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EFFECTIVENESS OF STEM CELL THERAPIES (CSCS) IMPROVING PATIENTS CLINICAL OUTCOMES IN THYROID CARCINOMA

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1. ABSTRACT

Emerging evidence has identified a distinct subset of cells, known as cancer stem cells (CSCs), as key contributors to tumor initiation, progression, recurrence, and metastasis. These cells possess the unique abilities of self-renewal and differentiation, making them central players in treatment resistance and tumor relapse. CSCs have been discovered in various tissues, including both healthy and malignant thyroid tissue, and their function is regulated by specific intracellular signaling pathways and microRNAs (miRNAs) that influence gene expression and cellular behavior (Vicari 2016). In thyroid cancer, CSCs are particularly concerning due to their high invasiveness and resistance to conventional therapies such as chemotherapy and radiotherapy. This resistance is thought to play a major role in cancer recurrence and the spread of disease after initial treatment. Therefore, understanding the biology and behavior of thyroid CSCs may offer promising new avenues for targeted therapies, especially for aggressive and treatment-resistant forms of the disease.

KEYWORDS: Emerging evidence, distinct subset of cells, cancer stem cells (CSCs), tumor initiation, progression.

1. INTRODUCTION

Thyroid cancer is the most prevalent form of endocrine malignancy globally, with a steadily increasing incidence over the past several decades. The most common subtype, differentiated thyroid cancer (DTC), encompasses papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), both of which are generally associated with favorable prognoses and high survival rates following treatment. Notably, PTC accounts for approximately 80–85% of thyroid cancer cases, while FTC comprises about 10%. Additionally, papillary thyroid carcinoma alone represents nearly 96% of newly diagnosed thyroid cancers (Stewart, 2014).

Despite its typically indolent nature and high curability, the incidence of thyroid cancer has surged by over 240% in the last 30 years, prompting concerns regarding potential etiologic factors. Although the majority of thyroid nodules identified in the general population are benign, the presence of vocal cord paralysis in a patient may indicate locally advanced or invasive thyroid malignancy. At the other end of the clinical spectrum lies anaplastic thyroid carcinoma (ATC), a rare but highly aggressive subtype characterized by rapid progression, poor prognosis, and resistance to conventional therapies (Stewart, 2014).

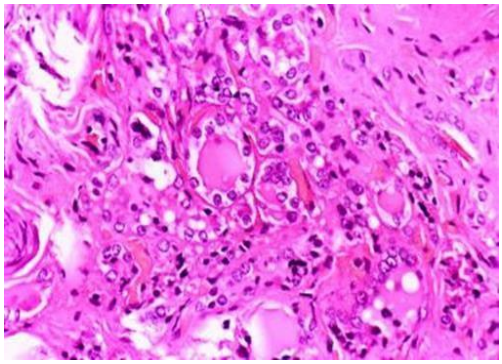
The discovery of CSCs in thyroid tumors has provided a new dimension to our understanding of thyroid tumorigenesis. CSCs are a subpopulation of tumor cells endowed with the capacity for self-renewal and multi-lineage differentiation, and they have been implicated in tumor initiation, therapeutic resistance, disease recurrence, and metastasis. Therefore, elucidating the

role of CSCs in thyroid cancer may offer novel therapeutic avenues, particularly for aggressive and treatment-refractory forms of the disease. This paper aims to examine the function of CSCs in thyroid cancer, with a particular focus on their contribution to treatment resistance and tumor relapse.

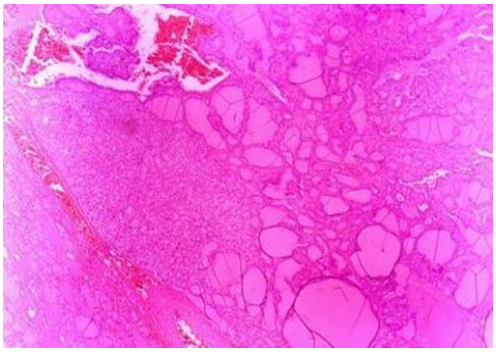
2. Anatomy and Pathology

2.1 Follicular and Pappilary Types

FTC is a well-differentiated malignancy that closely mimics the normal histological architecture of the thyroid gland. Arising from follicular epithelial cells, FTC is the second most prevalent type of thyroid cancer, following PTC. The papillary-follicular carcinoma, a mixed histological form, is classified as a variant of papillary thyroid carcinoma. Both follicular and papillary thyroid carcinomas are categorized as DTCs, collectively accounting for approximately 95% of all thyroid cancer cases. (Santacore, 2016).



