REVIEW

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Unfolding of Imminent Bio-Signatures in the Prognosis of Thyroid Cancer; The Emergence of Estrogen Related Receptor Gamma (ERRγ) as a Hurricane

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Abstract

Thyroid cancer's incidence has increased by leaps and bounds over the last years and accounts for 2.8% of new cases of cancers. This increasing bar is partially assisted by enormous screening to understand the sub-clinical status. Advanced tumor growth is the leading cause of thyroid cancer-associated death. However, the complete understanding of the underlying cause is still to be disclosed. The updated clinical assessment evidenced a few major oncogenes viz. RAS, BRAF, and RET as key drivers in the development and progression of thyroid cancer. The BRAF mutation, a major cause of aggressive tumor type in papillary thyroid carcinoma, is frequently reported. The characteristic oncogenic changes imply thyroid cancer to be clinically an ideal model for targeted therapy against RET, RAS, and BRAF mutation. Though the sensitive biochemical marker assay has been improvised, the diagnosis of thyroid follicular neoplasms is still a big challenge as the biopsy aspiration cannot define the nature of the tumor in 30% of the cases. The main hurdle is assisted distinction between follicular thyroid lesions. The discrimination between follicular thyroid adenomas and carcinomas is histologically accomplished. This strictly necessitates the identification of sensitive diagnostic/prognostic markers to mitigate the risk of thyroid cancer and to avoid the unnecessary hurdles of biopsy and surgery. An array of prognostic biomarkers is being used for the diagnosis of thyroid cancer. However, Estrogen Related Receptor Gamma (ERRγ) is setting a new benchmark among the clinical biomarkers. The dramatic expression of ERRγ in thyroid cancer enables itself not only to serve as a characteristic diagnostic marker but also as a therapeutic target. Recently, we have reported that ERRγ is upregulated in 96 papillary thyroid cancer (PTC) and 26 poorly differentiated/ anaplastic thyroid cancer (ATC) samples. Various synthetic ERRγ inverse agonists viz. GSK5182, DN200434, and 24e are fully proved to modulate ERRγ expression in ATC to attain partial cure. If this finding can be assayed on a larger scale the evaluation of this marker may be warranted and informative. This review article highlights the ascending sheds of clinical biomarkers of thyroid cancer. This also reveals the clinical importance of ERRγ as an evolving diagnostic and therapeutic target in thyroid cancer.

Keywords: ERRγ- thyroid cancer- genetic mutations- Lnc RNA- miRNA- biomarkers- Agonist/Inverse agonist

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Introduction

Thyroid cancer has become the most common endocrine disorder in the past few years (Chen et al., 2009). Depending on its origin, most thyroid cancers are derived from follicular epithelial cells such as papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), and Hurthle cell carcinoma. These cells are characterized as well-differentiated cancer, while anaplastic thyroid cancer (ATC) is a form of undifferentiated cancer. Notably, ATC is highly lethal in all forms. The inefficient diagnostic tools and partial knowledge of molecular mechanistic are predicted to be the prime reason for the failure of the clinical cure for PTC and ATC (Cabanillas et al., 2016). Therefore, a better understanding of the signalling pathways during thyroid cancer development and progression has stimulated interest in the identification of biomarkers. These sensitive biomarkers may catalyze the speed of prediction in cancer progression and thus may enable clinicians to decide the most appropriate course of treatment. The biomarkers define the effectiveness of surgery, chemotherapy, and radioiodine ablation. The progression in understanding of molecular alteration and exploration of the novel biomarkers for the diagnosis is

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substantially achieved. Presently, the multistep model of thyroid follicular carcinogenesis derived from Vogelstein's model for colon cancer is widely accepted for screening diagnostic biomarkers in colon cancer (Sameer, 2013). The discovery of thyroglobulin and calcitonin added a revolutionary change in the management of thyroid cancer by encompassing the early diagnosis and more intensive follow-up (Costante et al., 2009; Gianoukakis, 2015). Moreover, the improvisation of genomic and proteomic assays paves the way to identify multiple biomarkers at a glance and elucidate the molecular profile signature for different tumor samples at different stages (Srinivas et al., 2001). Essentially, a heap of biomarkers is in the race to prove their efficacy in the diagnosis of thyroid cancer. In the early stages, most of these biomarkers are employed regularly in the clinical screening of thyroid cancer. Though the picture of diagnostic markers keeps on changing according to the cup of tea, the accuracy and specificity of thyroid biomarkers seem to be a challenging conundrum for wide adoption in routine clinical practice. Hence, it is imperative to focus on finding a breakthrough for the early and accurate diagnosis of thyroid cancer. Meanwhile, the emergence of Estrogen related receptor gamma (ERRγ), an inducible transcription factor and member of the NR3B family, can modulate the activation of the endocrine and metabolic signals such as insulin and glucagon. The ligand-independent constitutive expression of ERRγ owing to its ligand-binding domain (LBD) furnishes a unique identity among the crowd of nuclear receptors (NRs). However, the expression of ERRγ is intrinsically controlled by some tissue specific co-regulators; co-activator, and co-repressor that modulate different endocrine and metabolic signals. The abundant expression of ERRγ is noted in various tissue like the heart, kidney, liver, and brain and thus masters crucial phenomena like hepatic gluconeogenesis, ion homeostasis, and mitochondrial biogenesis (Singh et al., 2019). Interestingly, the expression of ERRγ is upregulated in 40% of the patients encountered with ATC and 60% in poorly differentiated thyroid cancer (PDTC) (Kim et al., 2020).

