Cancer stem cells as a potential therapeutic target in thyroid carcinoma (Review)

LUISA VICARI[1], CRISTINA COLAROSSI[2], DARIO GIUFFRIDA[2], RUGGERO DE MARIA[3] and LORENZO MEMEO[1,2]

[1]Cell Biology Unit, IOM Ricerca Srl; [2]Department of Experimental Oncology, Mediterranean Institute of Oncology, Viagrande, I-95029 Catania; [3]Regina Elena National Cancer Institute, I-00144 Rome, Italy

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Abstract. A number of studies have indicated that tumor growth and proliferation is dependent on a small subset of cells, defined as cancer stem cells (CSCs). CSCs have the capability to self-renew, and are involved with cancer propagation, relapse and metastatic dissemination. CSCs have been isolated from numerous tissues, including normal and cancerous thyroid tissue. A regulatory network of signaling pathways and microRNAs (miRNAs) control the properties of CSCs. Differentiated thyroid carcinoma is the most common type of endocrine cancer, with an increasing incidence. Anaplastic thyroid carcinoma is the most rare type of endocrine cancer; however, it also exhibits the highest mortality rate among thyroid malignancies, with an extremely short survival time. Thyroid CSCs are invasive and highly resistant to conventional therapies, including radiotherapy and chemotherapy, which results in disease relapse even when the primary lesion has been eradicated. Therefore, targeting thyroid CSCs may represent an effective treatment strategy against aggressive neoplasms, including recurrent and radioresistant tumors. The present review summarizes the current literature regarding thyroid CSCs and discusses therapeutic strategies that target these cells, with a focus on the function of self-renewal pathways and miRNAs. Elucidation of the mechanisms that regulate CSC growth and survival may improve novel therapeutic approaches for treatment-resistant thyroid cancers.

Contents

Correspondence to: Dr Lorenzo Memeo, Department of Experimental Oncology, Mediterranean Institute of Oncology, Via Penninazzo 7, Viagrande, I-95029 Catania, Italy
E-mail: lorenzo.memeo@grupposamed.com

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1. Introduction

Thyroid cancer is the most common type of endocrine malignancy (1). According to the World Health Organization, thyroid cancer may be classified into four subtypes based on its histopathological characteristics: Papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC) and anaplastic thyroid carcinoma (ATC) (2). PTC accounts for 80-85% of all thyroid cancers, while FTC accounts for 10-15% of cases. PTC and FTC are well-differentiated cancers and, if detected early, exhibit a favorable prognosis. MTC arises from the parafollicular C cells of the thyroid and accounts for ~5% of all thyroid cancers. ATC, which is defined as undifferentiated thyroid cancer (UTC), is a rare, aggressive and lethal malignancy that exhibits rapid disease progression (3). Although ATC accounts for only 1% of all thyroid cancers, it accounts for >50% of mortalities that are associated with thyroid carcinoma, with a median survival time of 6-8 months (4,5).

Thyroid cancer treatments include surgery, radioiodine therapy and chemotherapy (6,7). Notably, survival rates have not significantly increased in recent years, which indicates that certain thyroid tumors are resistant to the current therapeutic modalities (8).

The identification of cancer stem cells (CSCs) in a number of tumor types represents a crucial step for the development of effective therapies (9,10). CSCs are a population of cells that have the capacity to self-renew and initiate tumors. Furthermore, when transplanted into immunodeficient nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice, CSCs lead to regeneration of the original cancer (11).

CSCs have been identified in a number of solid tumors and hematological malignancies (12-24). Similarly, CSCs have been identified in thyroid cancer; however, their function in this tumor type remains to be fully determined (25).

The resistance of CSCs to conventional treatments may provide a biological basis for understanding tumor recurrence (26). Eradication of CSCs is considered a primary goal

of cancer therapy (27). Therefore, increased understanding of the characteristics of thyroid CSCs and the identification of therapeutic agents that target the CSC population may lead to the development of novel treatments that specifically target ATC and advanced forms of differentiated tumors.