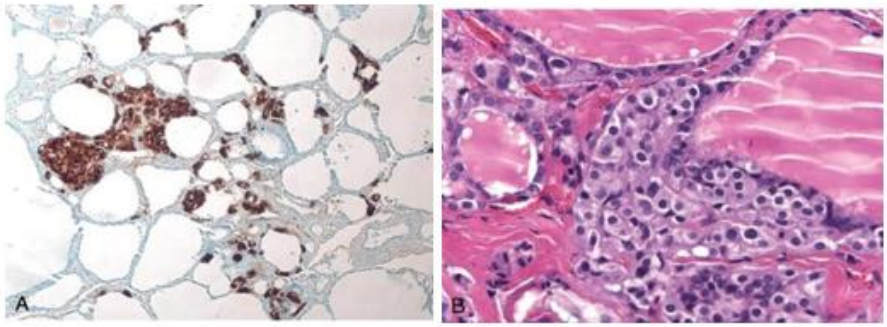
Histologic pattern of a mildly differentiated follicular thyroid carcinoma (250 X). Image courtesy of Professor Pantaleo Bufo at University of Foggia, Italy.



Histologic pattern of a rare lymph node metastasis of follicular thyroid carcinoma (140 X). Image courtesy of Professor Pantaleo Bufo at University of Foggia, Italy.

2.2 Medullary Thyroid Carcinoma

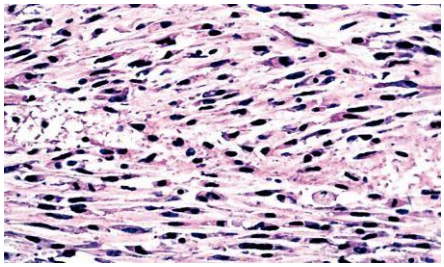
MTC is an uncommon thyroid malignancy of C-cell derivation representing approximately 3% to 12% of all thyroid cancers. It has a propensity for metastasis to regional lymph nodes. Although a majority of MTCs are acquired as sporadic tumors, 25% to 30% of cases are heritable, being associated with multiple endocrine neoplasia (MEN) 2A, MEN 2B, or with the familial medullary thyroid carcinoma syndrome (Etit et.al. p. 1767).



C-cell hyperplasia. A, both nodular and non-nodular forms of C-cell hyperplasia are present, as highlighted by immunohistochemical staining for calcitonin (calcitonin immunohistochemistry, original magnification 50). B, The C cells have delicate granular cytoplasm, round nuclei, and an even chromatin pattern (hematoxylin-eosin, original Magnification 400) (Etit et.al, p.1770).

2.3 Anaplastic Carcinoma

Anaplastic tumors are the least common and account for only 1% of all thyroid cancer cases. These are the deadliest of all thyroid cancers with a very low cure rate even when utilizing the very best treatments. The mortality rate for this type of cancer is within one year from the day they are diagnosed (Norman, 2014).



Anaplastic Carcinoma. Image courtesy of pathologyoutlines.com

3. Cancer Stem Cells (CSCs)

3.1 Cancer Stem Cell Theory

The CSC theory posits that within a heterogeneous tumor mass, only a subpopulation of cells possesses the capacity for sustained self-renewal and tumor propagation. These CSCs exhibit properties reminiscent of normal tissue-specific stem cells, including quiescence, multipotency, and the ability to initiate new tumor growth. Unlike bulk tumor cells, which are generally proliferative and more differentiated, CSCs are often relatively dormant, reside in specialized microenvironments or "niches," and are highly resistant to conventional therapies such as chemotherapy and radiotherapy (Borovski et al., 2011).

