Association of Cancer Stem Cells with Thyroid Cancer and Therapeutic Prospects

S. K. Dey and A. Khanam

Institute of Nuclear Medicine and Allied Sciences, Faridpur
Bangladesh Atomic Energy Commission

Abstract

Cancer stem cells (CSCs) are a small subpopulation of cells those have stem-like properties and can act as key factors for tumor initiation and recurrence. CSCs have been isolating from a different solid tumor and hematological malignancies. Correspondingly, CSCs have been identifing thyroid cancers also. The identification and characterization of CSCs largely depend on the presence of surface markers shared with normal stem cells. Conventional anticancer therapies target mature cancer cells, so cancer stem cells which are relatively quiescent and resistant, are not eradicated. As CSCs can efficiently repair DNA damage following exposure to radio or chemotherapy, they are capable of reconstituting the original tumor. So, the aim of anticancer therapy should be on destruction of CSCs by abruption of self-renewal pathways, promotion of differentiation of cancer cells and inhibition of survival mechanisms. Several drugs are under trial to target these pathways to develop ideal treatment. A better understanding of physiology, behavior and molecular pathology of cancer stem cells will lead to more effective therapeutic targeting of poorly differentiated and advanced form of primary and metastatic thyroid malignancies.

Keywords: Cancer stem cells (CSCs), Thyroid cancer

1. Introduction

Thyroid cancer is the most common type of endocrine cancer [1]. According to the World Health organization (WHO), thyroid cancer has been classified into four groups based on the histopathological characteristics: Papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), medullary thyroid cancer (MTC) and anaplastic thyroid cancer (ATC) [2]. Papillary thyroid cancer (80-85% of thyroid cancers) and follicular thyroid cancer (10-15% of cases) are known as differentiated thyroid cancer (DTC) and contribute to majority of thyroid cancers with a superior prognosis. Medullary thyroid cancer arises from the parafollicular C cells of the thyroid and accounts for about 5% of all thyroid cancers. ATC is a form of undifferentiated thyroid cancer (UTC) which is rare but aggressive and lethal cancer that exhibits rapid disease progression [3].

Though total thyroidectomy with radioiodine therapy is the treatment of choice for localized differentiated thyroid cancer at present, it fails to eradicate aggressive thyroid cancers. In contrast to differentiated thyroid cancers, anaplastic thyroid cancer (ATC) is more aggressively invade nearby structures and metastasize rapidly. It approaches to almost 50% of all thyroid cancer related deaths, the median survival being only six months [4]. Considering chemo and radio-resistant nature of aggressive thyroid cancers, researchers have been continuously trying to discover new treatment strategies to eradicate thyroid cancer cells. All these attempts led to the recent understanding of the role of a special type of cells called cancer stem cells (CSCs).

The cancer stem cells (CSCs) are a small biologically distinct subpopulation of cancer cells in each tumor that have self-renewal and multi-lineage potential and have the capacity for cancer initiation, metastases, recurrence and resistance to therapy [5]. These cells are now considered responsible for initiation of tumor, growth, recurrence and resistance to chemo and radiation therapy [6]. Moreover, if transplanted in to immunodeficient nonobese diabetic/ severe combined immunodeficiency (NOD/SCID) mice, CSCs lead to regeneration of the original cancer [7].

Cancer stem cells have been identified in a number of solid tumors and haematological cancers [8-17]. Their ability to resist conventional therapy may provide a biological basis for understanding tumor recurrence [18]. Eradication of cancer stem cells is now a primary goal of anti-cancer therapy [19]. Therefore, understanding of the characteristics of the thyroid cancer cells and development of new therapeutic agents that can eradicate cancer stem cell population might provide better management of anaplastic thyroid cancer as well as more advanced forms of differentiated thyroid cancer in future. This review depicts the characteristics of cancer stem cells and thyroid cancer stem cells, markers of CSCs, clinical implications as well as therapeutic prospects regarding eradication of cancer stem cells.

2. Cancer Stem Cells (Cscs)

There are two models considered by the cancer biologists which can explain heterogeneity of cancers. One is the stochastic model which explains that cancer is initiated by genetic mutations in single cancer cell followed by subsequent genetic events in different subpopulations of cells.

The second is the cancer stem cell (CSC) model which considers that there is a small biologically distinct subpopulation of cancer cells known as CSCs in each tumor that has self-renewal and multi-lineage potential. Though both the models can explain the cellular heterogeneity of cancers, the two models are not mutually exclusive. The cancer stem cell model can also address some other questions in cancer biology, such as recurrence, metastasis and resistance to therapy [5].

Corresponding author: drskdey707@gmail.com

Cancer stem cells are a small subpopulation of cancer cells characterized by self-renewal capacity to differentiate in to several tumor cells and metastasize [20]. CSCs may originate from normal stem cells or may result from de-differentiation of normal cells. In contrast to differentiated cancer cells, CSCs are relatively quiescent, exhibit a slow cycling rate and exist in a "stem cell niche" that regulates self-renewal and differentiation [21]. CSCs can survive in a serum-free conditions and can proliferate as cellular solid clusters called "tumor spheres" [22]. Moreover, CSCs can form tumors when injected in to immunodeficient mice [23-24].