The present review summarizes the characteristics of thyroid CSCs and discusses potential approaches for targeting thyroid CSCs that may be used in combination with the current therapeutic modalities to achieve long-lasting effects and avoid tumor recurrence and progression.

2. CSCs

Two major models account for the heterogeneity of tumors: The stochastic/clonal evolution and the CSC models (28).

The first model postulates that each cell within a tumor exhibits a tumorigenic potential that is uniquely driven by the acquisition of novel genetic mutations and epigenetic changes that favor clonal selection (29). The second model hypothesizes that only a subpopulation of tumor stem cells possess high tumorigenic activity, representing a hierarchical organization similar to that observed in normal tissue (30). The clinical implication of the CSC model is that the elimination of all CSCs arrests tumor growth, whereas the failure to eliminate CSCs will eventually lead to tumor relapse (28).

CSCs are a small subpopulation of cancer cells characterized by self-renewal, with the capacity to differentiate into several tumor cell types and metastasize (31).

In contrast to differentiated tumor cells, CSCs are relatively quiescent, exhibit a slow cycling rate and exist in a 'stem cell niche' that regulates self-renewal and differentiation (32).

CSCs survive in serum-free conditions and proliferate as cellular solid clusters termed 'tumor spheres' (10). Furthermore, CSCs form tumors when injected into immunodeficient mice (33,34).

Bonnet and Dick (12) first reported the existence of a CSC population in 1997. The authors identified a population of leukemic stem cells in human acute myeloid leukemia and demonstrated that CSCs initiated leukemia in NOD/SCID mice (12).

In 2003, Al-Haj *et al* (13) were the first to identify CSCs in a tumor of the breast. Subsequently, CSCs have been identified in a number of other solid tumors, including tumors in the brain (14), prostate (15), colon (16-18), head and neck (19), lung (20), melanoma (21), liver (22), ovary (23) and pancreas (24).

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 aldehyde dehydrogenase (ALDH).

A number of CSCs markers have been identified, including cluster of differentiation (CD)34+/CD38- in leukemia (12), CD44+/epithelial surface antigen (ESA)+/CD24- in breast cancer (13), CD133+ in brain (14), colorectal (18), lung (20) and endometrial cancer (35), CD44+/CD24+/ESA+ in pancreatic cancer (36), CD44+/CD117+ in ovarian cancer (37), CD44+/CD271+ in head and neck squamous cell carcinoma (38), CD90 in liver cancer (39), CD105 in kidney cancer (40), and CD271 in melanoma (41), hypopharyngeal carcinoma (42) and osteosarcoma (43).

In addition, recent evidence indicates that ephrin (Eph) tyrosine kinase receptors and their ligands, Ephs, sustain CSC self-renewal capacity, viability, invasiveness and tumorigenicity (44,45).

Recently, EphA2 has been reported to exhibit a significant function in the regulation of glioblastoma (46) and lung CSCs (47). However, the association between EphA2 and thyroid CSCs remains unclear.

A small subset of cells, termed SP cells, have been isolated from various solid tumors, including normal and neoplastic thyroid cells, using flow cytometric analysis (48). SP cells exclude the DNA binding dye Hoechst 33342, and are highly enriched for stem cells (49). The efflux of Hoechst dye is dependent on the expression of the adenosine triphosphate-binding cassette (ABC) family of membrane transporters; when overexpressed in cancer cells, they export anticancer drugs, leading to drug resistance (50).

ALDH is a nicotinamide adenine dinucleotide phosphate+-dependent enzyme that is involved in the detoxification of intracellular aldehydes to weak carboxylic acids (51). Recently, a high level of ALDH activity has been identified as a characteristic of CSCs, and the ALDEFLUOR™ flow cytometric assay has been widely used for the isolation and study of CSCs in various cancer types (52,53).

3. Thyroid CSCs

CSCs exhibit an important function in malignant progression, therapeutic resistance and recurrence of thyroid cancer (54-56).