Traditionally, the stochastic (or classical) model of cancer held that all tumor cells are biologically equivalent in their potential to drive tumorigenesis, and that any cancer cell has the capability to initiate a new tumor given the right conditions. However, accumulating evidence supports the hierarchical model, which suggests that tumors are organized similarly to normal tissues, with only a discrete subset of CSCs at the apex of the cellular hierarchy. These cells are responsible for maintaining tumor growth, while the bulk of the tumor consists of non- tumorigenic progeny with limited proliferative potential.

CSC origin remains a subject of investigation. They may arise from normal stem cells that acquire oncogenic mutations, or from more differentiated cells that undergo dedifferentiation a process whereby mature cells revert to a stem-like state. This transformation is driven by the activation of oncogenes and the inactivation of tumor suppressor genes, which collectively deregulate key pathways controlling cell cycle, differentiation, and apoptosis.

The tumor microenvironment (TME) comprising stromal cells, immune cells, extracellular matrix components, and soluble factors such as cytokines and growth factors plays a critical role in supporting CSC survival, self-renewal, and plasticity. Similar to the stem cell niche in normal tissues, the CSC niche maintains stemness and shields these cells from immune responses and cytotoxic therapies. Notably, signals from the TME can induce non-stem cancer cells to reacquire stem-like properties, further contributing to tumor heterogeneity and therapeutic resistance.

Furthermore, the TME may facilitate metastatic dissemination by promoting epithelial-to- mesenchymal transition (EMT), enhancing cell motility and invasiveness. Once disseminated, CSCs may exploit permissive microenvironments at distant sites termed the "pre-metastatic niche" to initiate secondary tumor formation.

Collectively, these insights highlight the dynamic interplay between CSCs and their microenvironment, suggesting that targeting both CSCs and the supportive components of the TME may be critical to achieving durable therapeutic responses and preventing relapse (Podberezin et al., 2013).

3.2 CSCs Markers

CSCs are detected and enriched using both in vivo and in vitro methods. In vivo assays, such as xenotransplantation of human tumor cells into immunodeficient mice, were pivotal in establishing CSC theory. These assays rely on the tumor-forming ability of rare CSCs under specific host conditions, and more refined techniques like serial dilution and serial transplantation can detect CSCs at the single-cell level. These methods remain the gold standard but are dependent on host factors like microenvironment compatibility and the timing of analysis. While highly sensitive, these methods demand substantial resources (Pastrana, et al., 2011).

To overcome the limitations of in vivo methods, several in vitro assays have been developed. These include colony-forming assays and microsphere (or neutrosphere) assays to assess stemness and proliferative ability. Additional techniques include the side population (SP) assay, which leverages CSCs' dye efflux capabilities, and aldehyde dehydrogenase (ALDH) activity assays, which detect high aldehyde dehydrogenase activity characteristic of CSCs (Marcato, 2011). The PKH26 label-retaining assay identifies quiescent CSCs through asymmetric dye dilution, and CSC-specific surface markers such as CD24, CD44, CD133, and ABCG2 are used for flow cytometric sorting (Lingala et al., 2010). While these in vitro assays are more accessible, they have limitations like inter-lab variability, potential marker alteration during processing, and sensitivity to environmental conditions. Together, these complementary techniques enable more accurate CSC detection and enrichment for research and therapeutic targeting.

Marker	Cancer Types Associated with CSCs	Function/ Significance
CD44	Breast, pancreatic, colorectal, prostate	Involved in cell adhesion, migration, and tumor initiation
CD24	Breast, pancreatic	Associated with cell adhesion and metastasis
CD133	Colorectal, prostate, hepatocellular, brain tumors	One of the most widely used CSC markers; marks highly tumorigenic cells
EpCAM	Colorectal, hepatocellular	Mediates cell-cell adhesion; associated with epithelial cancers
CD166	Colorectal	Cell adhesion molecule linked to tumor progression
CD34	Acute myeloid leukemia	Hematopoietic stem cell marker also found on leukemic CSCs
CD38	Acute myeloid leukemia	Typically used in combination with CD34 to define stem-like leukemic populations

The table highlights key surface markers associated with CSCs across various tumor types. These markers play crucial roles in processes such as cell adhesion, migration, and tumor initiation. Understanding the expression and function of these markers is essential for identifying CSC populations and developing targeted cancer therapies.

