Cancer Stem Cell Theory and Intratumor Heterogeneity in Thyroid Carcinogenesis

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Abstract

Intratumor heterogeneity is becoming a main concern not only biologically but also clinically, because it is critical to determine the cancer cells that have the potential to contribute to disease progression and to develop more effective cancer therapies. Although intratumor heterogeneity is also well recognized in thyroid cancer, it remains to be elucidated regarding how the heterogeneity arises in a tumor. The cancer stem-like cells (CSCs) are considered to cause the heterogeneous lineages of cancer cells that comprise the tumor. The epidemiologic study among Japanese atomic-bomb survivors and our animal experiments indicate that the presence of tumor-initiating cells (TICs) starts only in childhood and persists for over 50 years in thyroid gland, and these cells should be a subpopulation of immature thyroid cells possessing a life long-lasting or self-renewal capability, suggesting their stem cell-like properties. Although several attempts have been performed to identify thyroid CSCs, there is no convincing evidence demonstrating the presence of thyroid CSCs. We hypothesize that there may be hierarchically different CSCs that possess variable levels of tumorigenic potential and exert their tumorigenic potential depending on individually suitable microenvironment in a thyroid tumor.

Keywords: Thyroid cancer, cancer stem cell, heterogeneity, ALDH, EMT

Introduction

Two theories have been proposed in thyroid follicular cell carcinogenesis: the fetal cell carcinogenesis theory and the more common multistep carcinogenesis theory (1-3). In fetal cell carcinogenesis theory, cancer is regarded as an abnormal development of fetal thyroid cells and genomic alternations play an oncogenic role by preventing thyroid fetal cells from differentiating. According to the multistep carcinogenesis theory, most cancers are clonal in origin, arising from a single abnormal cell and progressing as a result of accumulation of inheritable molecular alterations (2). This multistep carcinogenesis model was originally shown convincingly in colorectal carcinoma by Vogelstein et al. (3). However, the hypothesis of another tumorigenic pathway, that of de novo carcinogenesis, suggesting the development of a cancer from normal colonic mucosa, without the intervening step, has been a matter of discussion for decades (4, 5). Indeed, colorectal de novo cancers account for a considerable proportion in Japan and the expected probabilities of developing de novo cancer are 18.6% for men, and 27.4% for women with early cancer (6). In de novo cancer theory, tumor-initiating cells (TICs) should be phenotypically malignant. There is accumulating evidence that solid tumors, including colorectal cancer, contain a distinct subpopulation of cancer stem-like cells (CSCs) or TICs that play important roles in cancer initiation, progression, recurrence, and metastasis. Therefore, identification and analysis of CSCs are fundamental for cancer study. We review here regarding the recent evidence of the CSCs presence in thyroid cancer and discuss about the possible involvement of CSCs during thyroid carcinogenesis.

Heterogeneity of thyroid cancers

Thyroid cancers of follicular cell origin can be classified as well differentiated carcinoma (WDC), such as papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), poorly differentiated carcinoma (PDC), and anaplastic carcinoma (AC) (7). Although the overall prognosis of WDC is excellent, PDC is moderately aggressive and AC is one of the most malignant human cancers with a significantly poor outcome (8). Molecular analyses have revealed the involvement of three main alterations including RET/PTC rearrangements, BRAFV600E, and RAS mutations in PTC, and two main alterations including RAS mutations and PAX8/PPARγ rearrangements in FTC (9-17). These molecular alterations are observed in a mutually exclusive manner and considered to contribute to the initiation of normal follicular cells during thyroid carcinogenesis. On the other hand, mutations of both TP53 gene and CTNNB1 gene encoding β-catenin are restricted to PDC and AC, suggesting their significance in the late stage of thyroid carcinogenesis. Thus, thyroid cancers of follicular cell origin are a heterogeneous entity on clinical, pathological, and genetic points of view.

Molecular dissection of the genome has clearly shown cancer heterogeneity within organ sites and within tumors (18, 19). Intratumor heterogeneity is well recognized also in thyroid cancers. For PTC, several studies with fluorescence in situ hybridization analysis for RET/PTC rearrangements revealed that only subpopulation of tumor cells exhibited this rearrangement in PTCs (20-22). Another study demonstrated overexpression of the genes encoding TGF-β and integrin pathway molecules in the invasive peripheral region compared to the central region of same
tumor, while these genes are known to be involved in epithelial-to-mesenchymal transition (EMT), (23). Also, a study on the AC cases with different microscopic appearances (e.g., spindle or giant cell morphology) has found that mutations in the CTNNBI gene are common but focal in AC (24). Intratumor heterogeneity is becoming a main concern not only biologically but also clinically, because it is critical to determine the cancer cells that have the potential to contribute to disease progression and to develop more effective cancer therapies. However, it remains to be elucidated regarding how heterogeneity arises in a tumor during thyroid carcinogenesis.