In this review, we have tried emphasizing the evolution of biomarkers along with their applicability in the effective diagnosis of thyroid neoplasms. This review enlists the popular and evolving biomarkers starting from Long non-coding RNAs (Lnc RNAs; ≥200 bps long), to molecular bio-signatures followed by mutation assisted biomarkers, Micro RNAs (mi RNAs), and serum based/Immunohistochemical markers (Figure 1, Table 1 and 2). The following segments is not meant to be exhaustive or to compile up the biomarkers reported to date but a flashback of established and recently updated biomarkers to make a comparative study for a better understanding of new loopholes in eliminating thyroid cancer. This article also addresses the dual features of ERRγ as a sensitive biomarker in cancer progression and a promising therapeutic target to win the cancer war.

Long non-coding RNAs

Long non-coding RNAs (lnc RNAs; ≥200 bps long) play a major role in epigenetics and regulation of gene expression at transcriptional or post-transcriptional levels. (Huarte, 2015; Batista and Chang, 2013). They are the chief regulator of cancer progression and therefore can serve as oncogenes or tumor suppressors (Chiu et al., 2018). Further, the expression of lnc RNAs has been reported in the tumorigenesis of thyroid cancers (Lan et al., 2015; Yang et al., 2016) thereby suggesting that they can serve as a potential biomarker in diagnostic analysis.

For instance, the basal levels expression of MIR 22 HG, associated with transcription, cell cycle regulation, mRNA splicing, and apoptosis, was downregulated in various stages of thyroid cancer (Qin et al., 2019). Later on, LUCAT1 was reported to be promptly involved in the development of PTC via cell-cycle regulation, proliferation, and epigenetic modifications while the subsequent knockdown of LUCAT1 reduces cell proliferation and invasion (Luzón-Toro et al., 2019). HOX transcript antisense intergenic RNA (HOTAIR) belongs to the recently discovered Long non-coding RNAs (lnc RNAs) that plays a critical role in cancer cell proliferation, survival, migration, drug resistance, and genomic stability (Tang and Hann, 2018). Moreover, HOTAIR has been delineated as a prognostic factor in colon (Kogo et al., 2011), and glioma cancer (Zhang et al., 2013). Alternatively, the expression of HOTAIR has been correlated with increased metastasis (Gupta et al., 2010) along with poor prognosis in human thyroid carcinoma compared with normal cells (Zhang et al., 2017). Noticeably, knockdown of HOTAIR in TPC-1 and SW579 thyroid cell lines showed inhibited cell growth and invasion of the cells. This strongly indicates that HOTAIR is a promising biomarker in patients with thyroid carcinoma (Zhang et al., 2017). However, the expression levels of HOTAIRM1 were significantly downregulated in PTC tissues (Li et al., 2020) whereas the in-vitro data evidenced the overexpression of HOTAIRM1 that leads to impairment of PTC cell invasion, proliferation, and migration. Furthermore, HOTAIRM1 acts as a negative regulator of miR-107 and thus diminishes the expression of TDG (Thymine- DNA glycosylase). This cumulatively suggests that HOTAIRM1/miR-107/TDG axis plays an important role in PTC progression and may be employed as a therapeutic target for PTC. On the contrary, in the case of ATC, the expression of HOTAIRM1 was found to be exclusively upregulated (Zhang et al., 2021) which firmly induces the expression of MET (receptor tyrosine kinase molecule) and further the AKT signalling pathway by repressing miR-144 biogenesis. The lnc RNAs as competing endogenous molecules, sponge the effect of their downstream micro-RNA targets. The mechanistic axis of lnc RNAs and their targets helps in the regulation of thyroid cancer progression or suppression.

Most recent studies have reported the upregulation of a few lnc RNAs such as LINC00488 (Xie et al., 2021), PVT1 (Feng et al., 2018), and lncRNAn384546 (Feng et al., 2019) in PTC. These lnc RNAs bind to their miRNA targets and downregulate their expression level that further variably regulate their immediate sub-targets. For instance, LINC00488 binds to miR-376a-3p and suppresses its expression, which later on negatively regulates PON2 (Paraoxonase-2) (Xie et al., 2021). Lnc RNA PVT1