3.3 Evidence of CSCs in Thyroid Tumors

Recent insights have significantly altered the traditional view of thyroid carcinogenesis. While it was once thought that thyroid cancer developed gradually through the accumulation of mutations in normal thyroid follicular cells, it is now evident that specific genetic alterations—such as BRAF, RAS, or RET/PTC rearrangements—can independently drive tumorigenesis. These mutations typically occur in a mutually exclusive manner, suggesting distinct molecular pathways and origins for different thyroid cancer subtypes (Shibru et al., 2008). Supporting this, the "fetal cell carcinogenesis" hypothesis proposes that thyroid cancer arises from remnants of fetal thyroid cells, rather than mature follicular cells. In this model, oncogenes sustain these fetal cells in an undifferentiated, proliferative state by inhibiting their differentiation, offering a robust explanation for both clinical diversity and molecular characteristics of thyroid tumors. This underscores the importance of identifying fetal thyroid cells, particularly thyroid stem cells (TSCs), to better understand thyroid tumor biology (Takano et al., 2006) Concurrently, efforts to define thyroid CSC populations have evaluated eight thyroid cancer cell lines for known CSC markers and ALDH activity. Four lines (FRO, KTC3, ACT1, and 8505C) demonstrated both thyrosphere formation and in vivo tumorigenicity in immunodeficient mice. Notably, lines enriched for $ALDH^+$ cells exhibited enhanced sphere-forming ability, providing functional evidence for the presence of CSCs in thyroid cancer (Shimamura et al., 2014).

In a study by Zito et al., researchers examined several anaplastic thyroid carcinoma (ATC) cell lines cultured in vitro to assess the expression of CD133, a surface marker commonly associated with CSCs. Two cell lines, ARO and KAT-4, exhibited a substantial population of CD133- positive cells, while the others showed minimal or no expression. These findings were validated using multiple methods, including flow cytometry and immunostaining.

Subsequent analysis focused on the ARO cell line. CD133-positive and CD133-negative subpopulations were isolated and compared. The CD133-positive cells demonstrated enhanced proliferation, clonogenicity, and self-renewal capacity, along with elevated expression of OCT-4, a transcription factor associated with stemness.

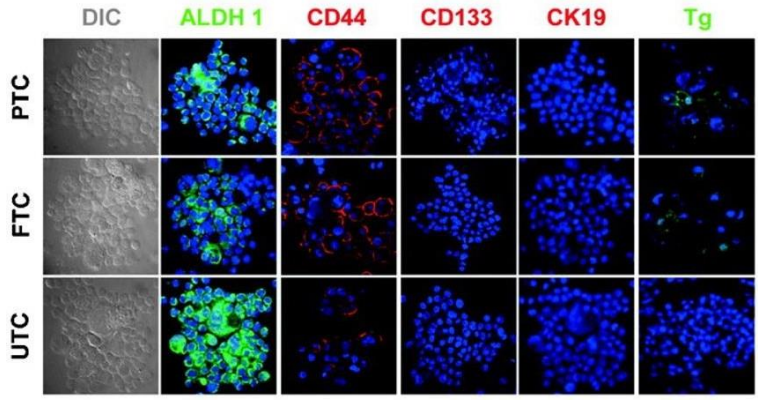
Moreover, CD133-positive cells showed increased resistance to conventional chemotherapeutic agents, implicating them in drug resistance. While these cells retained expression of TTF-1, a marker of thyroid lineage, they lacked other markers of mature thyroid cells, consistent with a less differentiated, stem-like phenotype.

