prostate cancers [14]. In UM cells, the expression of the EZH2 protein has been associated with increased proliferation. Furthermore, significantly higher levels of EZH2 expression have been detected in cell lines derived from both primary tumors and metastases. Jin *et al*. demonstrated a correlation between EZH2 concentration and the growth of UM cells and tumors in mice. In their studies, cell lines and induced tumors treated with the GSK126 inhibitor showed a marked decrease in UM cell viability and a reduction in tumor size [22].

Aldehyde dehydrogenase (ALDH) is an enzyme that plays a key role in cellular detoxification – by metabolizing byproducts such as aldehydes and retinoic acids – and in the protection against reactive oxygen species. Cancer tumors with a cell population rich in ALDH are characterized by enhanced growth and higher resistance to therapies [23]. In UM, there is no sufficient evidence to definitively identify ALDH as one of the markers of CSCs.

Some of the most commonly used markers of stem cells are SOX2 and nestin. A 2011 study by Thill et al. demonstrated SOX2 presence in several UM cell lines derived from both primary and metastatic tumors [20]. The authors noted that this protein was detected in the cytoplasm of UM cells. Histological examination results reveal the presence of SOX2 only on the periphery of the tumor. Whether SOX2 qualifies as a CSC marker remains inconclusive. While its low frequency in UM might support this designation, insufficient data exist to clarify the role of SOX2 in the development of UM tumors and their metastasis. Djirackor et al. reported elevated SOX2 levels in tissues derived from the primary tumor and linked this increase to poorer patient survival rates. Elevated nestin levels also correlate with changes in the primary tumor towards an epithelial phenotype, an increased mitotic index, and the changes in the chromosome 8q. Interestingly, high nestin levels are observed not only in tumors and tumor-derived cells but also in circulating cancer cells. Blood samples from patients with both skin melanoma and UM have shown the presence of cells with elevated nestin concentrations [21, 24].

Is it possible to define stem cells in UM?

Stem cells are characterized by their capacity to differentiate into various cell types. They range from embryonic stem cells, which are pluripotent and capable of differentiating into multiple cell types, through unipotent stem cells, which have a more limited differentiation potential, to induced pluripotent stem cells (iPSCs). Over the years, growing attention has been directed towards cancer stem cells, which, like normal stem cells, have the capacity for self-renewal and differentiation. They are thought to contribute to accelerated tumor growth and metastasis, as well as resistance to cancer therapies and post-treatment relapse. Research on CSCs involves a variety of tests aimed to characterize these cells, including evaluation of their ability to initiate and propagate tumors *in vivo* in NOD/SCID mice. *In vitro* studies show that CSCs form unique colonies and, more significantly, have the ability to develop into three-dimensional spheroids. Spheroid formation, along with differentiation capacity, is regarded as the gold standard for stem cell identification [25, 26]. *In vitro* tests also enable the determination of their growth kinetics, which varies depending on the cell type, as well as the comparison of proteins and other substances they secrete.

For the detection of CSCs based on markers, a range of tests can be utilized. For example, Real-Time PCR allows for the identification of specific markers at the mRNA level, while Western Blot provides information about the proteins present in cells. Flow cytometry, on the other hand, enables the differentiation of cell pools based on surface markers, as well as the sorting of these cells for further analysis. Cancer cells with stem cell-like properties exhibit increased resistance to therapeutic agents. For this reason, culturing cells with a specific compound at appropriate concentrations to select a resistant population is also used as a test for the presence of CSCs [27, 28].

Consequently, a single marker alone is insufficient to definitively confirm the presence of CSCs in cancer. Studies to date suggest that multiple stemness markers are typically associated with specific cancer types. This implies that such proteins should be considered when searching for CSCs. The same applies to uveal melanoma. The proteins EZH2 and ALDH show promise due to their significant impact on tumor development and aggressiveness, as described above. Furthermore, their presence appears to be interconnected. Another potential marker on the surface of UM stem cells is the ALCAM protein (CD133) and its variants, with its presence being correlated with increased aggressiveness of UM. The identification of stemness markers for melanomas is further complicated by their origin. Uveal melanoma originates from melanocytes, which, like tumors of the nervous system, are derived from the neural crest. Consequently, they share common markers, such as SOX2 and nestin. While these markers may suggest the presence of the CSC population in UM, they can also indicate high plasticity of these cells. This trait is characteristic of all cells originating from the neural crest, including UM cells, thus warranting further investigation. Potential candidates for stemness markers in UM are shown in Fig. 1.

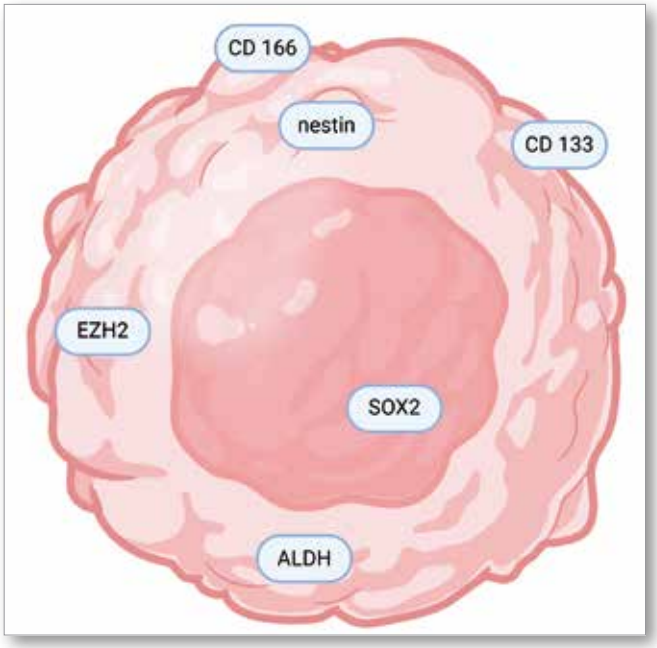

Fig. 1. Stemness markers in UM that could potentially be used to identify CSCs in UM.

Unfortunately, there are still too few studies aimed at identifying cancer stem cells in UM to definitively determine which of the proposed markers may indicate the presence of CSC populations. One of the challenges is the limited access to UM material. Samples obtained from primary tumors in patients are highly diverse, frequently necrotic, and contain heterogeneous cells. Furthermore, primary tumor cells exhibit instability in *in vitro* cultures, undergoing rapid phenotypic changes that make accurate characterization challenging. Additionally, the scarcity of material from UM metastases presents a significant difficulty, further limiting therapeutic options. In fine needle biopsy, the amount of collected material may be insufficient for obtaining cell lines. However, with the increasing amount of data, available materials, and advancements in therapeutic options, the state of knowledge regarding stem cells in UM is expected to increase. New research will enhance understanding of disease progression and its underlying mechanisms, potentially paving the way for novel diagnostic methods for metastasis and, in time, innovative therapeutic strategies.

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