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Review

Solid Organ Cancer Stem Cells

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Abstract

Recently, several reports presented compelling evidence supporting the existence of Cancer Stem Cells (CSCs). The CSC Theory has challenged the traditional views of carcinogenesis. Although, the cancer stem cells' theory is rooted in the 19th century, it is the first true conceptual frame shift in the study of cancer over the last 150 years. As such, it merits serious consideration. Investigators have suggested that CSCs are the key drivers for tumor growth, recurrence and metastasis. Moreover, based on some data, it has been proposed that CSCs are capable of self-renewal via symmetric or asymmetric cell division, differentiation, tumor initiation and importantly, metastasis and therapeutic resistance.

Elucidating the role of CSC and their mechanistic modus operandi is a prime goal for the next decade. Standard anti-tumor approaches such as cytotoxic chemotherapy or radiotherapy targeting differentiated cancer cells likely need to be coupled with targeted-CSC therapy in order to rid a host of tumor burden. Furthermore, the identification and targeted treatment of CSCs may also improve screening, early detection and treatment, and prognostication of solid organ malignancy. Non-malignant stem cells are relatively immunologically inert. It is important to understand the interactions of CSC with the immune system. This paper summarizes the immunological aspects of CSC.

Introduction – Cancer Stem Cell Concept

Self-renewal, a specific type of cell proliferation, is a fundamental characteristic of normal stem

cells, which permits the creation of at least one progeny with similar developmental potential [1, 2]. Understanding of the de-regulation of self-renewal in normal stem cells has led to a parallel association with the traditional model of tumorigenesis. For many years, the traditional model of tumorigenesis accepted by most researchers has been that any cancer cell is capable of tumor initiation and propagation [3, 4]. However, in recent years, evidence has emerged regarding a subset of self-sustaining cells with the ability of self-renewal and tumor maintenance [5]. Contrary to the concept that activation of self-renewal mechanisms creates cancers, the cancer stem cell (CSC) model supports a hierarchical framework in which only a small population of cells (CSCs) are capable of tumor propagation [3, 6].

In addition to self-renewal, there are several other defining characteristics of the potential of CSCs: (1) the capacity for differentiation, (2) tumor initiation, and (3) asymmetric cell-division via non-random chromosomal co-segregation (ACD-NRCC) [7, 8]. Nearly forty years ago, Carins introduced the concept of ACD-NRCC in which each chromosome in a stem-cell contains one DNA strand that is conserved throughout multiple asymmetric divisions [9]. This unique property of single-strand DNA conservation allows CSCs to avoid the accumulation of mutations from DNA replication errors such that replication-errors are transferred to daughter-cells destined to differentiate and ultimately be eliminated. For years, techniques capable of identifying CSCs that had undergone ACD-NRCC did not exist. As a result, using the fundamental properties of CSCs, investigators identified various membrane and cytoplasmic markers associated with putative CSCs in researching solid organ cancers such as breast, colon, liver, pancreas, and bladder cancer and melanoma [10-16]. However, the clinical prognostic value of these biomarkers remains unclear. Therefore, precise identification and adequate characterization of putative CSCs is imperative to the development of therapeutic targets.

Cancer and the Immune System

The interaction of the immune system and tumor cells evolves throughout all life period of cancer formation and progression. In 2002, Dunn et al. elegantly described the three phases of immunoediting or immunomodulation: (1) *elimination*, (2) *equilibrium*, and (3) *escape* [17]. Neoplastic changes are initially recognized by the innate immune system in which natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) produce interferon-γ (INF-γ), an essential mediator of immunosurveillance against tumors, to promote cytotoxic activity of macrophages [18]. The coordinated function of these effector cells leads to the destruction of the initial tumor cells, which constitutes the *elimination* phase of cancer immunomodulation [17, 19, 20]. As innate immune cells destroy cancer cells, tumor-associated antigens are released and recognized by dendritic cells (DCs). DCs possess the ability to process these antigens and create peptides bundled as major histocompatibility complex (MHC) class I and II molecules so that CD8 and CD4 T cells, respectively, can recognize them. Destruction of tumor cells (*elimination*) in conjunction with the release of pro-inflammatory cytokines by innate immune cells triggers tumor-specific T cells to execute anti-tumor activity [20]. However, the elimination phase of neoplastic cells may be incomplete leading to the second, *equilibrium* phase.