Another study investigating the tumorigenic potential of thyroid cancer cells, researchers identified a small subpopulation—approximately 1.2% to 3.5%—with high ALDH activity, exhibiting hallmark properties of CSCs. These $ALDH^+$ cells demonstrated self-renewal, formed thyrospheres in serum-free culture, and maintained tumorigenicity in immunodeficient mice.

Upon exposure to thyroid-stimulating hormone, both ALDH activity and sphere-forming capacity were suppressed, indicating that CSC behavior can be influenced by the microenvironment. To functionally assess their tumor-initiating ability, sorted $ALDH^+$, $ALDH^-$, and unsorted cells from primary thyroid tumors were subcutaneously injected into nude mice.

$ALDH^+$ cells efficiently initiated tumors in most animals—75% in PTC, 70% in FTC, and 80% in UTC—whereas $ALDH^-$ cells, even at higher doses, rarely formed tumors and only after a delay, likely due to minimal $ALDH^+$ contamination. The resulting xenografts closely resembled the original tumors in both morphology and genetic profile. Notably, BRAF-mutant tumors exhibited reduced ALDH activity, while tumors with combined BRAF and p53 mutations showed the highest ALDH levels, suggesting a link between genetic alterations and CSC enrichment. Immunohistochemical

analysis further confirmed the presence of CD44 in PTC and FTC, reinforcing its association with CSC populations (Todaro et al., 2010).



Confocal microscopy analysis of DIC (left) and immunofluorescence for the indicated antigens on thyroid cancer spheres (Todaro et.al, 8877)

3.4 Mechanisms of CSC-Mediated Therapy Resistance

CSCs exhibit multiple mechanisms of resistance that allow them to evade conventional therapies and contribute to tumor recurrence. One key strategy is their ability to enter a dormant or quiescent state, during which they are non-proliferative and thus less susceptible to treatments like chemotherapy and radiation, which primarily target rapidly dividing cells. Dormant CSCs can persist undetected for extended periods and later re-enter the cell cycle, driving tumor regrowth and metastasis. These cells often express stemness-associated transcription factors such as SOX2 and SOX9, which promote survival in hostile environments and enhance resistance to immune-mediated clearance (Malladi et al., 2016).

Another major resistance mechanism involves the overexpression of ATP-binding cassette (ABC) transporters—transmembrane proteins that actively efflux chemotherapeutic agents from the cell. Notable examples include ABCG2, which exports drugs like doxorubicin and methotrexate, and ABCB1 (P-glycoprotein), which is commonly found in drug-resistant tumors (Moitra et al., 2015). These transporters not only reduce drug accumulation in CSCs but are also regulated by developmental signaling pathways such as Hedgehog, further linking efflux capacity to broader CSC maintenance programs(Sari et al., 2018). Additionally, CSCs evade apoptosis through activation of the Rho-ROCK pathway, which enhances expression of survivin, a protein that inhibits programmed cell death (Yang et al., 2018). This pathway also promotes cellular motility and invasiveness, contributing to both resistance and metastatic behavior.

Beyond these mechanisms, CSCs are tightly regulated by non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), which influence gene expression without encoding proteins. For instance, miR-125b promotes chemoresistance in hematologic malignancies, while inhibition of miR-21 and miR-221 in pancreatic cancer reduces CSC frequency and aggressiveness. LncRNAs such as lncRNA-ROR and HOTAIR further enhance CSC survival and resistance, particularly under chemotherapeutic stress (Nahand et al., 2019) Collectively, these biological strategies—dormancy, drug efflux, apoptotic evasion, and ncRNA-mediated regulation—underscore the complexity of CSC-mediated therapy resistance and highlight the need for targeted interventions to overcome treatment failure and prevent tumor relapse.

4. Molecular regulations CSCs

4.1 Signaling Pathways

Several key signaling pathways that normally regulate survival, self-renewal, and differentiation in healthy stem cells are abnormally activated in thyroid CSCs, contributing to tumor maintenance, progression, and resistance to therapy. The Wnt/β-catenin, Sonic Hedgehog (Shh), and Notch pathways form an interconnected network that supports the stem-like properties of thyroid CSCs (Clara et al., 2020). Overactivation of the Wnt/β-catenin pathway enhances proliferation, spheroid formation in vitro, and resistance to treatments such as radioactive iodine. Epigenetic regulators like KDM1A further increase Wnt activity, reinforcing drug resistance (Zhang et al., 2022). Additionally, reduced expression of tumor suppressor genes such as DAPK1 leads to heightened Wnt signaling and increased expression of stem cell markers including OCT4, SOX2, and NANOG, contributing to a more aggressive phenotype (You et al., 2021).

