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Cancer stem-like cell behavior in anaplastic thyroid cancer: A challenging dilemma

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ABSTRACT

Aims: Anaplastic thyroid carcinoma (ATC) is an undifferentiated tumor of the thyroid which is characterized with poor prognosis, leading to its aggressive behavior and resistance to conventional therapies. Cancer stem cells (CSCs) are tumor cells that have self-renewal and clonal tumor initiation. Like other cancers, many studies have shown that ATC also has tumor cells with properties like stem cells. To evaluate the concept of cancer stem-like cell theory of ATC, we conducted this study to emphasize both on the concept of cancer stemness origin of these cells and target them for further therapeutic purposes. In the current study, we showed that two ATC cell lines, SW1736 and C643, have subpopulations (SP) that are similar to CSCs.
Materials and methods: Using MACS technique, cells positive for CD133 were isolated and subsequently validated with flow cytometry. For further analysis, expression of some stemness markers was evaluated.
Key findings: ABCG2, *CD133*, and *Sox2* were significantly up-regulated, while *Nestin* was down-regulated in $CD133^{pos}$ subpopulation compared to $CD133^{neg}$ cells. In contrast to previous reports that over-expression of *Nestin* was considered as a marker for thyroid CSCs, we noticed that expression of *Nestin* was declined in stem cell-like tumor cells, derived from ATC cell lines.
Significance: This study reconfirmed the concept of cancer stem-like cell identity of SW1736 and C643 cells. Indeed, the characterization of CSCs should not be merely based on surface markers. Cell origin and genetic background should be additionally considered on CSCs subpopulation of ATCs for therapeutics.

1. Introduction

Cancer is one of the most life-threatening diseases with no conclusive therapy so far. Although there is no agreement on the factors responsible for some cancers' features such as metastasis, relapse and drug resistance, it seems that tumor heterogeneity plays the major role. Indeed, tumor is a complex environment containing tumor cells and various infiltrating cells such as endothelial, hematopoietic and stromal cells. These exterior cells affect tumor environment (TME) by changing metabolic status of tumor cells (like creating hypoxic condition) [23].

Generally, two models have been used for explaining tumor heterogeneity, stochastic and hierarchical models. In one hand, stochastic model relies on presumption that cancer is the result of mutations in cell cycle's genes that lead to cell hyperproliferation. In this model, cells that over express oncogenes or down regulate tumor-suppressor genes are capable of producing cancer. On the other hand, hierarchical theory emphasizes that tumor cells are originated from a small population of cells (CSCs) that have similar characteristics to adult stem cells (Fig. 1). According to this model, carcinogenesis occurs when an adult stem cell bypasses regulation or even when a terminally differentiated cell (with cancerous properties) is dedifferentiated (Fig. 1A, B) [35]. However, these two models are not mutually exclusive and can be harmonized by considering cellular plasticity, genetic diversity and non-genetic influences [27] [32,35].

CSCs are tumor cells that have self-renewal, clonal tumor initiation and clonal long-term repopulation capacity (Fig. 1B) [35,37]. Also, CSCs are able to transit between stem cell and non-stem cell states, an ability that refers as plasticity. CSCs are able to be quiescent for a long time and metastasize to different organs. These cells are resistant to usual treatments [34]. CSCs anatomically and functionally do not resemble the original organs' adult stem cells. Actually, it has been shown that CSCs lose multipotency (generation of an entire tissue) and asymmetric cell division in comparison with adult stem cells [27]. Hence, many of

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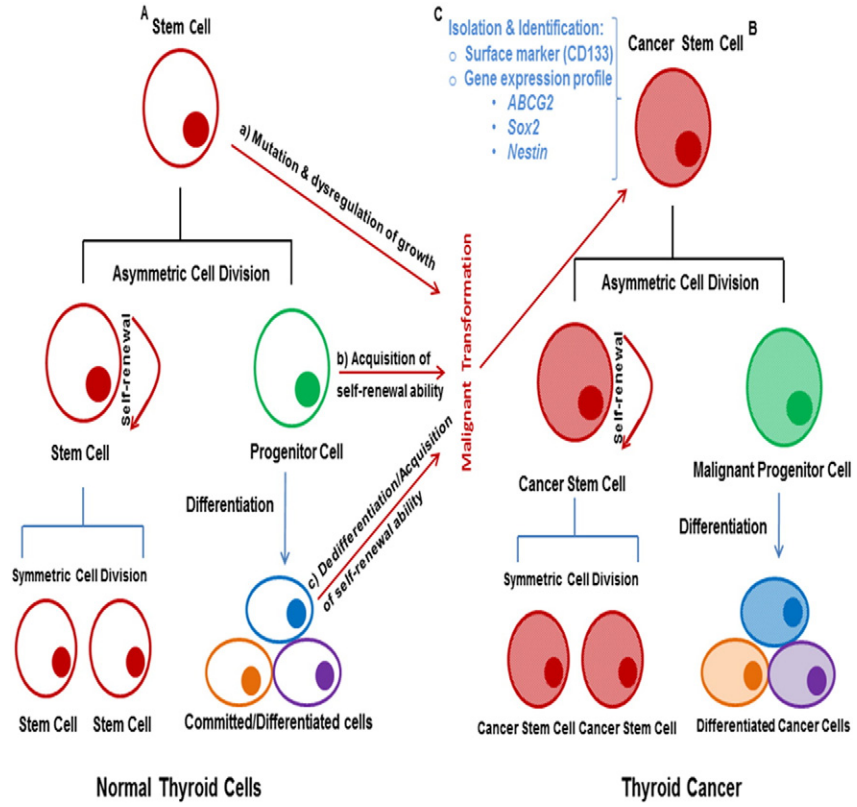

