Review Article

BRAF Mutation and its Effects on Radioiodine Uptake in Patients with Anaplastic Thyroid Carcinoma

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Abstract

Context: Anaplastic thyroid carcinoma (ATC) is poorly differentiated subtype of thyroid cancer which either resistant to radioactive iodine (RAI) therapy or conventional chemotherapy. Each process of the biological characteristics in normal thyroid cells, including iodide uptake by sodium-iodide symporter (NIS), synthesis of thyroglobulin (Tg), expression of thyroid peroxidase (TPO) and receptor for thyrotropin (TSHR), can be an onset stage for emerging thyroid carcinoma. Decrease or absence of NIS mRNA in thyroid carcinomas has well described for resistant to RAI therapy in these patients.

Evidence Acquisition: The original articles related to the role of the BRAF mutations on the sodium-iodide symporter functions and radioiodine uptake in patients with anaplastic thyroid carcinoma were found by a search in Scopus, PubMed, Science direct, Springer and some else with an emphasis on literature published in the recent years.

Results: The related studies disclosed that mutations in the mitogen-activated protein kinase (MAPK) pathway happen in more than 90% of thyroid cancer. Also serine/threonine-protein kinase BRAF is an important component of the MAPK pathway. Its mutations cause reduction of NIS mRNA compared to tumors with other mutations.

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Introduction

The most widespread endocrine malignancy auditing for 1% of cancers worldwide, is thyroid cancer [1]. The annual incident rate of thyroid cancer in various parts of the world is approximately about 0.5 to 10 in every 100,000 individuals [2]. Benign follicular adenomas, well-differentiated papillary and follicular carcinomas, and also aggressive anaplastic carcinoma are the phenotypes of tumors extracted from thyroid epithelial cells [3]. Well differentiated tumors are more than 95% of thyroid cancers that respond to thyroid hormone suppression and surgery followed by radioactive iodine (RAI) therapy. Although repeat occurrence of disease occurs in about 30% of cases [4].

Anaplastic thyroid carcinoma (ATC) is poorly differentiated subtype of thyroid cancer that accounts for 2% to 5% of all thyroid cancers [5] which either resistant to RAI or conventional chemotherapy [6]. This cancer may initiate de novo or evolve from papillary or follicular thyroid carcinomas [7]. Scilicet, in 50% of the patients, it may occur following a long-term history of goiter, thyroid adenoma, papillary or follicular carcinoma [8]. Swift growth and distribution with a rapidly growing neck mass are characteristic of the clinical course of anaplastic thyroid carcinoma. For most patients, complete surgical excision is not possible, and radiation and chemotherapy are not efficient. The time of morbidity to mortality in all anaplastic thyroid carcinoma patients are 2 to 7 months [9], with a 10-years survival of approximately 3 percent [10]. In this malignancy, tumor cells usually permeate into encircling tissues at the time of diagnosis, and have undesirable prognosticated. Among thyroid carcinomas, ATC is the third in frequency at a rate of 1.6% after papillary and follicular carcinomas [11]. It can be seen at any age; however, it is more common between 60 and 70 years old, and women are five times more susceptible to developing the cancer [12].

Each process of the biological characteristics in normal thyroid cells, including iodide uptake by Sodium-Iodide Symporter (NIS), synthesis of thyroglobulin (Tg), expression of thyroid peroxidase (TPO) and receptor for thyrotropin (TSHR), can be an onset stage for emerging thyroid carcinoma [13]. Survey the related studies demonstrated that expression of NIS, TPO, Tg and TSHR gradually decrease in thyroid cancers [14, 15]. Also, decrease or absence of NIS mRNA in thyroid carcinomas has well described [16]. While immunohistochemistry studies have investigated that NIS protein is overexpressed in the cytoplasm of many thyroid tumor cells [15].

Over all of, somatic gene mutations in components of the principal oncogenic pathways such as: mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinases (PI3K), Wnt/β-catenin pathway, hedgehog signaling pathway, etc. high occur in ATC [17]. The related studies exhibited that mutations in the MAPK pathway happen in more than 90% of thyroid cancer [18, 19]. Also serine/threonine-protein kinase BRAF is an important component of the MAPK pathway [20]. In this review article, we survey the mechanism of BRAFV600E mutation, its effects on sodium-iodide symporter (NIS) functions in thyroid cells and outcomes inhibition of this gene on radioiodine uptake by thyroid tumor cells in radioactive iodine therapy manner.