Notch and Hedgehog signaling pathways also play essential roles in thyroid CSC biology. The Notch pathway, particularly through the Notch1 receptor, promotes survival, self-renewal, and invasiveness, with downstream targets such as c-MYC facilitating tumor

growth (Doolittle et al., 2022). Inhibition of Notch1 has been shown to reduce thyroid CSC proliferation and tumor- forming capacity. Similarly, activation of the Shh pathway through the transcription factor Gli1 supports thyroid CSC maintenance and chemoresistance by inducing survival-promoting genes such as Snail and activating downstream effectors like AKT and c-Met, which enhance cell motility and metastatic potential(Wang et al., 2019). Disruption of these pathways decreases thyroid CSC viability and invasive behavior. Together, the dysregulation of these signaling mechanisms, along with support from the tumor microenvironment, underlies the persistence and resilience of thyroid CSCs. Targeting both the internal signaling abnormalities and the external cues that sustain them may offer a more effective therapeutic approach for advanced thyroid cancers.

4.2 Role of miRNA

MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression by controlling the activity of genes after they are transcribed into RNA. In thyroid cancer, miRNAs have become a significant focus of research because they influence tumor behavior and could improve diagnosis and management. Unlike proteins, miRNAs do not encode genetic information directly but modulate whether certain genes are turned on or off, affecting processes like cell growth, differentiation, and survival. Measuring miRNA expression patterns in thyroid tissue and fine needle aspiration biopsies has shown promise in distinguishing benign from malignant thyroid nodules, with some miRNAs demonstrating high accuracy in identifying specific cancer types such as follicular and Hurthle cell carcinomas (Raza et al., 2014). Thus, miRNAs represent potential biomarkers that could enhance diagnostic precision, especially when conventional methods yield uncertain results.

Beyond diagnosis, miRNAs also play a crucial role in regulating thyroid cancer stem cells, which are implicated in tumor initiation, progression, and treatment resistance. Specific miRNAs, including miR-146a/b, miR-221/222, and miR-21, are frequently upregulated in aggressive thyroid tumors and promote cancer cell proliferation, invasion, metastasis, and immune evasion. For example, miR-15 and miR-16, located in a frequently deleted chromosome region in other cancers like chronic lymphocytic leukemia, help regulate genes critical for cell growth control, and their loss may contribute to unchecked cell proliferation (Zhao et al., 2021). Thus, dysregulated miRNA expression disrupts normal gene networks in thyroid cancer stem cells, enabling these cells to maintain stem-like features and resist therapies. Understanding the complex roles of miRNAs in thyroid CSC biology not only offers insight into tumor behavior but also opens avenues for targeted therapies that could improve patient outcomes.

5. Clinical Implications and Therapeutic Strategies

5.1 Current Challenges in Treating CSC Driven Thyroid Cancers

Treating thyroid CSCs is particularly difficult because these cells are highly resistant to conventional therapies such as chemotherapy, radiotherapy, and radioactive iodine. CSCs can enter a dormant state, allowing them to evade treatments that target rapidly dividing cells. They also overexpress drug efflux transporters like ABCG2, which pump out chemotherapeutic agents, further reducing treatment efficacy. As a result, CSCs often survive initial therapy, contributing to tumor recurrence and metastasis, which complicates patient outcomes and long- term management.