Two models of thyroid carcinogenesis exist: Multi-step carcinogenesis and fetal cell carcinogenesis (57-59). The first model hypothesizes that thyroid cancer cells are derived from thyrocytes via genomic changes (57-59). The second model postulates that thyroid tumor cells originate from the remnants of three types of fetal thyroid cells that persist until early childhood: Thyroid stem cells, thyroblasts and prothyrocytes (57-59).

Cancer cells may also originate from more differentiated cells that have acquired stem-like characteristics via genetic alterations and epithelial-mesenchymal transition (EMT) (60). EMT is a mechanism that generates further CSCs with increased invasiveness and a metastatic phenotype (61). In addition, EMT exerts a critical function in tumor recurrence, and is associated with a loss of E-cadherin expression by genes that repress E-cadherin, including Snail, Slug, zinc-finger E-box-binding (ZEB)1, ZEB2 and Twist1 (62). During EMT, cells become less adherent, lose polarity and acquire an invasive phenotype (63).

Thyroid CSCs are identified due to their expression of biomarkers and their ability to form thyrospheres *in vitro* and tumors *in vivo* (64).

Zito *et al* (65) first attempted to isolate CSCs in 2008, and analyzed the expression of CD133 by flow cytometry in thyroid cancer cell lines. Subsequently, Friedman *et al* (66) demonstrated that the transplantation of CD133+ cells into immunodeficient NOD/SCID mice was sufficient to induce tumor growth *in vivo*. However, these results were considered controversial, as Schweppe *et al* (67) evaluated 40 thyroid cancer cell lines using short tandem repeat and single nucleotide polymorphism array analyses, and reported that a number of the cell lines tested were

redundant or did not originate from the thyroid. These results indicated that CSCs should be isolated from human thyroid biopsy specimens rather than cell lines.

Using ALDH as a marker, Todaro *et al* (68) were the first group to isolate thyroid CSCs from primary thyroid carcinomas. These cells are most common in UTC (5%), followed by PTC (2%) and FTC (1-2%). Todaro *et al* (68) expanded such thyroid CSCs populations as thyrospheres, which retain tumorigenic potential and ALDH1 and CD44 expression, but are negative for CD133 expression.

The authors demonstrated that cells exhibiting high ALDH expression ($ALDH^{high}$) were capable of self-renewal and thus, unlimited replication (68). As UTC CSCs are highly malignant, these cells undergo a higher number of symmetric divisions than PTC and FTC CSCs (68). Therefore, thyrospheres may present a potential tool for preclinical studies. Todaro *et al* (68) also demonstrated that orthotopic injection of 100 thyroid CSCs into the mouse thyroid gland recapitulated the behavior of the parental tumor, including local spreading and formation of distant metastases. As expected, the migration capability of $ALDH^{high}$ cells derived from UTC was higher than that of FTC or PTC (68). Furthermore, $ALDH^{high}$ UTC cells aggressively invaded the trachea and esophagus, and metastasized to distant locations, including the lung (68). By contrast, FTC and PTC CSCs developed moderately invasive tumors (68). The authors further demonstrated that the cell migration capacity was associated with increased c-Met (hepatocyte growth factor receptor) and Akt (a serine/threonine protein kinase) expression (68). These results indicated that c-Met and/or Akt may represent potential therapeutic targets in thyroid cancer.

Malaguarnera *et al* (69,70) also identified CSCs in PTC, and demonstrated that CSCs, isolated as thyrospheres, expressed octamer-binding transcription factor 4, sex determining region Y-box 2 (SOX2), Nanog, CD133 and CD44 stem cell markers. Additionally, the expression of the insulin receptor (IR) isoforms (IR-A and IR-B), insulin-like growth factor (IGF)-IR, IGF-I and IGF-II was higher in CSCs than in differentiating cells.

Li *et al* (71) isolated tumor stem cells from anaplastic thyroid cancer cell lines (THJ-11T, THJ-16T, THJ-21T and THJ-29T), and demonstrated that a subpopulation of these cells expressed the stem cell markers POU class 5 homeobox 1 (POU5F1) and Nanog, and formed tumors in immunodeficient mice. Notably, none of the cell lines expressed CD133.