The *equilibrium* phase constitutes the longest phase in immunoediting, which the host immune system, and any tumor cell variant that has survived, exist in a dynamic *equilibrium*. T

cells and cytokines such as IFN-γ are able to contain, yet not destroy, all cells within a tumor bed. As a result, genetically unstable tumor cells remain and undergo rapid division of genetically mutated cells. This constant selective control of the immune system can maintain tumors at a subclinical stage (*equilibrium*) for an extended period of time, which creates tumor cells that are highly resistant to a host's immune system. It has been demonstrated that immune rejection is an essential component for tumors to become clinically detectable, which has been defined as the third phase of immunoediting, *escape* from immune control [18, 21]. In the *escape* process, while tumor cells persist with acquired resistance to immunologic detection and elimination, the immune system remains engaged. Genetically mutated cancer cells create neoantigens containing epitopes recognized by T cells, which may contribute to decelerated tumor progression [22, 23]. Therapeutic strategies, such as radiotherapy, have been developed to stimulate the release of tumor neoantigens in order to recover effective, innate immune system destruction of tumor cells. These therapies have proven effective against rapidly dividing tumor cells, yet CSCs remain resistant to such therapy as well as innate, immunoediting mechanisms.

Cancer Stem Cells: Escape from the Immune System

It has been postulated that CSCs play a key role in a tumor's ability to evade the immune system. To date, there is no evidence to suggest that CSCs are recognized by the immune system. Various tumor immunology models are centered on whole tumor cell antigens based on the "bulk" tumor, which permit cells to escape immune system recognition. CSCs are thought to capitalize on this mechanism to escape detection [24, 25]. More evidence is mounting in which CSCs may be responsible for solid organ tumor resistance to therapeutic modalities. For instance, following treatment with Trastuzumab, an antibody targeting cells that express the Her-2-neu antigen, breast cancer cells develop immune-resistant cells. Within this subpopulation there is a highly tumorigenic CSC fraction with a new reduction in HER2 expression. These CSCs not only evade immunoselection, but possess the ability to create new population of cells that represent those present before immunotherapy that are strikingly more tumorigenic than the initial tumor [26].

Cancer Stem Cell: Epithelial-Mesenchymal Transition

The epithelial-mesenchymal transition (EMT) is a phenomenon consisting of a series of events, beginning with alterations in cell-cell junctions and the cytoskeleton, which was first defined as a component of embryogenesis. However, more compelling data has been shown in the translation of early stage tumors into invasive cancer [27]. Acquiring the EMT phenotype has been linked to tumor progression in which cells have the ability to infiltrate surrounding tissues and transform these cells in order to metastasize to distant site [28]. The correlation between cell proliferative properties for both CSCs and EMT-cells is not obvious. Typical EMT-cells do not proliferate and CSCs proliferate slowly. This could explain CSCs' ability to escape chemoradiotherapy while maintaining an EMT-state. However, CSCs also have the potential to create the most aggressive, highly proliferating tumor cells, suggesting that the overlap of CSC- and EMT-properties may not be simultaneous [29]. It is now believed that the emergence of CSCs is linked to EMT, and that this relationship is the key to targeted therapy, particularly in the case of drug resistance [30, 31].

Cancer Stem Cells: Vaccines

Increasing attention has been paid to the CSC model due to the potential implications for targeted therapy. Traditional tumor vaccines have targeted antigens selectively expressed on differentiated tumor cells. These modern therapies target the progeny of CSCs that make up most of the tumor burden, yet they fail to target the actual CSCs. This likely explains the development of drug resistance and eventual failure to eradicate a tumor. As a result, there has been a paradigm shift to evaluating immunogenicity induced by purified CSCs by using CSCs as the source of antigen in the priming of DCs.

Studies have found that CSC-based vaccines confer protective anti-tumor effects by directly targeting CSCs through the induction of complement-dependent cytotoxicity (CDC) and CTLs [32]. Xu et al. showed that vaccination with DCs loaded with glioblastoma multiforme-derived CSCs, activated CTLs against CSCs, could prolong survival in animal models as well as human brain tumor patients [33]. A major issue with CSC-based vaccines alone is that CSCs compromise only a small sub-population of the tumor bulk. However, targeting CSCs in conjunction with traditional treatment modalities may improve survival and decrease cancer recurrence by enhancing the effectiveness of first-line cancer therapy [34]. T-cell vaccines targeting CSCs could represent a potential CSC-targeted therapy in which a specific pool of cells from which a tumor replenishes are depleted.

Cancer Stem Cells: T-Cells

Cytolytic functions of tumor-specific CTLs require antigen recognition in the context of MHC class I on antigen-presenting cells (APC) or target cells. Identification of a universal CSC marker remains the rate-limiting step to understand the susceptibility of CSCs-derived from solid-organ tumors to CTLs. Until a better, or universal, CSC-tumor antigen is established, various techniques will continue to be explored to trigger immune responses without CSC-tumor antigens [35-37].