Heterogeneity and CSC model

Three different mechanisms are considered to cause the intratumor heterogeneity including: i) stochastic clonal evolution through genetic or epigenetic changes, which involves intrinsic differences; ii) environmentally determined effects which confer phenotypic and functional differences upon cancer cells in different locations; and iii) CSC model (25, 26). CSC could be defined as a small subset of cancer cells that self-renew to generate additional CSC as well as to differentiate to phenotypically diverse cancer cells including non-tumorigenic cells with limited proliferative potential, creating a hierarchical organization. The CSC model is based on the presence of such undifferentiated cells, which are primarily responsible for cancer growth and disease progression. The presence of only mature differentiated cells in residual tumor masses after chemotherapy is a favorable prognostic factor, while the presence of residual undifferentiated cells predicts disease recurrence (27). CSCs have been initially identified in acute myeloid leukemia (28). Compelling data support the CSC model in various human solid cancers including breast cancers, brain cancers, colon cancers, pancreatic cancers, and ovarian cancers (29-34), suggesting several cancers consist of phenotypically and functionally heterogeneous cancer cells. However, the thyroid CSC remains to be confirmed.

Evidence for the presence of TICs showing stem cell-like properties in thyroid

It is well established that exposure to ionizing radiation, especially during childhood or adolescence, is a risk factor for thyroid cancer. Japanese atomic-bomb (A-bomb) survivors have been a critical source of information for insight into radiation-related thyroid cancer risk. Recently, a long-lasting thyroid cancer risk was reported for A-bomb survivors who were exposed to bombing at age of younger than 20-year-old (35). The risk decreased sharply with increased age at exposure and there was little evidence of increased thyroid cancer rate for those exposed after age 20. This study estimated that the excess thyroid cancer risk associated with childhood exposure has persisted for over 50 years after exposure, suggesting that TICs only occur in childhood and persist for several decades. Furthermore, our animal experiments with local irradiation (8 Gy) to the anterior neck of rats confirmed the frequent occurrence of thyroid cancers in childhood (4- and 7-week-old) exposure group but not in adulthood (7-month-old) exposure group (Fig. 1). This study also found that there were no significant differences in the number of DNA double strand breaks of follicular cells at acute phase (within 24 hours) after irradiation between both groups (Fig. 2), indicating that a high dose irradiation can easily induce DNA damage in follicular cells in any age but no TICs in adulthood. Another study by us with transgenic mouse demonstrated that although the expression of BRAFV600E in all thyroid cells from the fetal could lead to tumorigenesis, the postnatal expression of BRAFV600E in a small population of thyroid cells is not able to induce thyroid cancer (36). We speculate that the cell-of-origin for TIC is restricted to a certain subpopulation of postnatal thyroid gland and the BRAFV600E is required to be specifically expressed in such cells to induce thyroid cancers. When considering the difference between young and adult thyroids, we should take into account the involvement of normal stem/progenitor cells for thyroid carcinogenesis. Taken together these findings, TICs should be a subpopulation of immature thyroid cells possessing a life lasting and/or self-renewal capability, suggesting their stem cell-like properties.

![Figure 1: Macroscopic and histologic features of radiation-induced thyroid cancer at 16 months after exposure of a 7-week-old rat to 4 Gy local radiation.](image1)

![Figure 2: Alteration of the number of DNA double strand breaks in rat thyroid follicular cells after irradiation by immunofluorescence analysis of the 53BP1, a DNA damage response molecule. There are no significant differences in the number of 53BP1 nuclear foci at each time point (control, 6 hours, and 24 hours) after irradiation among 4-week-, 7-week-, and 8-month-old rats.](image2)
Figure 3: Hypothesis of a variable model to understand the range of disorders from indolent (e.g., latent cancer) to slowly (e.g., poorly differentiated carcinoma) or rapidly progressive (e.g., anaplastic carcinoma) and the intratumor heterogeneity. There may be hierarchically different CSCs, which possess variable levels of tumorigenic potential and exert their tumorigenic potential depending on individually suitable microenvironment (CSC niche) in a single tumor.