Ahn *et al* (72) isolated thyroid CSCs from the TPC-1 PTC cell line and 11 human specimens. These CD44+CD24- cells expressed the stem cell marker POU5F1, and a higher percentage of these cells were identified in clinically aggressive recurrent PTCs compared with less aggressive primary PTCs. Furthermore, only 20% of mice inoculated with these CD44+CD24- CSCs generated tumors, as PTC is significantly less tumorigenic than undifferentiated ATC.

Furthermore, to identify specific thyroid CSC markers, Shimamura *et al* (73) investigated 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 activity represents a candidate marker.

4. Therapeutic strategies

At present, the molecular pathogenesis of thyroid carcinoma remains unclear and in particular, little is known regarding the development of ATC. Conventional therapies target mature cancer cells and thus, thyroid CSCs, which are relatively quiescent and intrinsically resistant, are not eradicated (74,75). CSCs efficiently repair DNA damage following exposure to cytotoxic injury, and are therefore capable of reconstituting the original tumor (74). In consequence, it is important to identify novel therapeutic approaches that target thyroid CSCs (76-78).

Possible strategies to destroy thyroid CSCs and overcome chemo- and radioresistance may involve the following: Increasing sensitization of CSCs directly using agents that kill CSCs specifically or promote their differentiation; targeting and blocking important CSCs signaling pathway components, 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 (79).

STAT3 is a transcription factor that regulates cell cycle progression, matrix cellular invasion and angiogenesis (80). A previous study revealed that STAT3 is constitutively activated in numerous cancer types, indicating that it exhibits an important function in resistance to chemoradiotherapy (81).

Tseng *et al* (82) demonstrated that the STAT3 signaling pathway is required for the self-renewal of CD133+ ATC cells. The authors revealed that combined treatment with cucurbitacin and radiochemotherapy blocked STAT3 activity, which suppressed CD133+ cell survival and tumorigenesis *in vitro* and *in vivo* (82).

c-Met, an oncogene that encodes the c-Met protein, controls the motility and mitogenesis of epithelial cells, including cancer cells (83). In thyroid cancer, c-Met activation induces proliferation, invasion and angiogenesis, contributing to tumor growth and cell spreading (84). Todaro *et al* (68) revealed that silencing of c-Met completely abrogates the metastatic capacity of thyroid CSCs. These results indicate that c-Met is a potential therapeutic target in thyroid cancer treatment.

Metastatic MTC responds poorly to conventional treatments with chemotherapy and radiotherapy (85). The RET signaling pathway is involved in stem cell maintenance and the development of MTC (86). Inhibition of RET expression reduces MTC sphere formation, indicating that this may represent a possible therapeutic objective (87).

ABCG2 and ABCB1, members of the ABC transporter family, are involved in multiple drug resistance (88). Zheng *et al* (89) revealed that the failure of doxorubicin treatment in ATC may be explained by the upregulation of ABCG2 and ABCB1 in thyroid CSCs. The development of therapeutic strategies that directly target ABC transporters may present a useful method for killing thyroid CSCs.

CSCs exist in a specific stem cell niche, which has a special microenvironment that controls their self-renewal and maintains their undifferentiated state (32). The CSC niche is composed of various stromal cells types, and includes a vascular system, mesenchymal and immune cells, an extracellular matrix, and soluble factors (90). These stromal cells maintain the CSCs in a quiescent state, and regulate their self-renewal and differentiation via the modulation of several

signaling pathways (90). In addition, the CSC niche appears to be important for metastasis formation via induction of EMT (32). Furthermore, it enhances therapy resistance by protecting CSCs from various genotoxic insults (32).

Knowledge regarding the microenvironment of the CSC niche remains limited and requires further investigation. The vasculature is a promising target in the microenvironment of the CSC niche, as inhibition of angiogenesis and depletion of blood vessels may destroy CSC niches and inhibit tumor growth (91). Understanding the association between CSC niches and their microenvironments may lead to the development of innovative thyroid cancer treatments (92).