Fig. 1. A schematic representation of stem cell and cancer stem cell concept in thyroid cells. A) Stem cell is capable of asymmetric cell division. It sequentially generates a hierarchy of different differentiated cells. Meanwhile, some symmetrical cell divisions occur that generate two stem cells, thus ensuring homeostatic control. B) Cancer stem cell may arise from one of the following pathways: a) a stem cell undergoes mutation and losses of growth regulation, b) a progenitor cell undergoes several mutations, c) a differentiated cell undergoes dedifferentiation (epithelial to mesenchymal transition, EMT), thus acquiring self-renewal capability. Like stem cells, cancer stem cells are capable of dividing asymmetrically and symmetrically. C) Series of in vitro strategies with specific biomarkers have been used to isolate and identify cancer stem cells, derived from patients with thyroid cancer or from certain thyroid cancer cell lines. These include the CD133 cell surface marker and gene expression profile for *ABCG2*, *Sox2*, and *Nestin* genes.

researchers prefer to use "tumor-initiating cells (T-ICs)" term instead of CSCs. However, phenotypes and gene-expression patterns of the cell of origin may differ considerably from that "CSCs" [35].

Most of signaling pathways that are important for maintaining stemness in stem cells, are similarly vital for CSCs such as Oct4/Sox2, Notch, and Wnt. It has been shown that disrupting CSCs' signaling pathways, leading to differentiation of them, can inhibit cancer drug resistance and relapse (known as differentiation therapy) [5,20,36].

First, CSCs were isolated from leukemia followed by their isolation from many solid tumors [1,28]. Generally, techniques that are used for CSCs' isolation are based on the enrichment of CSCs via Hoechst dye or surface markers [18]. CD44 and CD133 are two common cell surface markers that have been used for the isolation of CSCs from many tumors [45,51]. In addition, some studies have been successful in isolating CSCs from cancer cell lines [10,18,49]. Isolating CSCs from cell cultures can facilitate studies since they are more accessible than clinical samples.

Based on differentiation state, there are two types of thyroid cancers; differentiated thyroid cancer (papillary thyroid carcinoma and follicular thyroid carcinoma) and undifferentiated thyroid cancer (ATC). Differentiated thyroid cancers show good survival rate and are the most frequent type of thyroid cancers [7]. In contrast, ATC is an undifferentiated, aggressive and most deadly of all thyroid cancers. This type of cancer has a very low rate of cure and most patients die one year after diagnosis [24]. It is believed that chemo- and radio-resistant nature of ATC is due to the presence of stem cell-like tumor cells within the ATC. Therefore, many attempts have been done to isolate and characterize CSCs in this type of tumor [7,26,31]. Here, we observed that two ATC cell lines, SW1736 and C643, have SP that resembles CSCs. $CD133^{pos}$ cells, derived from these two cell lines, have gene expression pattern similar to CSCs and express stemness factors (Fig. 1C).

To evaluate the concept of cancer stem-like cell theory of ATC, we conducted this study to emphasize both on the concept of cancer stemness origin of these cells and target them for further therapeutic purposes.

2. Materials and methods

2.1. Cell lines and cell culture

SW1736 and C643 are ATC cell lines (CLS Cell Lines Service GmbH, Germany) which were analyzed by STR (Supplementary Figs. 1 & 2) to confirm their cellular integrity and were subsequently cultured in RPMI high glucose (Gibco) plus Pen/Sterp 50 μg/ml (Gibco) and 10% heat-inactivated fetal bovine serum (Gibco) at 37 °C in a CO_2 incubator.

The Institutional Review Board of Endocrinology and Metabolism Research Institute approved this study (IRB No. 00207-2012-07-17).

2.2. Sorting cells by MACS and evaluation of $CD133^{pos/neg}$ cells by flow cytometry

SW1736 and C643 cells were trypsinized and labeled with primary CD133 Biotin and Biotin-Microbeads (Indirect CD133 cell Isolation Kit, Miltenyi Biotec), according to the manufacturer's instructions and then cell sorting was performed by Magnetic Activated Cell Sorting (MACS) technique. Cell purity was estimated by flow cytometry using phycoerythrin (PE)-conjugated anti-human CD133/2 (clone AC141, Miltenyi Biotec) by BD FACSCalibur (Becton Dickinson, San Jose, CA, USA).