Evidence acquisition

The original articles related to the role of the BRAF mutations on the sodium-iodide symporter functions and radioiodine uptake in patients with anaplastic thyroid carcinoma were found by a search in Scopus, PubMed, Science direct, Springer and some else. We used related keywords like: BRAF mutation, BRAF signaling, anaplastic thyroid carcinoma, sodium-iodide symporter with an emphasis on literature published in the fifteen recent years (2000-2015).

Results

(A) MAPK signaling

Mitogen-activated protein kinase (MAPK) signaling system be made up of distinct pathways that act to control several dissimilar cellular processes such as gene transcription, synaptic plasticity motility, cell proliferation, apoptosis, metabolism and long-term memory. MAPK pathway includes three main signaling pathways: Extracellular-signal-regulated kinase (ERK) pathway, c-Jun N-terminal kinase (JNK) pathway and p38 pathway that able to activate various downstream effectors. Furthermore, these different pathways are joined by combining parts from an extensive mitogen-activated protein kinase (MAPK) signaling toolkit [21].

The MAPK kinase kinases (MAPKKKs), MAPK kinases (MAPKKs), MAPKs, MAPK scaffolding and target proteins as diverse transducers are composed of each mitogen-activated protein kinase (MAPK) signaling toolkit [22]. The rapidly accelerated fibroblast (RAF) family of serine/ threonine kinases, the MAPK/extracellular-signal-regulated kinase MEK1/2, and ending with the extracellular signal-regulated kinase (ERK), are components of the major group of the kinases in the MAPK pathway [23]. Activation of the MAPK pathway is a frequent event in many cancers [24].
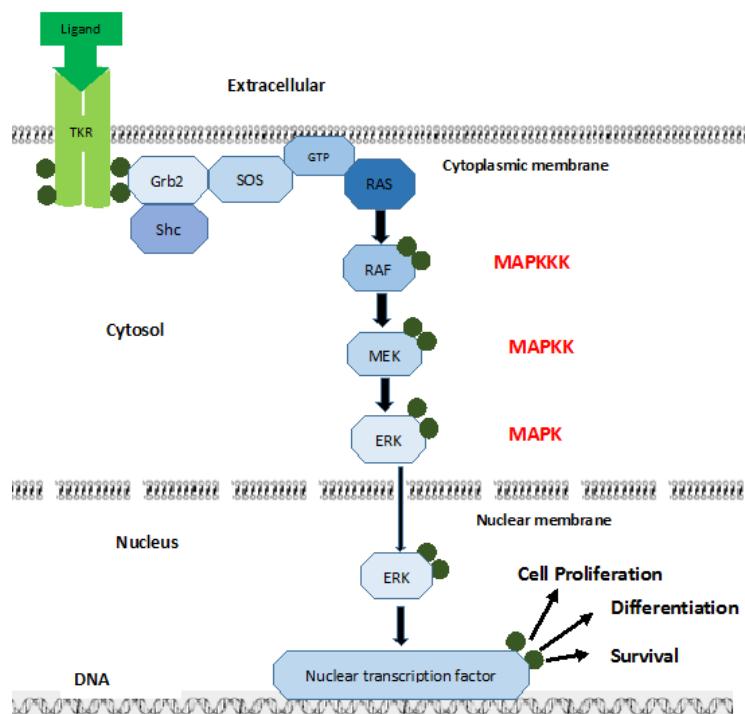

Figure 1. Schematic overview of ERK pathway activation. Both protein tyrosine kinase-linked receptors (PTKRs) and G protein-coupled receptors GPCRs capable activate this pathway. For the PTKRs, ligand binding usually cause receptor dimerization, which allows the cytosolic tyrosine kinase domains to come together and to phosphorylate each other. These phosphorylated residues, then function as docking motifs to pull in signaling components such as Shc, growth factor receptor-bound protein 2 (Grb2) and Son-of-seven less (SoS) that then activate the small GTP binding protein RAS. Then, activated Ras interacts with the protein kinase RAF, which initiates the phosphorylation cascade of the ERK pathway. The activated phospho-ERK1/2 leaves the plasma membrane to diffuse into the cytoplasm and then into the nucleus, where it phosphorylates and activates a number of transcription factors that control the expression of genes, which are required for proliferation, differentiation and survival [Reviewed by ref. 26, 27].