Finally, microRNAs (miRNAs or miRs), which are small non-coding RNAs (~21 nucleotides), are aberrantly expressed or lost in a variety of cancers (93). Generally, miRNAs bind to the 3'-untranslated regions of target messenger RNAs (mRNAs), resulting in mRNA degradation or inhibition of translation (94).

miRNAs that are upregulated in cancer may function as oncogenes and promote cancer development by negatively regulating tumor suppressor genes (95). By contrast, miRNAs that are frequently downregulated may function as tumor suppressors to inhibit cancer development via downregulation of oncogenes (96).

Recent studies have indicated that miRNAs may represent potential targets for novel therapies in aggressive thyroid carcinomas (97). The function of miRNAs in various thyroid tumors is unclear; however, certain miRNAs may represent useful diagnostic tools (98,99).

It has been demonstrated that numerous miRNAs are transcriptionally upregulated or deregulated in PTC compared with healthy thyroid tissue (100-109), in FTC compared with follicular adenoma (110-112); in MTC (113,114) and in ATC (115-117).

Furthermore, the use of miRNAs as circulating biomarkers in thyroid cancer is gaining increasing attention (118). Lee *et al* (119) demonstrated that plasma levels of miR-222 and miR-146b are associated with PTC recurrence.

Several miRNAs regulate CSCs properties, including self-renewal ability, tumorigenicity and drug resistance (120-123). CSCs have generally high levels of oncogenic miRNAs and low levels of tumor suppressive miRNAs (122).

To the best of our knowledge, no miRNAs have been identified in thyroid CSCs. Notably, a recent study demonstrated that miRNAs control CSC functions and regulate cancer progression by affecting EMT (124). Therefore, a more detailed understanding of how miRNAs control the EMT process and regulate CSC function may lead to the clinical application of miRNAs in thyroid cancer diagnosis, treatment and prognosis.

Finally, investigation of thyroid stem cell regulation by Eph receptors and Ephs may lead to the identification of novel targets for therapeutic intervention in thyroid cancer.

5. Conclusion

Thyroid cancer remains a major public health issue (8). Of all thyroid cancers, >90% of cases are well-differentiated and generally exhibit a favorable prognosis (3). By contrast, ATC, an UTC, is associated with a poor prognosis (3).

At present, surgery, radiation therapy, chemotherapy and hormonal therapy are used to treat thyroid cancer; however, these treatments often exhibit limited efficacy (7).

Conventional therapies target highly proliferating cells that form the majority of the tumor mass, but they are ineffective against slowly proliferating or quiescent CSCs, which are responsible for drug resistance, metastasis and recurrence (125).

Therefore, an ideal therapy would eliminate both CSCs and their progeny. Although certain advances have been made, the absence of important information regarding the underlying mechanisms of increased expansion and survival of thyroid cancer cells has prevented the development of reliable treatments.

The ability to identify, isolate and study thyroid CSCs has a number of implications with potential novel therapeutic consequences: i) Their molecular characterization and elucidation of the relevant signaling pathways will allow the identification of novel diagnostic markers and therapeutic targets; ii) an extensive analysis of gene expression levels may reveal the existence of unknown regulatory pathways that control CSCs; iii) CSCs may be useful for preclinical therapeutic screening and for monitoring the effects of novel biological therapies; and iv) knowledge of CSC behavior within the four subtypes of thyroid cancer may lead to the development of subtype specific treatments.

Future studies that investigate the molecular pathways responsible for thyroid CSC survival and expansion are required to increase the understanding of thyroid CSCs and to identify relevant therapeutic targets. Drugs that specifically target thyroid CSCs with minimal side effects are required. In addition, elucidating the associations between thyroid CSCs, differentiated tumor cells and the microenvironment is essential.

Finally, considering the importance of thyroid CSCs, this field of study has become an essential step for the development of targeted, selective and individualized treatment to achieve complete eradication of thyroid cancer.

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