Efforts have been made in utilizing DC-CSC fusion vaccines to induce polyclonal CTLs. In ovarian cancer (OVCA), a subset of OVCA-initiating cells (OCICs) with a CD44+ phenotype, were isolated from patients with OVCA based on the capacity of self-renewal and spheroid formation in serum-free culture, as well as their ability to grow from a low-cell inoculum in immunocompromised mice models. Weng et al. demonstrated that the induction of OCIC-targeted CTLs led to preferential destruction of CD44+ OCICs [36]. Todaro et al. used Zoledronate, a bisphosphonate, to sensitize malignant colon CSC-targets to CTL cytotoxicity [37]. However, a major drawback of this approach is the possible cross-reactivity these T cells may have with normal cells, thereby triggering autoimmune disease.

Cancer Stem Cells: CXCL12-CXCR4 and Metastasis

CXCL12 (also known as stromal cell derived factor-1 or SDF-1) is a chemokine that binds to its receptor C-X-C chemokine receptor type 4 (CXCR4). The CXCL12-CXCR4 signaling pathway has been shown to be critical in the retention and homing of hematopoietic stem cells in the bone marrow environment and in lymphocyte-trafficking. The CXCR4 receptor has been found to be a prognostic CSC marker for various solid organ malignancies such as breast, lung, prostate

and pancreas carcinoma. These cancers commonly metastasize to lung, bone, liver and lymph nodes, which are areas of high CXCL12 secretion.

Secretion of CXCL12 at these sites of metastasis serves as a chemoattractant for CXCR4+ primary tumor cells, and has been shown to be a key factor in the development of metastatic disease [38]. Hypoxic environments have been shown to up regulate CXCR4 expression; thus promoting tumor survival and metastatic invasion. Currently, several DNA damage-based chemotherapy regimens induce these hypoxic environments, which may have a paradoxical effect on the ability of CSC to metastasize, while the chemotherapy simultaneously destroys differentiated tumor cells. Therefore, a better understanding of the metastatic potential of CSCs is needed in order to develop effective therapies.

Cancer Stem Cells: Melanoma Immunotherapy

While early stage of the melanoma is associated with a favorable prognosis following surgical resection, metastatic melanoma still carries a bleak prognosis. Unlike other visceral solid organ tumors such as colon and breast cancer, metastases from melanoma are less predictable. Brain, lung, small bowel and liver are common sites of distant disease in melanoma. This complex metastatic profile and poor prognosis have driven years of rigorous research in an effort to establish more efficacious treatment.

Over the years, response rates to traditional, systemic chemotherapy for melanoma have been variable with limited impact on survival, yet associated with considerable toxicity. While immunotherapies such as IFN-α and Interleukin-2 (IL-2) share the limitations and efficacy of drugs such as Darcarbazine, two new novel systemic therapies received FDA-approval for metastatic melanoma in 2011: Vemurafenib (a BRAF inhibitor) and Ipilimumab (an anti-CTLA4-blocking monoclonal antibody). Unfortunately, only 50% of melanomas carry the BRAF mutation. For those with the mutation, Phase III trials have demonstrated up to 50% treatment response rate, but the durability of this response remains marginal [39]. Unlike Vemurafenib, Ipilimumab, a potent stimulator of CTL cytotoxicity, has demonstrated more durable response rates, but has been associated with significant autoimmune toxicities [40].

Many studies to-date have provided definitive proof-of-principle that the immune system can be manipulated to produce an efficacious treatment response. Regardless of the promising early results, the relatively low, durable response rates have forced researchers to further elucidate what prognostic factors and biomarkers are indeed essential to optimally harnessing the immune system to fight cancer with minimal side-effects. Studies have demonstrated the presence of melanoma stem-like cells that demonstrate self-renewal, have tumorigenic capability and the ability to form metastases suggestive of melanoma CSCs [41, 42]. Using various selections methods such as CD133 expression, radioresistance or the ability to form melanospheres, Pietra et al. used IL-2 activated NK cells to kill malignant melanoma enriched with putative CSCs [43]. This study, like many others, suggests that strategies for designing effective immunotherapy for melanoma must incorporate an additional component that can target melanoma CSCs.