(B) ERK pathway

The extracellular-signal-regulated kinase (ERK) pathway is one of the major signaling cassettes of the MAPK signaling pathway. Control of cell proliferation and the synaptic plasticity responsible for learning and memory are important example of its signaling functions [25].

Both protein tyrosine kinase-linked receptors (PTKRs) and G protein-coupled receptors GPCRs can activate this pathway. For the PTKRs, growth factors such as platelet-derived growth factor (PDGF) or epidermal growth factor family (EGF) usually cause receptor dimerization, which allows the cytosolic tyrosine kinase domains to come together and to phosphorylate each other. These phosphorylated residues, then function as docking motifs to pull in signaling components such as Shc, growth factor receptor-bound protein 2 (Grb2) and Son-of-seven less (SoS) that then activate the small GTP binding protein Ras. Then, activated Ras interacts with the protein kinase RAF, which initiates the phosphorylation cascade of the ERK pathway. The activated phospho-ERK1/2 leaves the plasma membrane to diffuse into the cytoplasm and then into the nucleus. Afterward, it phosphorylates and activates a number of transcription factors [26] such as the E26 transformation specific (ETS) family, as well leads to genes expression that promote cell growth, differentiation and survival (Fig. 1), [27].

(C) BRAF

BRAF gene located on chromosome 7q34 and encodes a cytoplasmic serine threonine kinase protein. This protein is trans-located on the cell membrane after being bound and activated by RAS, which results in the phosphorylation and activation of mitogen activated protein kinase (MAPK) and other downstream targets of the MAPK signaling pathway. It accountable for controlling cell proliferation and differentiation through the MAPK pathway and has an important role in the regulation of the MAP kinase/ERK signaling pathway [23, 28].

In mammalian cells, three isoforms of the serine threonine kinase RAF, containing ARAF, BRAF and CRAF (RAF1) with different tissue expression rates, are reported [29]. Mutation and improper activity of this gene may result in a pro-mitogenic force, causing apoptosis resistance, tumor progression, abnormal differentiation and proliferation of many human cancers such as hairy cell leukemia [30,31], multiple myeloma [31,32], colorectal cancer [31], colon cancer [33], non–small-cell lung cancer (NSCLC) [34], ovarian carcinomas [35], 1–3% of lung cancer [36], melanoma [37], more than 50% of papillary thyroid carcinoma [38,39] and 25% of anaplastic thyroid carcinoma [5,40].

At the first time, a mutation in BRAF gene has been described in 2002 [31]. Most often, mutation occurred in either exon 11 or 15 across all malignancies and at a thymine-to-adenine transversion at nucleotide 1799 (originally listed as 1796), which leading to a valine to glutamic acid change at codon 599 (V599E). At a later time, because of a nomenclature change, it renamed to V600E [31, 41]. Naturally, the most important BRAF V600 mutation is V600E and other mutations such as V600K, V600M, V600R, V600D and V600G are less common [42].

Hydrophobic interactions between the activation loop and the ATP binding site maintain the dephosphorylated wild-type BRAF protein in an inactive structure. Conformational change, due to V600E mutation, is believed to mimic the phosphorylation in the activation segment. Indeed, by insertion of an acidic residue closes to a site of regulated phosphorylation (at serine 598), cause the G-loop segment activation of BRAF and capable bind to MEK and ERK as a monomer [43]. Therefore mutated BRAF, persists continuous phosphorylation of MEK and elevation ERK phosphorylation and resulting in target genes transcription. Whereas, it is resistant to negative feedback signals such as MAPK phosphatase 1 (MKP1) that attempt to counterbalance the ERK activation [44].