Bonnet and Dick [25] first reported the existence of CSC population in 1997. The authors identified a population of leukemic stem cells in human acute myeloid leaukemia and demonstrated that CSCs initiated leaukemia in NOD/SCID mice [25].

Al-Haj etal [8] first identified CSCs in breast cancer. Later on, CSCs have been identified in brain cancer [9], cancer in prostate [10], thyroid cancer [11], colon cancer [12-13], head and neck cancer [26], lung cancer [27], melanoma [14], cancer in liver [15], ovary [16] and pancreas [17].

The definite way to confirm the presence of cancer stem cells is by isolating cells and then serially injecting them in to immuno-deficient mice to identify tumor initiation. CSCs may be isolated by flow cytometry according to the expression of several markers, detection of side- population (SP) phenotypes by Hoechst 33342 exclusion and expression of cytoprotective enzymes such as aldehyde dehydrogenese (ALDH) [28]. A number of CSCs markers have already been identified including CD44+/epithelial surface antigen (ESA)+/CD24- in breast cancer [8], cluster of differentiation [CD] 34+/CD38- in leukemia [25], CD133+ in brain cancer [9], colorectal cancer [13], lung [27] and endometrial cancer [29], CD44+/CD24+/ESA+ in cancer of the pancreas [30] CD44+/CD117+ in ovarian cancer [31], CD44+/CD271+ in head and neck cancer [32], CD90 in liver cancer [33], CD105 in renal cancer [34] and CD271 in melanoma [35], hypopharyngeal carcinoma [36] and osteosarcoma [37].

Aldehyde dehydrogenage (ALDH) is a nicotinamide adenine dinucleotide phosphate dependent enzyme that is responsible for detoxification of intracellular aldehydes to weak carboxylic acids [38]. Recently, researchers have identified a high level of ALDH activity as a characteristic of CSCs and the ALDEFLUORTM flow cytometric assay has been widely used to isolate and study CSCs in various cancers [39-40].

3. Thyroid Cancer Stem Cells

Organogenesis of thyroid gland depends on specific transcription factors responsible for differentiation of progenitor cells. Certain cell-specific transcription factors namely thyroid transcription factor (TTF) 1, TTF 2, Hhex factor, pax 8, fgfr-2 and Eya1 are known to play definitive roles in thyroid development [38, 41]. But expression of these factors through a controlled regulation is essential to carry out cellular differentiation and expression of thyroid specific genes.

Mature thyroid cells express a number of markers of differentiation such as thyroglobulin (Tg), thyroid peroxidase (TPO) and thyroid stimulating hormone (TSH) receptor.

The way of thyroid carcinogenesis has been documented in various literatures. There are two models of thyroid carcinogenesis namely multi-step carcinogenesis and fetal cells carcinogenesis [42-44]. The multistep carcinogenesis proposes that well differentiated thyroid cancer cells of follicular origin are transformed into undifferentiated cells through sequential events during maturation of thyroid epithelial cells [45]. Though there are opinions that well-differentiated follicular cells rarely proliferate and thus there is limited chance of accumulated mutations in the cells. Moreover, the genetic mutations happened in well-differentiated cancers are not seen in anaplastic cancers [46].

The model of fetal carcinogenesis proposes that thyroid cancer cells originate from abnormal transformation of fetal cells which are (1) Fetal thyroid stem cells, the primitive cells that express onco-fetal protein responsible for the origin of ATC (2) Thyroblasts, which express fetal protein and Tg and give rise to PTC and (3) Prothyrocytes, which are differentiated cells responsible for FTC/follicular adenoma [42, 44]. Once these cells undergo malignant transformation, they lose their ability to differentiate further and become a potential source of CSCs.

Thyroid CSCs are identified by their expression of surface biomarkers and their ability to form thyrospheres in vitro and tumors in vivo [5]. Zito etal [47] first attempted to isolate CSCs and analyzed the expression of CD133 by flow cytometry in thyroid cancer cell lines. Subsequently, Friedman etal [48] demonstrated that transplantation of CD133+ cells into immunodeficient NOD/SCID mice can induce tumor growth in vivo.

Todaro etal [49] first isolated thyroid CSCs from primary thyroid carcinomas using ALDH as a marker. These cells are most common in UTC (5%), followed by PTC (2%) and FTC (1–2%). Todaro etal [49] expanded such thyroid CSCs populations as thyrospheres, which retain tumoriogenic potential and ALDH1 and CD44 expression but are negative for CD133 expression. Malaguarnera etal [50-51] identified CSCs in PTC and demonstrated that CSCs expressed octamer-binding transcription factor 4, sex determining region Y-box 2 (SOX2), Nanog, CD133 and CD44 stem cell markers.