Cancer Stem Cells: Breast Cancer Immunotherapy

As observed for melanoma, breast cancer recurrence and metastases are thought to be a result of the limitations of current systemic and radiotherapy treatments to eliminate breast CSCs, a sub-population with potent tumor-initiating-capacity and resistance to apoptosis. Currently, the breast cancer literature contains conflicting results regarding the characterization and identification of putative breast CSCs both *in vitro* and *in vivo* [13].

Overexpression of the proto-oncogene, Her-2/neu, occurs in nearly 25% of all newly diagnosed breast cancers. Trastuzumab, a humanized monoclonal antibody, targets the extracellular domain of HER2 protein and has been shown to improve both disease-free and overall survival in patients with all stages of Her-2/neu+ breast cancers [44]. However, initially responsive tumors typically become resistant to Trastuzumab, and the etiology of this resistance remains controversial. One possible explanation for this could be increased resistance to breast CSCs rather than resistance to the antibody-dependent cell-medicated cytotoxicity (ADCC) evoked by Trastuzumab [26]. Once again, this recapitulates the concept that current therapies must target normal cancer cells as well as CSCs for breast cancer.

Cancer Stem Cells: Colon Cancer Immunotherapy

Preliminary studies have shown that colon CSCs are susceptible to NK cell-mediated lysis. More importantly, putative colon CSCs express high levels of NK cell ligands (NKp30, NKp44 and NKp46), whereas differentiated colon cancer cells have near undetectable levels of these ligands [45]. Undetectable levels of MHC class I molecules exist among colon CSCs, and high levels are found on differentiated colon cancer cell lines. While current chemotherapy agents can sensitize tumor cells to immune cell-mediated destruction, current immunotherapy modalities are designed to stimulate adaptive immune responses [46]. Therefore, additional studies are needed to further capitalize on the effective NK cell-mediated lysis of colon CSCs in order to augment current immunotherapy strategies for colon cancer.

Cancer Stem Cells: Potential Therapeutic Applications

The ongoing controversy with the development of CSC-targeted therapy revolves around the lack of universally accepted criteria and methodologies to isolate CSCs, given the inability to precisely identify and adequately characterize putative CSCs. Various methods have yielded differing results among solid organ cancer cells: (1) side population analysis utilizing a stem cells' ability to efflux Hoechst dye due to transport ATP-binding G2 expression; (2) isolation of sub-population of cells that express various biomarkers associated with stem-like properties such as CD133+ or ALDH+ cells; and, (3) spherosphere formation in stem-cell enriched media. We recently demonstrated that surface markers or SP alone are insufficient to define putative CSCs due to their heterogeneity [12]. Additionally, recently developed techniques, such as the isolation of label-retaining cancer cells (LRCCs), have permitted the isolation of cells that have undergone ACD-NRCC [7, 8]. Based on the fundamental stem cell principle, these techniques provide a unique ability to identify and isolate putative CSCs, or LRCCs. Therefore, further investigations to characterize LRCCs from solid organ tumors could explain resistance to current therapies and cultivate new CSC-targeted therapies.

Conclusions

In recent years, compelling evidence has emerged supporting the CSC theory for solid organ tumors. The CSC theory has challenged the traditional views of tumor growth and consequently, stimulated innovative therapeutic strategies. By definition, CSCs are capable of self-renewal (via symmetric or ACD-NRCC), differentiation (allowing for reconstitution of all cell types of the original tumor), and tumor initiation (propagating tumors when transplanted to a separate environment). Many studies have suggested that CSCs are the main reason for tumor growth, recurrence and metastasis [47].

Understanding the mechanisms by which solid organ tumors develop or acquire resistance to currently established treatment modalities is essential to curing aggressive and metastatic cancers. Standard anti-tumor approaches such as cytotoxic chemotherapy or radiotherapy targeting differentiated cancer cells likely need to be coupled with targeted-CSC therapy in order to rid a host of all tumor burden. Furthermore, the identification and targeted treatment of CSCs may also improve screening, early detection and treatment, and prognostication of solid organ malignancies [48].

List of abbreviations

CSC: cancer stem cell

ACD-NRCC: asymmetric cell-division via non-chromosomal co-segregation

INF-γ: interferon gamma

DC: dendritic cells

MHC: major histocompatibility complex

EMT: epithelial-mesenchymal transition

CTL: cytotoxic T lymphocytes

APC: antigen-presenting cells

OVCA: ovarian cancer

OCIC: ovarian cancer-initiating cells

CXCL12: stromal derived factor-1 or SDF-1

IL-2: interleukin-2

NK: natural killer

ADCC: antibody-mediated cell-mediated cytotoxicity

LRCC: label-retaining cancer cells

Competing Interests

The authors have no competing financial or non-financial interests to disclose.

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