Table 1: Studies on identifying cancer stem-like cells in thyroid cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>CSC marker</th>
<th>Subjects</th>
<th>Self-renewal</th>
<th>In vivo tumor xenograft</th>
<th>Sphere colony assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitsutake N Endocrinology (2007)</td>
<td>Side population</td>
<td>Non-thyroid cell line: ARO</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thyroid cell line: FRO, TPC, WRO</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Zito G PLoS one (2008)</td>
<td>CD133</td>
<td>Non-thyroid cell line: ARO, KAT-4</td>
<td>Yes</td>
<td>No data</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thyroid cell line: KAT-18, FRO</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Friedman S PLoS one (2009)</td>
<td>CD133</td>
<td>Non-thyroid cell line: ARO</td>
<td>Yes</td>
<td>Yes</td>
<td>No data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thyroid cell line: FRO, TPC</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Todaro M Cancer Res (2010)</td>
<td>ALDH, CD133, CD44</td>
<td>Primary thyroid cancer</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Shimamura M Endocrine J (2013)</td>
<td>ALDH, CD326</td>
<td>Thyroid cell line: FRO, KTC3, ACT1, 8505C</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

and CD44+ for gastric cancer (39). In thyroid cancer, as shown in Table 1, side population (40) and CD133+ (41, 42) are originally reported as CSC markers by using ARO and KAT-4 cells. However, these two cell lines are now thought to be identical to the colon cancer cell line, HT-29 (43). Recently, Todaro et al. demonstrated that a small subpopulation with high aldehyde dehydrogenase (ALDH) activity showed CSC-like features/tumorigenic ability by using primary thyroid cancer cells (44). Furthermore, Shimamura et al. reported that three (FRO, KTC3, and ACT1) out of the four AC-derived cell lines with a high ALDH activity showed CSC-like features, while none of the WDC-derived cell lines including the KTC1, TPC, and WRO, did not show SC-like features regardless of the ALDH activity (45). This study also evaluated whether ALDH-high fraction exhibited a symmetric division, a hallmark of CSC. As the results, ALDH-high fraction possessed both self-renewal and differentiation capacities generating both same and different heterogeneous populations, however, ALDH-low fraction also gave rise to both two populations. Thus, these subpopulations did not follow a classical hierarchical model. ALDH activity is probably a major candidate marker to enrich thyroid CSCs but not universal. Furthermore,
Yasui et al. demonstrated that the ACT1 (an AC-derived cell line) exhibited EMT phenotype; the number of ALDH-positive fraction decreased; and the ALDH-negative fraction gained greater tumour-forming ability than ALDH-positive fraction by induction of SNAIL expression (46). EMT is thought to play a critical role in the invasion and metastasis of cancer and to be associated with CSC properties (47, 48). Given that expression of SNAIL is restricted at the invasive fronts of thyroid cancers with an EMT phenotype (23), the role for ALDH in CSC properties may be dependent on the EMT status. In thyroid cancer, therefore, the state of CSC might be also flexible in certain environments. There may be different CSC markers depending on individual cases. Further characterization is needed to understand the whole view of thyroid CSCs.

Summary

A model of cancer progression that is more suited to the current understanding of cancer biology is one of variable progression depending on tumor type and stromal microenvironment (49). In thyroid carcinogenesis, a variable model should be more suitable to understand the range of disorders from indolent (e.g., latent cancer, papillary microcarcinoma, and conventional WDC) to slowly (e.g., aggressive variants of WDC and PDC) or rapidly progressive (e.g., AC) and the intratumor heterogeneity (50). We hypothesize that there may be hierarchically different CSCs that possess variable levels of tumorigenic potential and exert their tumorigenic potential depending on individually suitable microenvironment (CSC niche) even in a single tumor (Fig. 3). For instance, latent cancer or papillary microcarcinoma may be mainly consistent with no/low tumorigenic cells in the indolent niche, while AC may be arisen when high tumorigenic CSCs activate under the rapidly progressive niche. Indeed, our studies suggest that high tumorigenic CSCs are isolated only from AC-derived cell lines but not from WDC-derived cell lines, and their tumorigenic potential appears to be influenced by acquisition of EMT characteristics, which are strongly associated with circumstances in a single tumor (45, 46). Although several attempts have been performed to date to identify thyroid CSCs, there is no convincing evidence demonstrating the presence of thyroid CSCs. Studies on thyroid CSCs are still limited and further researches are definitely required.

Acknowledgement

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References