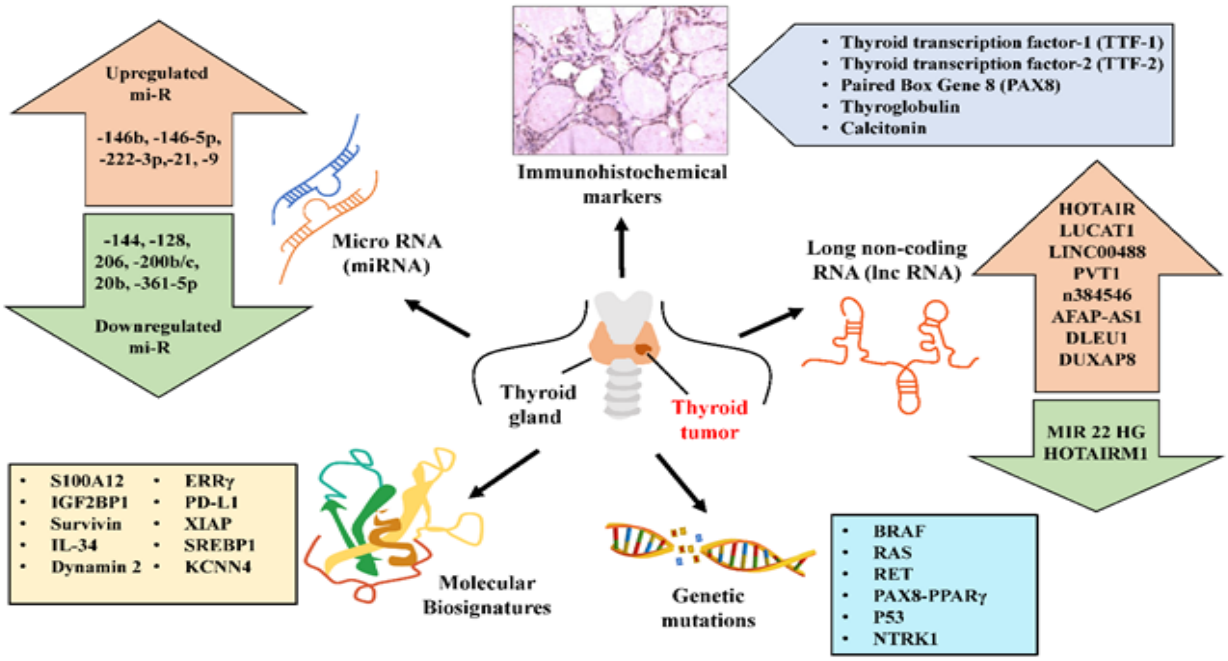

Figure 1. Representation of Updated Biomarkers for Thyroid Cancer

competitively binds to miR-30a and thereby enhances the expression of IGF1R (insulin-like growth factor 1 receptor) (Feng et al., 2018). Lnc RNA n384546 sponges the activity of miR-145-5p (Feng et al., 2019), thereby regulating its downstream target and AKT3.

Similarly, other long non-coding RNAs such as DLEU1 (Li et al., 2020) (deleted in lymphocytic leukemia 1) and DUXAP8 (Liu et al., 2021) are found to be up-regulated in PTC cell lines and tissues. Nevertheless, it was observed that knockdown of DLEU1 suppresses PTC cell proliferation and invasion in vitro, and inhibits xenograft tumor growth in the nude model mice that elicit PTC progression by sponging miR-421 with elevated expression of ROCK1. Likewise, the silencing of DUXAP8 suppressed the migration, proliferation, and invasion activity of PTC cells by targeting miR-223-3p and the concomitant downstream target, CXCR4 (Liu et al., 2021). Furthermore, the downregulated expression level of MIR22HG is correlated with higher age, lymph node metastasis, residual tumor status, N/T stage, and Grade in TC whereas upregulation of MIR22HG leads to increased disease-free survival in TC. To our interest, MIR22HG acts as a modulator of the apoptotic process, transcription, mRNA splicing, cell-cycle progression, and the Hippo signaling pathway in TC (Qin et al., 2019). These facts suggest that MIR22HG may be considered a prognostic marker of TC.

DANCR OR ANCR, lnc RNA (anti-differentiation non-coding RNA), is responsible for de-differentiation of epidermal cells (Malakootian et al., 2018), favours tumor growth and metastasis in hepatocellular carcinoma (HCC) (Wang et al., 2018), induces osteosarcoma progression upon activation of AXL and suppression of miR-33a-5p (Jiang et al., 2017), and facilitates cisplatin insensitivity via modulation of AXL/PI3K/Akt/NF-Kb axis in glioma cells (Ma et al., 2018). Even, few scientific reports have affirmed a direct relationship between DANCR expression and clinicopathologic features of PTC patients (Lu et al., 2020). These clinical findings are sufficient to assume DANCR as a potent biomarker for detecting the cancer spread in different types of tissues or organs. An extensive study to unfold the function of SNHG12 was conducted on different cancer types including bladder cancer, cervical cancer, osteosarcoma, breast neoplasia, and hepatocellular carcinoma (Li et al., 2020). All of these reports cordially signify the upregulated expression of SNHG12 and its associated consequences in tumor size, tumor node metastasis (TNM), and a poor survival rate along with cell proliferation and invasion in vitro. Eventually, SNHG12 was reported to be upregulated in PTC and contribute to cell proliferation and subsequent metastasis (Li et al., 2020).

In ATC, the current findings revealed overexpression of lnc RNA AFAP-AS1 (actin filamentin-1 antisense RNA) reduction of which inhibited the expression of ATC through the miR-155-5p/ETS1/Erk pathway (Ning et al., 2021). Concludingly, we can assume that with the advancement in precision medicine and genetic diagnosis lncRNA is expected to become a target in gene-targeted therapy, but great challenges in research into lncRNA for better treatment strategies remain a conundrum.

Molecular bio-signatures

Screwing up of undefined molecular biomarkers of follicular thyroid carcinoma is assisted to advance the diagnosis of follicular neoplasm, as these tumors do not give access to the definitive diagnosis of cancer. Recently updated biomarkers have been enlisted to promote the ease of diagnosis before the worse prognosis of thyroid cancer.

Rap2A

In a scientific study, the assessment of the relative expression of different genes like CCND2, PLAB, PCSK2, TSHR, and IGF-1Rin in archived thyroid tissue through

Table 1. Summarized Outline of Updated Biomarkers in Different Types of Thyroid Cancer