With the view to identify specific thyroid CSC markers, Shimamura etal [52] studied the expression of nine cell surface markers (CD13, CD15, CD24, CD44, CD90, CD117, CD133, CD166 and CD326) as well as ALDH activity and the ability to form spheres in vitro and tumors in vivo, in eight thyroid cancer cell lines (FRO, KTC1/2/3, TPC1, WRO, ACT1 and 8505C). The results indicated that ALDH represents a candidate marker for thyroid cancers.

4. Metastatic Potential of Cancer Stem Cells

Thyroid cancers invade adjacent structures and metastasize through lymphatic or hematogenous channels. Todaro etal. [49] described aggressive metastatic features of CSCs derived from undifferentiated thyroid carcinomas after transplantation into immuno-compromised mice. This finding throws the question of whether metastases arise directly from CSCs [53]. Another concept has been documented by various authors that points out the metastatic potential of CSCs secondary to epithelial-mesenchymal transition (EMT) and the inverse mesenchymal-epithelial transition (MET) at an advanced stage of the disease [54-56]. This findings described a strong association between EMT/MET and CSCs suggesting that EMT increases epithelial plasticity, confers tumor progression and therapeutic resistance to cancer cells. These transformed cells, then behave like stem-cells similar to those seen in normal thyroid tissue.

Vasko etal [55] described in a study that papillary thyroid ccarccinoma was associated with EMT due to over-expression of vimentin which led to regional lymph node invasion by the tumor [55]. EMT is also said to be associated with loss of E-cadherin, SNAIL, Twist and activation of β-catenin gene expression which make cells lose their adhesion and thus facilitate metastasis [56]. Various authors have documented the role of micro RNAs in this transition process which causes CSCs undergo unlimited proliferation and makes capable of initiating tumor growth at metastatic sites [54-55, 57].

5. Clinical Implications and Therapeutics

Conventional anticancer therapies target mature cancer cells, so thyroid CSCs, which are relatively quiescent and resistant, are not eradicated [58-59]. As CSCs can efficiently repair DNA damage following exposure to cytotoxic injury, they are capable of reconstituting the original tumor [58].

It has been recently demonstrated that chemotherapy with doxorubicin that fails to eradicate anaplastic thyroid cancer cells or to stop tumor progress might be due to the failure of this drug to target CSCs effectively [17]. Though the main population of cancer cells was killed by chemotherapy, the CSC fraction was relatively resistant to doxorubicin. The poor outcome of treatment with doxorubicin in undifferentiated thyroid cancer cells may be explained by up-regulation of different multi-drug resistance transporters of the ABC gene family including ABCG2 and MDR1 that exported the drug out of CSCs and conferred resistance to these cells sustaining tumor growth [17]. It has been observed that inhibition of stem cell marker ABCG2 protein by verapamil restores thyroid cancer cell response to verapamil [56].

Indeed, it is important to identify novel therapeutic approaches that target thyroid CSCs [60-62]. Possible strategies to eradicate thyroid cancer stem cells and overcome resistance to current chemo and radiotherapy may involve the following: Increasing sensitization of CSCs using agents that kill CSCs specifically or promote their differentiation; targeting and blocking important CSCs signaling pathway, including signal transducer and activator of transcription 3 (STAT3), c-Met, SOX2, rearranged during transfection (RET), CD44, ABC sub-family G member (ABCG)2 and ABCB1; and destroying CSC niches [63].

For radioiodine resistant undifferentiated thyroid cancers, therapeutic modalities have to be developed that not only target thyroid cancer cells but also eradicate CSCs that are responsible for tumor progression and recurrence. Thus, the isolation of CSCs may be a useful tool to improve the diagnosis of thyroid cancers, analyze the efficacy of new drugs and development of novel treatment for undifferentiated thyroid cancers that are rather resistant to conventional therapy.

Finally, an ideal therapy should eliminate both CSCs and their progeny. Although strategy have been improved, inadequate information about the underlying mechanisms of expansion and survival of thyroid cancer cells has prevented the development of reliable treatments. Several drugs are currently under investigations to target specific pathways. It would be interesting if such drugs would be able to re-differentiate thyroid cancer cells and restore their ability to uptake radioiodine.

6. Conclusion

Recent advances in tumor cell biology and genetics provides increasing evidence that a specific subpopulation of tumor cells plays a crucial role in tumor initiation, invasive growth and metastasis in several malignancies. These cells, known as cancer stem cells (CSCs) have the ability of asymmetric cell division and self-renewal, can give rise to progenitor cells that progressively differentiate into heterogeneous tumor cell mass. In-spite of low mortality rate of thyroid cancer, poorly differentiated and anaplastic thyroid cancers are deadly cancers due to their resistance to conventional therapy. As CSCs show a low proliferation rate, easily enter into quiescent state, are therefore very resistant to conventional therapy. A better understanding of the characteristics of thyroid CSCs will provide more effective targeting of poorly differentiated and advanced forms of thyroid malignancies.

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