Furthermore chromosomal rearrangement that leads to BRAF fusion to AKAP9 can also BRAF

activation, which similar to the BRAFV600E mutation [45]. Moreover, another mechanisms, for example: RET gene mutation, which expresses RET proto-oncogene as a transmembrane receptor tyrosine kinase, can activate the RAF-MEK-ERK signaling pathway [46]. We investigate this gene in medullary thyroid carcinoma in several studies [47-50].

(D) Sodium-Iodide Symporter

The iodide supply of the thyroid gland involves a two-step transport process. The first step of talent transport and concentrate iodide within the thyroid gland is defined in the production of the iodine-containing thyroid hormones. In the polarized thyroid follicular cells, iodide uptake occurs across the basolateral membrane protein, which is named sodium-iodide symporter or SLC5A5, in an active iodide transport manner during directed Na^+ gradient [51]. The second step is passive transport across the apical plasma membrane. The proteins insuring in this step are two potential apical iodide transporters belonging to the sodium/glucose co-transporter SLC family. One of them is a 110-kDa protein which named pendrin. It is a chloride/bicarbonate or formate exchanger and encoded by the PDS gene or SLC26A4 [52]. Another protein for the apical iodide transporter is AIT. It is a 69-kDa protein that encoded by the SLC5A8 gene and sharing 46% identity with human Na^+/I^- symporter [53].

Sodium-iodide symporter (NIS), is one of the thyroid iodide-metabolizing proteins. It is an integral plasma membrane glycoprotein with 13 putative transmembrane domains and expressed at the highest level in the thyroid cells and lactating breast [51, 54]. Regulation of NIS mRNA expression in thyroid follicular cells, is binding of transcription factors such as PAX8 and cAMP responsive element binding protein to the NIS upstream enhancer in response to TSH stimulation [55]. The effectiveness of radioactive iodine (RAI) ablation in RAI therapy after thyroidectomy is dependent on active intracellular transport and trapping of iodine by the NIS protein [56].

Based on the related studies, 25% of primary well-differentiated thyroid tumors such as papillary thyroid carcinoma (PTC) and 50% of aggressive anaplastic thyroid carcinoma are RAI resistant (RAIR). Therefore, efficiency of medical therapies after thyroidectomy are limited [57]. In addition, the loss of radioactive iodine (RAI) uptake by thyroid cancer cells for survival and mortality rates, is important [58]. Most often the mechanism of iodine radiotherapy resistance begins through decreased expression of thyroid iodide-metabolizing genes such as: thyroperoxidase, thyroid stimulating hormone (TSH) receptor, sodium-iodide symporter (NIS) and thyroid transcription factor 1 in the process of dedifferentiation [15, 59, 60].

(E) BRAF V600E and Sodium-Iodide Symporter

A result of conditional activation of BRAFV600E is effected on sodium-iodide symporter gene expression in thyroid cells [60-62]. BRAF gene mutations cause reduction of NIS mRNA level compared to tumors with other mutations or with no identifiable genetic changes in human thyroid cancers [63]. Several regulatory mechanisms of NIS gene expression in thyroid carcinomas have been evaluated. One of them has been described in activation of TGFβ/Smad signaling. In normal thyroid cells, activation of BRAF induced TGFβ/Smad signaling activation. Transforming growth factor (TGF) β acting via Smad is a potent repressor of NIS gene expression in normal thyroid epithelial cells. Therefore, impact of BRAF mutation and severity activation of TGFβ/Smad signaling are noticeable reasons for NIS gene repression induction [64].

CpG island methylation at promoter region, is another regulation mechanism of NIS gene expression in thyroid carcinomas. It is an important epigenetic alteration to silence several genes in various cancers [65], such as: colorectal cancer [66], pancreas cancer [67], and papillary thyroid carcinoma [68].

In addition, reports of related studies demonstrated that BRAF mutations impair the NIS protein targeting to the plasma membrane [69]. This occur is justifiable evidence for loss of RAI avidity and resistant to RAI therapy in patients with BRAF mutations [60, 70].