Marker	Expression	Cancer Type	References
Long non-coding RNA (LncRNA)			
LUCAT1	Upregulated	TC	(Luzón-Toro et al., 2019), (Xiong et al., 2020)
HOTAIR	Upregulated	PTC	(Zhang et al., 2017)
HOTAIRM1	Downregulated	PTC	(Li et al., 2020)
MIR22 HG	Downregulated	PTC	(Qin et al., 2019)
PVT1	Upregulated	PTC	(Feng et al., 2018), (Lin et al., 2020)
HOTAIRM1	Upregulated	ATC	(Zhang et al., 2021)
n384546	Upregulated	PTC	(Feng et al., 2019)
DLEU1	Upregulated	PTC	(Li et al., 2020)
LINC0048	Upregulated	PTC	(Xie et al., 2021)
DUXAP8	Upregulated	PTC	(Liu et al., 2021)
AFAP-A1	Upregulated	ATC	(Ning et al., 2021)
Molecular Biosignatures			
S100A12	Upregulated	PTC	(Wang et al., 2020)
ERRγ	Upregulated	ATC, PTC	(Singh et al., 2019)
IGF2BP1	Upregulated	PTC	(Haase et al., 2021)
Survivin	Upregulated	ATC	(Werner et al., 2017)
XIAP	Upregulated	FTC	(Werner et al., 2017)
IL-34	Upregulated	FTC	(Zhang et al., 2020)
SREBP1	Upregulated	PTC	(Li et al., 2020)
KCNN4	Upregulated	DTC	(Wen et al., 2020)
Dynamin 2	Upregulated	PTC	(Ren et al., 2020)
PD-L1	Upregulated	PTC	(Fadia et al., 2020; Girolami et al., 2020)
MMP-9	Upregulated	PTC	(Zarkesh et al., 2018)
CHI3L1	Upregulated	PTC	(Luo et al., 2017)
Micro RNA (miRNA)			
361-5p	Downregulated	PTC	(Li et al., 2018)
128	Downregulated	PTC, FTC	(Cao et al., 2019)
206	Downregulated	PTC	(Liu et al., 2019; Wang et al., 2019)
200b/c	Downregulated	PTC	(Zhou et al., 2020)
20b	Downregulated	PTC	(Hong et al., 2016)
146b	Upregulated	PTC	(Chou et al.,2017; Hardin et al., 2014)
146b-5p	Upregulated	PTC	(Jiang et al., 2020), (Hardin et al., 2014)
222-3p	Upregulated	PTC	(Jiang et al., 2020)

RT-PCR analysis evidenced a considerable change in three genes like PSCK2, RAP2A, and PLAB in carcinoma as respect to the adenoma. However, RAP2A expression was significantly elevated (25.9 folds, P=0.039) during IHC (immunohistochemistry) analysis of invading carcinoma cells through the thyroid capsule into the blood than that of the cancer cells just under the capsules (Prabakaran et al., 2011). Hence, RAP2A is worthy of further assessment as biomarker is involved in the invasion of thyroid follicular cells.

Leptin

It is an adipose-tissue related hormone that functions to regulate the intake of food, and energy-expenditures along with other biological activities like cell division, proliferation, and cell growth. Interestingly, the recent research reports have beautifully correlated the expression pattern of leptin with the progression of thyroid cancer. For which, 83 patients having papillary thyroid cancer were screened for serum thyroxin, thyrotropin, and leptin with respect to the 90 healthy subjects as control (Hedayati et al., 2011). The serum level profiling of leptin in thyroid cancer revealed an acceptable correlation that may facilitate a definite diagnosis of thyroid cancer besides other specified tumor markers.

HIF-1 (Hypoxia-Inducible Factor1)

Low oxygen tension (intratumoral hypoxia) is a key driver of aggressive disease and poor prognosis. HIF-1; is a hypoxia-induced transcription factor that leads to the reprogramming of genes to support tumor cell survival, metastasis, and hence resistance to chemo/radiotherapy.

Table 2. Brief Outline of the Immunohistochemical Biomarkers for Thyroid Cancer

Immunohistochemical Biomarker	Cancer type	References
Thyroid transcription factor-1 (TTF-1)	100% expressed in PC, FC, and FA%; 88% in MC Few cases of ATC	Katoh et al., 2000; Ordóñez, 2000; Matoso et al., 2011
Thyroid transcription Factor-2 (TTF-2)	100% expression in PC, FA, FC, PDC, and 75% case of MC. 7% in ATC	Nonaka et al., 2008
Paired Box Gene 8	19% of FC and 4% FA 100% expressed in PC, FC, PDC, and FA and 75% MC. 75%-80% in ATC	Lacroix et al., 2004 Bishop et al., 2011; Nonaka et al., 2008
Thyroglobulin	100% expression of TGB in PTC, 75% to 96% in FTC, 57% to 92% in PDC.	Bejarano et al., 2000; Cimino-Mathews et al., 2011; Harach and Franssila, 1988
Calcitonin	Medullary thyroid cancer	Brutsaert et al., 2014; Chernyavsky et al., 2011; Dora et al., 2008

Eventually, in thyroid cancer MAP-K (mitogen-activated protein kinase) and PI3K (phosphatidylinositol kinase), signaling cascade contributes to the activation of HIF-1 (Burrows et al., 2011). This highlights the aspect that direct or indirect targeting of HIF-1 via synergistic inhibition of MAP-K/ PI3K in assistance of radiotherapy may provide a new potential therapeutic approach to improvise the clinical response of thyroid cancer with the diminished metastatic burden.

IL-8 (interleukin8) & IFN-α (interferon-α)

The clinical assessment says that during the progression of chronic or autoimmune diseases, the serum level of IL-8 and IFN-α are expectedly high and so in thyroid patients too. Patients with any disease of the thyroid gland are well discriminated from the healthy person by clinical profiling of IL-8 and IFN-α due to the prevalent pathogenesis of thyroid cancer. Also, IL-8 helps to differentiate between early and advanced stages of the disease where, unlike IFN-α, IL-8 is shown to be predominantly associated with advanced stages of thyroid cancer. Thus, IL-8 may be exploited as a predictor of tumor stage and simultaneously serve as a putative target for therapeutic strategies (Kobawala et al., 2011).