Another significant challenge is the plasticity and heterogeneity of CSCs and their interaction with the TME. The TME not only protects CSCs but can also induce non-CSC tumor cells to regain stem like properties, increasing tumor complexity and therapeutic resistance. Moreover, genetic and molecular differences across thyroid CSC populations, involving pathways such as Wnt, Notch, and Hedgehog, lead to variable treatment responses. The lack of exclusive CSC markers, combined with regulatory factors like microRNAs that influence CSC survival and resistance, limits the development of effective targeted therapies. Overcoming these challenges requires innovative approaches that simultaneously target CSCs, their supportive niches, and the signaling pathways driving their persistence.

5.2 Therapeutic Strategies

Therapeutic strategies aimed at eradicating thyroid CSCs have increasingly focused on disrupting the molecular and microenvironmental mechanisms that underlie their survival, treatment resistance, and capacity for relapse. Central to these efforts is the inhibition of key signaling pathways such as Wnt/β-catenin, Notch, and Hedgehog that are critical for maintaining CSC stemness and self-renewal (Zeng et al., 2023). Preclinical models have demonstrated that targeting these pathways can reduce CSC proliferation, invasiveness, and resistance to therapies like radioactive iodine. Additionally, the role of microRNAs in regulating CSC behavior is gaining attention. Aberrant expression of oncogenic microRNAs promotes resistance and survival, while the loss of tumor-suppressive microRNAs contributes to unchecked CSC activity. Therapeutic modulation of microRNA networks holds potential for re-sensitizing CSCs to conventional treatments and disrupting their regulatory circuits.

Beyond intracellular signaling, effective therapeutic intervention must also address CSC-driven resistance mechanisms such as dormancy and drug efflux. Targeting quiescent CSCs, which evade treatment by entering a non-proliferative state, is critical for preventing recurrence.

Inhibitors of ATP-binding cassette transporters, including ABCG2, are under investigation to improve

intracellular drug accumulation and enhance cytotoxic efficacy (Begicevic et al., 2017). Furthermore, strategies that disrupt the tumor microenvironment by impairing stromal support or inhibiting signals that promote CSC plasticity may diminish CSC viability and their ability to repopulate tumors.

Future research is increasingly focused on targeted metabolomics, an approach that profiles small molecules within cells to identify new substrates transported by ABC proteins, thereby deepening our understanding of the broader biological functions of ABC transporters in cancer stem cells (CSCs). Determining which ABC transporters are selectively overexpressed in CSCs may facilitate the development of precision therapies targeting the most resistant and tumorigenic subpopulations. Rather than concentrating solely on inhibiting drug efflux, a more comprehensive strategy involves exploring the diverse roles these transporters play in CSC survival, dormancy, and plasticity. Elucidating the molecular mechanisms underlying ABC transporter activity could enable the design of interventions that not only increase CSC sensitivity to cytotoxic agents but also disrupt the pathways supporting their persistence and ability to drive relapse (Begicevic et al., 2017). Incorporating these targeted strategies alongside conventional therapies offers a promising avenue to enhance treatment efficacy and long-term outcomes in patients with aggressive or treatment-resistant thyroid cancers.

6. CONCLUSION

Cancer stem cells play a pivotal role in the pathogenesis and progression of thyroid cancer, contributing significantly to therapeutic resistance and disease relapse. Their unique capabilities of self-renewal, differentiation, and quiescence enable them to evade the cytotoxic effects of conventional treatments such as chemotherapy, radiotherapy, and radioactive iodine. These properties facilitate tumor persistence and metastasis, particularly in aggressive thyroid cancer subtypes, thereby posing a major challenge to effective clinical management. The intricate molecular pathways and regulatory networks governing CSC maintenance and survival further complicate therapeutic targeting, underscoring the necessity for novel approaches that address these underlying mechanisms.

The elucidation of CSC functions in thyroid cancer emphasizes their critical contribution to tumor initiation, progression, and resistance to therapy. Investigating the interplay between CSCs and the tumor microenvironment, as well as the influence of signaling pathways and non-coding RNAs, provides valuable insight into the mechanisms driving treatment failure and relapse.

Advancements in the identification of specific CSC markers and regulatory molecules offer promising potential for the development of targeted therapies. Such strategies are essential to overcome the limitations of current treatment modalities and to improve long-term patient prognosis by preventing tumor recurrence and metastasis.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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