(F) Targeting BRAF signaling pathway as therapeutic targets

The standard treatment of thyroid carcinoma is surgery followed by radioiodine remnant ablation [71]. Usually BRAF mutations cause tumor cells to be dedifferentiated and to have lost the expression of the sodium–iodide symporter (NIS), so they cannot concentrate radioiodine on RAI therapy [72]. However the results of immunohistochemistry studies verified that, in some thyroid cancer samples NIS protein is over expressed intracellular and cannot localize in plasma membrane [73]. In addition BRAF mutations bring about up-regulate various tumor-promoting molecules and cause thyroid carcinomas to be so aggressive and mortality [74]. Indeed, it is strongly associated with poor prognosis of thyroid cancer, including hostile pathological characteristics, increased repetition rates and failure of treatment [75].

Strategy of target therapy was interfering in a specific molecular target, which has a critical role in tumor growth and progression without harming normal cells. Current knowledge about molecular biology of thyroid carcinomas can be useful for target therapies and better treatment in advanced thyroid carcinomas such as ATC [76]. Usually this type of treatment has been applied especially to oncogenic protein kinases [77], and targeting BRAF in thyroid cancer [78]. The results of related studies have been shown that BRAF V600E mutation is associated with preferential sensitivity to MEK inhibition in human cancer [79].

Several molecules, including antisense drugs [80], histone deacetylase inhibitors [81] such as: valproic acid [82], retinoic acids [83], and also BRAF inhibitors like as: sorafenib, vemurafenib (PLX4032), RAF265, PLX4720, XL281 with different selectivity, have been developed. Treating the patients at the based on inhibition of BRAF signaling able to induce reexpression of NIS gene. These drugs can inhibit the cancer cells proliferation, survival, motility, and their invasion in vivo and in vitro [84-86]. Besides, MAPK inhibitors can persuade to restore the expression of NIS gene in patients with BRAF mutations and enhanced radioiodine uptake in RAI therapy [87]. Selumetinib (AZD6244; ARRY-142886) is a tight-binding, uncompetitive inhibitor of MEK1/2 that develop in clinical currently. It is one of the MAPK inhibitor, which can inhibit BRAF [88], and enhanced radioiodine uptake in advanced thyroid cancer [89].

In 2008 Nikolas K. Haass and colleagues indicated that in a two-dimensional cell culture, AZD6244 was cytostatic and decreased the growth of melanoma cells in a dose-dependent manner through the induction of G1-phase cell cycle arrest. Also, in the three-dimensional spheroid model, the effects of AZD6244 was largely cytostatic and reversible, with drug washout leading to spheroid regrowth. Finally, AZD6244 treatment reduced phospho-ERK in the tumors and meaningfully suppressed tumor growth. Furthermore followed these studies, they revealed that co-administration of MEK inhibitor and chemotherapy drug, potentially lead to tumor regression [90]. In addition, AZD6244 is a potent and effective MEK1/2 inhibitor. It can revoke activation of ERK1/2 in response to activation of transient expression of KRASV12. Also it can repeal the BRAF600E, KRAS12V or KRAS13D genes expression in tumor cells [91].

Conclusion

Chemotherapy drugs have failed in clinical trials for anaplastic thyroid carcinoma. Therefore, novel approaches to ATC therapy are needed. Surgery followed by radioiodine remnant ablation is the standard care for thyroid cancer patients. Radiation therapy is very specific and has a low rate of

adverse effects. Sometimes iodine uptake options are few and survival is poor. NIS protein, which has important role and is necessary for iodine uptake, is regulated at different levels, such as: transcriptional, translational, posttranslational, targeting and intracellular distribution. Nevertheless, NIS protein levels in thyroid cancers were reported to be higher than in normal tissue, impaired iodide uptake were seen in some thyroid carcinoma patients. Therefore NIS dysfunction can be caused by absent or decreased NIS gene expression and by damaged targeting plasma membrane localization and often not polarized but present at the apical and basal membrane. Recent advances studies in the genomic alterations of ATC and NIS regulation have brought about the possibilities of new therapeutic approaches for patients which are resistant to radioactive iodine in RAI therapy. Usage target therapy can be beneficial in selective target therapy for effective treatments and increase the survival of ATC patients.

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