Miscellaneous protein bio-markers

In recent years with the advancement in proteomics and genomics, certain protein-based biomarkers have emerged for the diagnosis of thyroid cancer. For instance, CHI3L1 (chitinase 3-like 1 or YKL-40), a glycoprotein secreted by endothelial cells, inflammatory cells as well as cancer cells and is assigned to regulate inflammation, and cell growth, apoptosis, and angiogenesis (Morera et al., 2019). It has been evidenced to be associated with 20

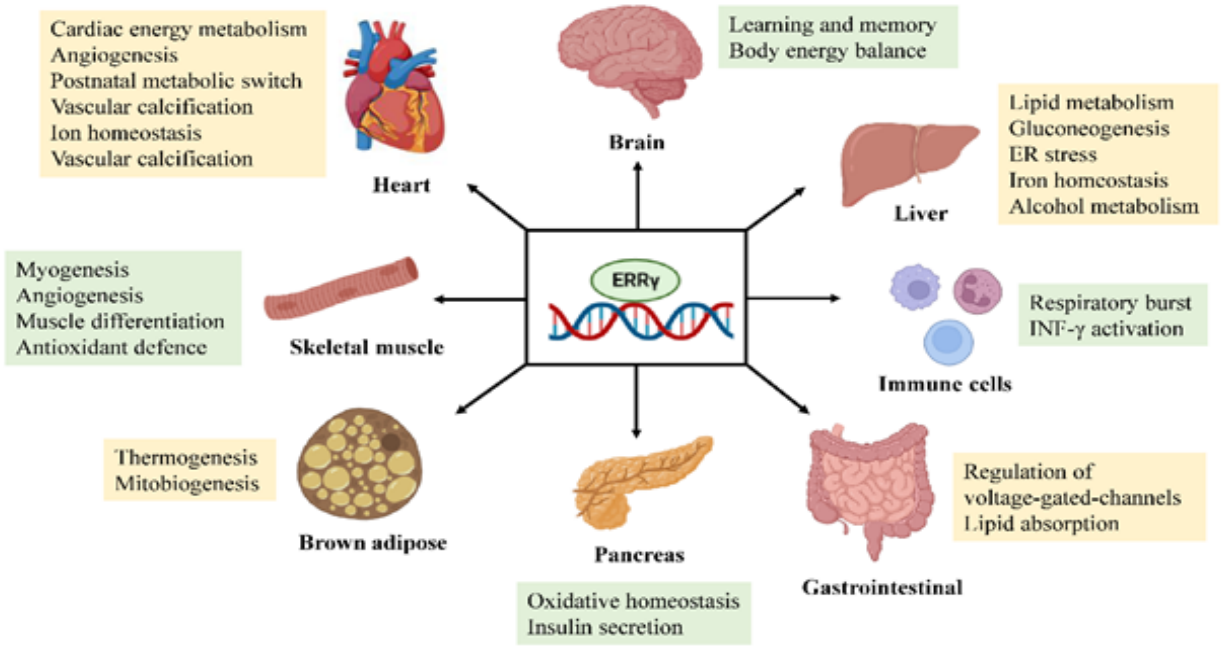

Figure 2. Physiological Role of ERRγ in Different Human Organs/Tissues/Cells

types of tumor metastasis and was elevated in 26 patients with different types of cancer (Liu et al., 2020; Luo et al., 2017). Recently, CHI3L1 has been well characterized for its elevated expression in serum samples of clinical TNM stages of PTC that strongly correlates with clinical stages and hence the survival rate of PTC patients (Luo et al., 2017). These findings indicate that CHI3L1 is an important diagnostic marker and a potential therapeutic target to uproot PTC (Dimitrova et al., 2021). Similarly, S100A12 (Wang et al., 2020), a calcium-zinc and copper-binding protein that plays a crucial role in the regulation of inflammatory processes and immune response increased significantly when examined in 109 PTC patients via immunohistochemistry and western blotting. Moreover, the inhibition of S100A12 led to the suppression of cell proliferation, invasion, and migration in PTC cell lines (TPC-1 and K1) while in-vivo data suggested reduction of tumor growth in xenograft mice model. Hence, it is worthy to conclude that S100A12 can serve as a novel diagnostic marker in PTC. To our interest, analysis of anaplastic thyroid cancer (ATC) protein biomarker via RNA-sequencing data was reported in 36 ATC, 18 poorly differentiated, 132 papillary, and 55 FTC, as well as 124 paired and unpaired normal thyroid tissues. Further, immunohistochemical analysis confirmed the selective de novo expression of IGF2BP1 protein in ATC (Haase et al., 2021) while IGF2BP1 and MAGEA3 expression distinguishes ATC from poorly differentiated thyroid carcinoma. Also, IGF2BP1 represents the foremost promising single-gene marker available for ATC, followed by MAGEA3. IL-34 is reported to be an oncogene in PTC (Zhang et al., 2020) owing to its upregulation in tissue and serum samples. The qRT-PCR and immunohistochemistry technique presented SREBP1 (sterol regulatory element-binding protein 1) as another upregulated biomarker in DTC (Li et al., 2020) and knockdown of which resulted in the impairment of cell proliferation and migration along with check down of cell-cycle progression in the G2 phase. Hence, SREBP1 as an oncogene may serve as a potential biomarker in thyroid cancer.

Similarly, microarray profiling of Survivin and XIAP was investigated in 44 FTC tissue samples (Werner et al., 2017) and corresponding non-neoplastic thyroid specimens. Survivin or XIAP knockdown leads to downregulation of cell viability and proliferation, activated caspase 3/7, and decreased tumor growth in vivo. Both molecules demonstrate significant functional implications in the oncogenesis of FTCs and thus may be employed as viable targets in patients with advanced FTC. In recent studies, it has been observed that ion channels of the plasma membrane play a significant role in various cancers (Pedersen and Stock, 2013) such as ovarian (Asher et al., 2010), prostate (Han et al., 2019), and also thyroid tumor (Mato et al., 2015), (Gong et al., 2018). Reportedly, KCNN4 (Potassium Calcium-Activated Channel Subfamily N Member 4) is upregulated in the case of PTC (Wen et al., 2020), silencing of which restricts the proliferation, invasion, and migration in PTC cells. It was evidenced that KCNN4 induces PTC progression by suppressing apoptosis and inducing epithelial-mesenchymal transition. Thus, KCNN4 may be used as a diagnostic biomarker in PTC.

Dynamin 2 and Programmed Death-Ligand 1 (PD-L1)

The expression profiling of Dynamin 2 (an apoptotic protein) among 112 patients of PTC (Ren et al., 2020) revealed that recurrence and poor prognosis are positively associated with the expression of DNM 2. Therefore, DNM 2 expression may help in the screening of the patients at high risk for clinical therapy. Programmed death-ligand 1 (PD-L1) is a type I transmembrane protein that has been emerging as a favourable predictive biomarker in anti-programmed cell death receptor therapy (Dell'Aquila et al., 2020) or checkpoint inhibitor therapy in different cancers (Girolami et al., 2020). PDL1 expression is also associated with concurrent autoimmune chronic lymphocytic thyroiditis and BRAFV600E mutation-driven PTCs (Girolami et al., 2020). Immunohistochemical data suggests that PD-L1 expression was higher in PTC with LT as compared to PTC without LT (Fadia et al., 2020) while its subcellular cytoplasmic and membranous localization is associated with decreased disease-free survival in PTC patients suggesting PD-L1 as a potential prognostic biomarker (Chowdhury et al., 2016).

ERRγ

Estrogen Related Receptors (ERRs) are the first orphan nuclear receptors identified based on sequence similarity with the classical estrogen receptors (ERα, ERβ, and ERγ) (Hummasti and Tontonoz, 2008). ERRs are significantly expressed in the brain, heart, liver, pancreas, kidney, and placenta (Heard et al., 2000). Even the receptors (ERRs) play a central role in energy homeostasis, cellular metabolism (Figure 2) and cancer (Kim et al., 2016). Estrogen related receptor gamma (ERRγ) is a member of this orphan nuclear receptor family and is expressed in the vital organs. Selectively ERRγ is involved in the development of metabolic diseases such as type 2 diabetes mellitus, liver injury, microbial infection, alcohol-induced oxidative stress (Kim et al., 2016), and impaired iron metabolism. Moreover, ERRγ plays a vital role in the transcription of metabolic pathways based on the circumstances of the cellular environments of the cancer cells. Currently, ERRγ is widely getting acceptance as a potential biomarker in various cancers including liver cancer (Kim et al., 2016), breast cancer (Riggins et al., 2008), prostate cancer (Audet-Walsh et al., 2017), and gastric cancer (Kang et al., 2018). Furthermore, immunohistochemistry (IHC) screening of 36 normal, 96 PTC and 26 ATC thyroid tissue samples unveiled that ERRγ shows higher expression in ATC tissue (Singh et al., 2019). Thus, the screening of ERRγ expression in human TC tissues and its functional studies on TC tissues and cell lines may help us to validate the efficiency of ERRγ as a prognostic biomarker and a therapeutic target for thyroid cancer.

Epigenetic bio-signatures

The heritable genetic alterations that happen independent of DNA sequence is called 'Epigenetics' that exclusively includes histone protein modifications, DNA methylation, and microRNA silencing. All these

mechanisms contribute in DNA regulation by gene silencing.

Functional aberrations of these mechanism lead to defective gene expression that finally results in different types of human cancer. However, the most interesting aspect of epigenetic alteration is that unlike genetic mutation, it is reversible as per the convenience of therapeutic need through anti-cancer agents. This in turn promoted the screening of epigenetic bio-signatures and their clinical status in thyroid cancer to perform primitive detection of chance of cancer formation before it worsens the clinical correction.

Micro RNAs (mi RNAs)

MicroRNAs (miRNAs) are small endogenous non-coding RNAs of approximately 22 nucleotides in length. They have key roles in post-transcriptional regulation of genes by repressing translation and/or degrading their messenger RNA targets in the cytosol, as well as in the alteration of gene expression in the nucleus. Interestingly, they can either act as oncogenes or tumor suppressor. Even, a single miRNA can modulate the expression of an array of genes. The dysregulated levels of many miRNAs are also involved in the metastasis and invasion properties of cancers (Celano et al., 2017; de la Chapelle and Jazdzewski, 2011; Lu et al., 2005). The very primitive study of miRNA expression profile in thyroid cancer was published in 2005 and reported the over expression of seventeen miRNAs whereas six miRNAs were found to be under expressed. PTC (papillary thyroid cancer) being the most prevalent case of thyroid cancer is getting clinical concern. It has been encountered with the upregulation of miR-146b (Chou et al., 2017; Hardin et al., 2014), miR-155 (Lee et al., 2015; Zhang et al., 2013), miR-181b, miR-221, miR-222 (Kondrotienė et al., 2021), and miR-224-5p (Zang et al., 2020). In contrast, downregulation of miR-361-5p (Li et al., 2018), miR-128 (Cao et al., 2019), miR-206 (Zhang et al., 2015), miR-200b/c (Wang et al., 2019), and miR-20b is reported in PTC and restoration of which leads to the inhibition of proliferation, invasion, and migration of cells.

In a recent study, it is found that expression levels of miR-361-5p were downregulated in PTC tissues (Li et al., 2018). In-vitro investigation suggested that there was significant inhibition in migration, proliferation, and invasion in TPC-1 cells when miR-361-5p was overexpressed, also tumor growth was suppressed in-vivo by targeting ROCK1 (Rho-associated coiled-coil kinase 1). miR-128 was found to be downregulated in PTC & FTC tissues (Cao et al., 2019) and was suggested as a potential therapeutic target. The restoration of miR-128 expression leads to the increased apoptotic activity and cell cycle arrest in G0/G1 phase similarly cell migration and invasion activity were also reduced which ultimately inhibited thyroid cancer progression. The expression analysis of miR-206 and miR-200b/c (Zhou et al., 2020) was significantly downregulated in PTC tissues and cells as reported in current investigations. It has been reported that miR-206 negatively regulates MAP4K3 (Liu et al., 2019) (Mitogen-activated protein kinase kinase) and Rap1B (Wang et al., 2019), hence overexpression of miR-206 in PTC cells leads to inhibition of proliferation, invasion, and migration of cells. Furthermore, overexpression of miR-200b/c negatively downregulates the function of Rap1B thereby inducing apoptotic effect. From this data, it can be concluded that miR-206 and miR-200b/c can serve as a potential therapeutic application in the treatment of thyroid cancer.

miR-20b acts as a tumour suppressor in PTC (Hong et al., 2016) as its expression is downregulated. It has also been reported that it inhibits the activity of the MAPK/ERK signalling pathway by targeting SOS1 and ERK2, suggesting its role as a potential therapeutic target. The upregulated expression of miR-222 (Huang et al., 2018) in PTC tissues promotes cell migration and invasion by targeting PPP2R2A (Protein phosphatase 2 regulatory subunit B alpha) suggesting that it can serve as a potential therapeutic target for thyroid cancer. The circulating exosomal miR-146b-5p (Jiang et al., 2020) and miR-222-3p (Jiang et al., 2020) have been reported as indicators of LNM in PTC for the first time, as they were found to be significantly upregulated thereby enhancing the migratory and invasive activities of the cells. It has been reported that the expression of miR-146b-5p and PRRX1(an EMT marker) (Hardin et al., 2014) was up-regulated in PTC cells and the knockdown of miR-146b-5p had an inhibitory effect on invasion and cell proliferation of cells, suggesting a therapeutic potential of miR-146b-5p in PTC (Chou et al., 2017). The multivariate analysis of the expression levels of miR-9 and miR-21 (Sondermann et al., 2015) were found to be significantly increased and could be considered as prognostic factors in patients with recurring PTC. The expression of miR-144 (Guan et al., 2015) was significantly down-regulated in the thyroid cancer tissue, and also the invasion and migration capability of thyroid cancer cells was inhibited by miR-144, suggesting it acts as a tumour suppressor in thyroid cancer.

As per the fascinating concept that expression levels of various miRNAs can pave the way for the early diagnosis of thyroid cancer, the circulating miRNAs are emerging as promising biomarkers because of their stability and tissue-specificity (Celano et al., 2017). Though miRNA expression profiling has been established as a feasible diagnostic marker in analysis of FNA samples, the clinical trials on larger scale is still demanding.

Serum based/Immunohistochemical Markers

Serum based/Immunohistochemical bio-markers belongs to the first generation of thyroid biomarkers. Preferably, serum biomarkers are likely to be sensitive and specific and thereby provides high certainty of clinical diagnosis with ease of measuring. Though bioinformatics assisted analytical tools have taken over serum-based biomarkers, the importance of these markers is still consistent over several decades owing to their simple measurement, reproducibility, availability, low cost-effect, high sensitivity and specificity. Instead, further is demanding for understanding several phenomena of thyroid carcinogenesis and this seems to be unachievable by targeting single serum biomarkers approach (Jensen et al., 2008).

Thyroid transcription factors (TTFs)

Immunohistochemistry reflects an outline in the way of prognosis, prediction, and genetic predisposition regarding thyroid pathology and the contingent emergence of new immunohistochemical biomarkers has led to an efficient diagnosis of thyroid lesions. In several studies, it has been reported that Thyroid transcription factors (TTFs) such as Thyroid transcription factor-1 (TTF-1), Thyroid transcription factor-2 (TTF-2), paired box protein-8 (Pax8), and homeobox protein Hhex (Kimura, 2011; Fernández et al., 2015) facilitates the development of the thyroid gland by adjusting the expression of thyroid-stimulating hormone receptor (TSHr), thyroglobulin, and the thyroid peroxidase (TPO) (Baloch et al., 2018). Hence the genetic alteration in TTFs can lead to the development of tumors and thyroid dysgenesis (Ordonez, 2012; Sequeira et al., 2001). TTF-1 shows a strong nuclear co-localization as observed in 5 differentiated follicular tumors (FC), 13 papillary carcinomas (PC), and 15 follicular adenomas (FA) (Katoh et al., 2000). Moreover, the expression levels of Pax8, TTF-1, and TTF-2 in anaplastic carcinoma (AC) were 79%, 18%, and 7% respectively while in medullary carcinoma (MC) were 75%, 88%, and 75% respectively. From their study, they concluded that Pax8 could serve as a useful marker for the diagnosis of anaplastic carcinomas (Nonaka et al., 2008). The radioiodine therapy of thyroid cancer can be more effective if cotransduction of TTF-1 and Pax-8 gene takes place as it results in NIS-mediated radioiodine accumulation and TPO and Tg-mediated radioiodine organification and intracellular retention. (Mu et al., 2012). It has also been reported that co-expression of both TTF-1 and PAX-8 can induce pro-tumorigenic effects (Dupain et al., 2016).

Thyroglobulin

Thyroglobulin, the precursor molecule for the synthesis of thyroxin (T4) and triiodothyronine (T3), is produced by thyroid follicular cells and is stored within the extracellular colloids in a highly concentrated form. It is organised by normal, hyperplastic and neoplastic thyroid tissue. Unfortunately, thyroglobulin tests are unlikely to be performed when patients receive thyroid hormone suppressive therapy. Additionally, in benign thyroid conditions like thyrotoxicosis, iodine deficiency, benign adenoma, and thyroiditis thyroglobulin assessment may provide an inaccurate reading in terms of rise in thyroglobulin level. However, the careful primer selection of Tg mRNA and TSHr enhances the sensitivity and specificity for detecting recurrence of thyroid cancer in the patients undergoing thyroid hormone suppressive therapy or the patients encountered with circulating anti-thyroglobulin antibodies. Interestingly, thyroglobulin is observed to be elevated in both neoplastic and non-neoplastic lesions of the thyroid gland. Thyroglobulin is significantly increased in patients suffering from follicular–derived thyroid cancer rather than those with benign conditions (Lin, 2008). The poorly differentiated thyroid carcinoma TG expression is decreased relatively while in ATC it always remains absent.

Calcitonin

Calcitonin is secreted by the Para-follicular cells in the thyroid gland, is an anti-hypercalcaemic hormone that opposes the effect of parathyroid hormone (PTH) and reduces blood calcium levels (Vriens et al., 2009). The exclusive increased expression of calcitonin is not only restricted to the medullary thyroid cancer (MTC), as it has been disclosed in other circumstances viz. C-cell hyperplasia, thyroid nodules of follicular cells, increased body-mass index and age, smoking, breast feeding and lung carcinoma (SCLC) but with prevalence of merely 5% cases.

Consequently, the preference of calcitonin as a diagnostic marker for MTC has switched from 'mass-screening' paradigm to a particular condition. On the basis of clinical progression MTC has been categorized into familial type (~25%) and a sporadic type (75%). The expression level of calcitonin in MTC is directly proportional to the development of tumor burden as manifested by tumor-size coupled with lymph node or distant metastasis. In contrast, patients diagnosed with poorly differentiated or metastatic MTC are encountered with low expression level of calcitonin because of the heterogeneity of tumor-cells. This type of clinical status is considered to be more aggressive in nature. Altogether, calcitonin assay is employed as a predictor of tumor aggressiveness. Calcitonin assay is frequently coupled with provocation stimulation or genetic test using RET mutation to add sensitivity and specificity

ERRγ; a novel therapeutic approach of thyroid cancer

A smart move in medicinal chemistry to adapt synthetic strategies for fine-tuning of ERRγ expression in various cancers is flourishing bit by bit. Several reports are available in favour of synthetic drugs that can modulate the expression of ERRγ in different cancers for better output in clinical war. For instance, agonist/inverse agonists of ERRγ like DY131 against gastric (Huang et al., 2021; Kang et al., 2018), uterine endometrial cancer (Yamamoto et al., 2012) and prostate cancer (Yu et al., 2007); GSK126 along with DY131 against gastric cancer (Huang et al., 2021); GSK4716 derived SLU-PP-1072 (inverse agonist) against prostate cancer (Schoepke et al., 2020); 24e, GSK5182, and DN200434 against thyroid cancer are successfully enlisted (Kim et al., 2020). Briefly, GSK5182 acts as an inverse agonist for ERRγ and induces an augmentation of iodine uptake in CAL-62 and BHT-101 through modulation of sodium iodine symporter (NIS). Reportedly, GSK5182 can increase the NIS trafficking in ATC cells via the regulation of ERRγ and the MAP kinase signalling pathway. Even, the synergistic effect of GSK5182 with I131 exemplifies the clinical potential of ERRγ in ATC (Singh et al., 2015). Moreover, DN200434, an inverse agonist of ERRγ, increases NIS expression in mouse model that is prone to restrict the tumor growth upon radio-iodine therapy (Singh et al., 2019). Thus, ERRγ may be exploited as dual character as in a sensitive biomarker in the assessment of thyroid cancer as well as an instant therapeutic target for the cure.

In Conclusion, in this review we have updated the evolving biomarker to set a background for a comparative

study on the present scenario of diagnostic tool in thyroid cancer. With respect to the special point of references we could demonstrate the rising of bio-markers involved at the molecular level during the development of thyroid cancer. The extract of the literature indicates that very selective bio-signatures can be employed as thyroid markers in routine clinical practice while application of other remains suspicious. We have also introduced ERRγ with its clinical features and adaptations in thyroid cancer whereby it is fair to designate ERRγ as promising biomarker along with a potent therapeutic target to uproot thyroid cancer. Undoubtedly, the staging, gradation, and screening of thyroid cancer warrant the need of sensitive cytological/histological bio-markers that will signal the early progression of thyroid cancer. However, the feverish context of drilling the promising biomarker of thyroid cancer needs utmost the care till date.

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The authors state that for this investigation no experiments have been performed on humans or animals. Confidentiality of data. The authors state that they have followed the protocols of their institute for the publication of patient data. Right to privacy and informed consent. The authors state that in this article there are no patient data.

Abbreviations

TC; Thyroid Cancer
PTC; Papillary Thyroid Cancer
ATC; Anaplastic Thyroid Cancer
FTC; Follicular Thyroid Cancer
ERRγ; Estrogen Related Receptor Gamma
LncRNA; Long-non coding RNA
miRNA/miR; Micro RNA
HOTAIR; HOX transcript antisense intergenic RNA

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